

Optic Disc Imaging in Conscious Rats and Mice

Bruce E. Coban,¹ Andrew C. Pearch,¹ Pentti T. Jokelainen,² and David F. Bohr³

PURPOSE. To determine whether useful images of the optic discs of conscious rats and mice can be obtained by using a photo slit lamp and a modified Goldmann-type fundus contact lens.

METHODS. Testing was performed with a photo slit lamp equipped with two 2x teleconverters and a digital camera through a Goldmann-type fundus contact lens that was fabricated for the rodent eye.

RESULTS. Images of the rat and mouse optic discs were obtained that are comparable to those used by ophthalmologists to assess optic neuropathy in glaucoma, a key part of the standard of care and of clinical investigation of this disease. The cup in the optic disc image of these rodents is darker than the neural rim of the disc, rather than lighter, as it is in humans.

CONCLUSIONS. In addition to the application of this imaging method to studies of the effect on optic disc cupping of induced increased intraocular pressure in rats and mice, by detecting and documenting the onset and the course of optic neuropathy, it should be valuable in identifying animal models of glaucoma, in studying neuropathogenic mechanisms, and in assessing the effects of experimental therapies. (*Invest Ophthalmol Vis Sci.* 2003;44:160-163) DOI:10.1167/iov.02-0105

The identification in 1997 by Stone et al.¹ of a human gene for primary open-angle glaucoma (POAG), a major cause of irreversible blindness worldwide, led naturally to the anticipated² development of an animal model of this disease in a rodent system. Recently, a second causative gene for POAG has been described.³ However, a laboratory animal model of POAG is still not available. One factor in the delay was resolved by a reliable, noninvasive method for measurement of intraocular pressure (IOP) in conscious rats⁴ and mice,⁵ because increased IOP, also called ocular hypertension, is the most important risk factor in human POAG. Still missing for phenotypic assessment of a rodent mimic of this disease is a reliable method for documenting in the living animal the sine qua non of POAG—its characteristic optic neuropathy.

The present report describes a method for obtaining images of the optic disc in conscious rats and mice that are comparable to those routinely available to ophthalmologists for detecting the onset and monitoring the course of optic neuropathy in patients with POAG. This is a key part of both the current standard of care and of clinical investigation of this disease.⁶ By obtaining documenting images of the optic discs over time in rodent models of POAG, changes in the discs' appearance that

are comparable to those observed clinically in humans can be correlated with the histology and histochemistry of the optic nerve head.

MATERIALS AND METHODS

The experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, the tenets of the Declaration of Helsinki, and the guidelines of the University Committee on the Use and Care of Animals (UCUCA) of the University of Michigan. Optic disc images were obtained in Brown Norway rats (Harlan Sprague-Dawley, Indianapolis, IN) and in CS7 Black mice (Jackson Laboratories, Bar Harbor, ME). Initial experiments were performed under general anesthesia with intraperitoneal pentobarbital sodium (50 mg/kg). Subsequently, images were obtained in conscious, untrained rats and mice that were gently restrained in modified rat or mouse polyethylene cones (Decapicone; Braintree Scientific, Inc., Braintree, MA) as has been described for Goldmann applanation tonometry in awake mice.⁵ Briefly, the apex of the cone was trimmed so that the animal's head could protrude until the cut edge of the cone crossed temporally midway between the eyes and ears. As in humans, the animals' pupils were dilated with drops of tropicamide (Mydracil 1%; Alcon Laboratories, Inc., Ft. Worth, TX), and their eyes were topically anesthetized with drops of proparacaine 0.5%. The animals were positioned at a photo slit lamp (900 P-BQ; Haag-Streit, AG, Köniz, Switzerland) on a Plexiglas platform supported by aluminum posts that fit into the holes for the chin-rest paper pins.

