

ANIMAL MODELS and EYE ORGAN CULTURE

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Ultimately the development of new therapies and improving our understanding of visual system function relies on transferring work done *in vitro* to animal models. The goal of the Animal Model and Eye Organ Culture core module is to provide users with assistance in using animal models. To facilitate the proposed research and to establish relevance to humans it is essential to have a resource that specializes in non-human primates. Preliminary to non-human primate studies, many similar research studies may be carried out in rats or mice. Within the Animal Model and Eye Organ Culture module, there will be expertise in primate anesthesia and monitoring, experimental glaucoma (ExpG) induction, imaging of anterior and posterior segments, retinal and central function, and anterior and posterior ocular segment physiology. This expertise will aid the efforts of vision researchers in assessing the effects of their agents, manipulations and vectors/constructs on ocular function in rodents and primates.

Animals

Adolescent or young adult cynomolgus (*Macaca fascicularis*) or rhesus (*Macaca mulatta*) monkeys of both sexes but primarily female weighing 2-12 kg. Long-Evans rats (or other species, where appropriate), or mice (albino, outbred or transgenic), ranging from postnatal day 2 to adult.

Anesthesia

Monkeys:

- Experiments/procedures < 1 hour – i.m. ketamine 10 mg/kg + i.m. diazepam 1 mg/kg or acepromazine 0.2-1 mg/kg; or medetomidine (30-75µg/kg i.m. or i.v.) or xylazine i.m. (0.4-0.6 mg/kg) to further reduce eye movement.
- Experiments/procedures > 1 hr – i.m. ketamine 10 mg/kg in conjunction with i.v. pentobarbital-NA 15 mg/kg. ONT surgery is done under isoflurane inhalation anesthesia.
- Euthanasia – i.m. ketamine 10 mg/kg in conjunction with i.v. pentobarbital 15 mg/kg followed by perfusion-exsanguination or pentobarbital i.v. overdose. Absence of a heartbeat is confirmed, consistent with recommendations of AVMA Panel on Euthanasia.

Rats:

- Experiments/procedures – i.p. ketamine 80 mg/kg with xylazine 8 mg/kg. For experiments involving laser treatment of the eye a drop of 0.5% proparacaine also will be used to anesthetize the cornea.
- Euthanasia – i.p. beuthanasia 100 mg/kg, diluted 1:1 with water) or CO₂.

Mice:

- Experiments/procedures – i.p. ketamine 80 mg/kg with xylazine 16 mg/kg. Neonatal rat (P2-4): Hypothermia (ice). Neonatal rat (P4-13): halothane (vapor only; animals will be physically separated from the halothane by a mesh screen or gap to avoid skin irritation)
- Euthanasia – i.p. beuthanasia 100 mg/kg or CO₂.

Refraction

Hartinger coincidence refractometer is used to measure the power of the lens in response to changes in ciliary muscle contraction. Other techniques that can be employed