Goldmann-Type Fundus Lens with Hruby Preset Lens-Type Holder

A special Goldmann-type fundus contact lens (Fig. 1) was fabricated for the rodents: The human fundus contact lens (Haag-Streit) was modified in a 5.7-mm diameter zone centered on its posterior surface that had a 2.5-mm radius of curvature (for the human it is 7.6 mm). A 7-mm diameter flat zone was machined centered on the anterior surface, and a coverslip with a broadband, antireflection coating was cemented to it. A Hruby preset lens holder (Haag-Streit) was modified to support this contact lens.

The contact lens, with a minute drop of 2.5% methylcellulose on the contact area, was placed on the cornea. One person adjusted the animal's position until the disc came into view for the other person, the photo slit lamp operator. The Hruby lens-type holder supported the contact lens. Its control lever was used for alignment, adjusting light reflexes away from the disc by slight turning and tilting of the contact lens, and maintaining focus by avoiding the movement of these small animals that a finger-held contact lens can cause.

Digital Image Acquisition

This photo slit lamp has a mirror housing that incorporates a front surface mirror that swings into the optical path and closes its flash contact when its shutter release bar is activated. The photo slit lamp settings in this study included: Galilean power changer at 25x, mirror housing diaphragm at 3, slit illuminator spotlight diameter at 3 mm and filter at heat-absorbing, and light source flash at high. Attached to the mirror housing was the objective tube for monophotography, an adapter (model F; Nikon, Melville, NY), two teleconverters (TC-201 2x; Nikon) and the camera.

A digital camera (FinePix S1 Pro; Fujifilm, Elmsford, NY) was used for the imaging. Its picture element is a 23.3 × 15.6-mm charge-coupled device (CCD) with primary color filter with 3.4 megapixels in

From the ¹Eye Research Fund Laboratory, and the Departments of ²Cell and Developmental Biology and ³Physiology, Medical School of the University of Michigan, Ann Arbor, Michigan.

Supported in part by research grants from the Lloyd and Mabel Johnson Foundation and from Keith A. Kobet, MD.

Submitted for publication January 30, 2002; revised July 2, 2002; accepted July 29, 2002.

Commercial relationships policy: N.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Bruce E. Coban, Eye Research Fund Laboratory, 2350 Washtenaw Avenue, Ann Arbor, MI 48104; becohan@umich.edu.

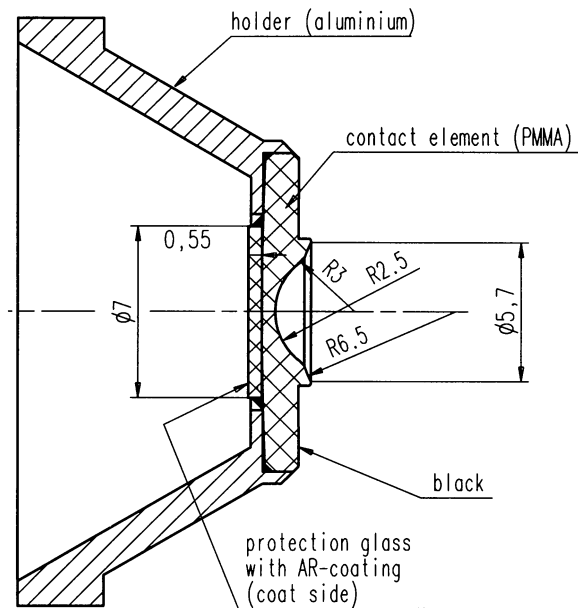


FIGURE 1. Rodent Goldmann-type fundus contact lens.

an interwoven pattern. The camera was tethered to the USB port of a personal computer, a hot shoe-to-PC adapter was mounted to the camera's accessory shoe and connected by a sync cord to the photo slit lamp flash socket. The camera settings were: ISO (equivalent film speed), 320; shutter speed, 1.5 seconds; white balance, auto; and tagged information file format (TIFF) red-green-blue (RGB) and image size 3040×2016 pixels. Based on this, the camera's calculated pixel density is 9354 pixels/mm^2 . A foot switch was wired in parallel to the left button of a computer mouse to activate the camera, swinging its

mirror out of the optical path, releasing its shutter, and closing its flash contact.

When satisfactory focus, centering, and light reflex location are obtained, the operator activates the camera with the foot switch and then, within the 1.5-second window, presses the shutter release bar of the photo slit lamp. Because the two flash contacts are in series, the flash fires. The exposure time of the image is effectively the flash duration. The image is saved to the hard drive of the computer.

After the imaging method was developed, additional series of images were obtained in conscious rats and mice with a second digital camera (D1x; Nikon). Its picture element, according to Nikon specifications, is a $23.7 \times 15.6 \text{ mm}$, 12-bit RGB charge-coupled device (CCD) with 5.47 megapixels and an effective pixel count of 5.33 megapixels (4024×1324 pixels). Based on this, the camera's calculated pixel density is $14,410 \text{ pixels/mm}^2$.

Histologic Correlation

The eyes of anesthetized rats and mice were perfusion fixed by injection of 2.5% glutaraldehyde in 0.1 M Sorensen's buffer through a needle in the left ventricle. The eyes were then harvested and embedded in paraffin or Epon. Serial longitudinal sections were obtained through the optic nerve heads.

RESULTS

Figure 2A is an image of the optic disc of a conscious Brown Norway rat, and Figure 2B is an image of a conscious CS7 Black mouse optic disc, both obtained with a digital camera (FinePix S1 Pro; Fujifilm). (The image detail obtained with the Nikon D1x digital camera was essentially identical.) The cup in the optic disc images of these rodents, unlike the human cup that is pale against the neural rim, is dark against a lighter background. The horizontal diameter of the image of the rat optic disc is approximately one quarter larger than that of the

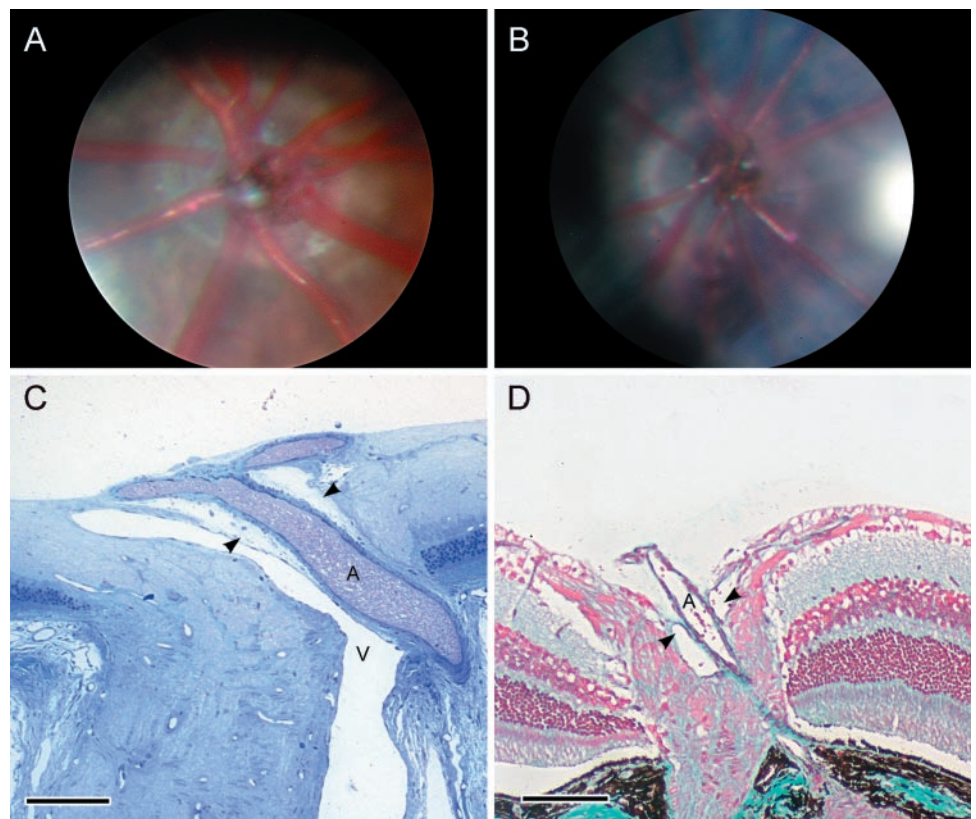


FIGURE 2. Images of optic discs of conscious rodents from digital originals obtained with a modified Goldmann-type fundus contact lens and a photo slit lamp at identical settings: (A) Brown Norway rat, (B) CS7 Black mouse. Longitudinal sections of optic nerve heads. Optic cups (arrowheads); central retinal artery (A); central retinal vein (V). (C) Brown Norway rat. Evans blue stain. (D) CS7 Black mouse. Gomori trichrome stain. Bar, $100 \mu\text{m}$.

mouse. In mice the observation was made that the pigmentation of the fundus increased with age, so that the difference in color between the disc and the surround became more pronounced as the animals grew into adults.

From the longitudinal serial sections of the optic nerve head of the rat (Fig. 2C) and of the mouse (Fig. 2D) representative examples are included to show the configuration of their cups and the sizes of their optic nerve heads. Both cup orientations are oblique to the disc surface and their shape is narrow and funnel-like. The rat optic nerve head is almost three times the diameter of the mouse at the level of the retinal pigment epithelium.

DISCUSSION

Cupping of the optic disc, the unequivocal clinical feature of human POAG, was first described almost 150 years ago and was soon followed by histologic studies that showed that this distinctive change in the appearance of the surface of the optic nerve head was the clinical manifestation of the neuropathy that characterizes this disease. Early in the 20th century the development of tonometry and perimetry led clinical POAG research to emphasize, respectively, increased IOP—its most important risk factor—and visual field defects—its irreversible functional effect.

Forty years ago clinical POAG research shifted toward cupping of the optic disc, and this focus endures today, because cupping is the crux between elevated IOP and visual field defects, both at the onset and during the course of the neuropathy. However, clinical studies of the optic disc in POAG have the burden of limitations inherent in research in patients and in addition the usual slow progress of this disease. Furthermore, histologic studies of the optic neuropathy of POAG are fraught with limitations of quantity and quality of available human tissue. Reports on these scarce specimens, poignant contributions by patients and their families, are unevenly distributed across the course of the disease, but almost never from the period of greatest interest, its onset. Moreover, the usefulness of these specimens, unavoidably acquired hours after death, declines to the extent research methods increasingly require fresh tissue.

These barriers to research on the optic neuropathy of POAG led to the adaptation of a basic tool of biomedical investigation, the animal model, in experiments in which IOP was elevated in the monkey^{7,8} by laser damage to the pathway of aqueous humor outflow. A series of productive studies of the optic nerve head in this animal was followed by comparable experiments in rats, after elevated IOP was induced by obstructing the venous outflow from the eye.⁹⁻¹¹

Fundus photography has been used in the rat,¹² and its use in mice has been described.¹³ Recently, the mouse disc has been imaged with a photo slit lamp through a gonioscopes.¹⁴ Two of the studies of the effect of increased IOP in the rat included optic disc photographs, one using a fundus camera with the cornea flattened by a coverslip,¹⁵ and one with a photo slit lamp through a contact lens.¹⁰

In a comparative study of the microscope of a photo slit lamp and a fundus camera for in-air resolution and for their ability to record fine vessel detail in normal human optic discs, the instruments performed comparably.¹⁶ However, because fundus cameras are telescopic systems, they can bring the optic disc into focus independent of its distance from the camera, and consequently disc image magnification varies with the refractive power of the eye.¹⁷ Further, although flattening the rodent cornea with a coverslip overcomes its aberrations, this can introduce optical distortion. Using the rodent Goldmann-type fundus contact lens with the microscope of the

photo slit lamp eliminates the effects of both corneal refractive power and corneal aberrations.¹⁸ Finally, a mirror in a photo slit lamp instead of a beam splitter maximizes illumination efficiency, both for viewing and imaging. A digital camera provides immediate evaluation of the image for exposure, centering, focus, and the location of light reflexes.

The quality of the rodent optic disc images acquired with the method described improved with practice, but the detail in the images presented of these optic discs, with diameters of approximately 300 μm in rats and 100 μm in mice, probably approaches the current limit of the eye-optical-camera system. Increasing the pixel density of the digital camera did not enhance the quality of the rodent optic disc image, because at the magnification of image acquisition and the image size presented in Figures 2A and 2B, pixel density is not the limiting factor in resolution. Even as advances in digital camera technology increase dynamic range and decrease signal-to-noise ratio, which will extend the contrast range and suppress blooming, achieving significantly more detail in these images is unlikely.

In images of these rodent's optic discs obtained with the same system settings, the horizontal diameter of the rat disc was only approximately one quarter larger than in the mouse. However, in the longitudinal histologic sections the size of the optic nerve head at the level of the pigment epithelium in the rat is almost three times larger than in the mouse. Because the effect of the cornea on image magnification was eliminated by the fundus contact lens and the difference in the axial lengths of these species' eyes is relatively moderate, the closeness of the disc image size is probably due to a difference in their crystalline lenses. In any event, the surprisingly large optic disc image in the mouse is an additional advantage of this most powerful system for investigating mammalian genetics as a tool toward understanding human POAG.

The shape and orientation of the cup in the rat and mouse optic nerve head—narrow, funnel-shaped, and oblique to the disc surface¹⁹—result in a shadowed-cup appearance in the optic disc images: dark against the lighter neural rim of disc tissue. In contrast, the human cup is typically relatively broad and shallow and in images is paler than the surrounding rim—characteristics that have contributed to the challenge of disc surface contour quantitation.¹⁸ Perhaps the different shape of the rodent optic cup will facilitate cup measurements in images of their discs, at least in two dimensions, for detection of cup size differences over time. In assessing rodent cup shape in the histologic material it was noted that it can be affected by the quality of fixation. Nerve fiber swelling can encroach on the cup if fixation is sub-optimal in eyes immersed in fixative.²⁰ Although better fixative access to the optic nerve head can be achieved by opening the freshly enucleated eye, the gold standard is by perfusion fixation, a technique obviously not applicable to human POAG material.

Optic disc imaging in conscious rodents obviates the loss of lens transparency that corneal drying under anesthesia can lead to, especially in the mouse. Although this is reversible, the phenomenon ends the imaging session. More important, repeated sessions in a conscious animal to obtain serial disc images will be performed without the risk of death from multiple anesthetics. This risk is significant in rodents, especially in mice, and would obviously be progressively greater in models to the extent the phenotype is expressed in aging animals.

Three such models are the goal of genetic engineering in a rodent system: elevated IOP without neuropathy, to mimic ocular hypertension in humans; elevated IOP with neuropathy, comparable to classic human POAG; and neuropathy without elevated IOP, which corresponds to human normal-tension glaucoma. Optic disc imaging will be an integral part of iden-

tifying each animal model, of deciding when to obtain optic nerve head tissue for study of the fundamental mechanisms of the neuropathy, and for assessing the effects on the neuropathy of experimental therapies.

Acknowledgments

The authors thank employees of Haag-Streit International (Köniz, Switzerland): Jürg H. Schnetzer, for arranging for Ulrich Dürr to provide the specially fabricated rodent Goldmann-type fundus contact lens and the photo slit lamp; Gerd Ulbers for providing the Fujifilm FinePix S1 Pro digital camera, arranging to have the antireflection coated coverslip applied to the contact lens, and providing the modified Hruby-type lens holder. They also thank Nancy Dressler of Nikon USA, Inc., for providing the D1x digital camera; Toby Donajkowski for machining the zone of the lens for the coverslip; James L. Beals for providing technical advice on the images; and Melissa S. Aniol for providing technical assistance in restraint of the rats and mice.

References

1. Stone EM, Fingert JH, Alward WLM, et al. Identification of a gene that causes primary open-angle glaucoma. *Science*. 1997;275:668-670.
2. Vogel G. Glaucoma gene provides light at the end of the tunnel. *Science*. 1997;275:625.
3. Rezaie T, Child A, Hitchings R, et al. Adult-onset primary open-angle glaucoma caused by mutations in optineurin. *Science*. 2002;295:1077-1079.
4. Cohan BE, Bohr DF. Goldmann applanation tonometry in the conscious rat. *Invest Ophthalmol Vis Sci*. 2001;42:340-342.
5. Cohan BE, Bohr DF. Measurement of intraocular pressure in awake mice. *Invest Ophthalmol Vis Sci*. 2001;42:2560-2562.
6. Nicolela MT, Drance SM, Broadway DC, Balwantray CC, McCormick TA, LeBlanc RP. Agreement among clinicians in the recognition of patterns of optic disk damage in glaucoma. *Am J Ophthalmol*. 2001;132:836-844.
7. Gaasterland D, Kupfer C. Experimental glaucoma in the rhesus monkey. *Invest Ophthalmol*. 1974;13:455-457.
8. Quigley HA, Hohman RM. Laser energy levels for trabecular meshwork damage in the primate eye. *Invest Ophthalmol Vis Sci*. 1983;24:1305-1306.
9. Moore CG, Milne ST, Morrison JC. Effect of glaucoma drops on unilateral experimentally elevated IOP in rats [ARVO Abstract]. *Invest Ophthalmol Vis Sci*. 1995;36(4):S738. Abstract nr 3412.
10. Morrison JC, Moore JC, Deppmeier LMH, Gold BG, Meshul CK, Johnson EC. A rat model of chronic pressure-induced optic nerve damage. *Exp Eye Res*. 1997;64:85-96.
11. Shareef SR, Garcia-Valenzuela E, Salierno A, Walsh J, Sharma SC. Chronic ocular hypertension following episcleral venous occlusion in rats [letter]. *Exp Eye Res*. 1995;61:379-382.
12. Slakter JS, Spertus AD, Weissman SS, Henkind P. An experimental model of carotid artery occlusive disease. *Am J Ophthalmol*. 1984;97:168-172.
13. Hawes NL, Smith RS, Chang B, Davisson M, Heckenlively J, John SWM. Mouse fundus photography and angiography: a catalogue of normal and mutant phenotypes. *Mol Vis*. 1999;5:22.
14. Smith RS, Korb D, Heckenlively J, John SWM. A gonioscopy for clinical monitoring of the mouse iridocorneal angle and optic nerve. *Mol Vis*. 2002;8:26-31.
15. Neufeld AH, Sawada A, Becker B. Inhibition of nitric-oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma. *Proc Natl Acad Sci USA*. 1999;96:9944-9948.
16. Cohan BE, Peach AC, Anderson SA. Comparison of photo slit lamp and fundus camera photography of the optic disc. *Arch Ophthalmol*. 1979;97:1462-1464.
17. Rudnicka AR, Frost C, Owen CG, Edgar DF. Nonlinear behavior of certain optic nerve head parameters and their determinants in normal subjects. *Arch Ophthalmol*. 2001;118:2358-2368.
18. Cohan BE. Multiple-slit illumination of the optic disc. *Arch Ophthalmol*. 1978;96:497-500.
19. Jokelainen PT, Bohr DF, Cohan BE. Structural differences between human and rat optic nerve head [ARVO Abstract]. *Invest Ophthalmol Vis Sci*. 2001;44(4):S826. Abstract nr 4431.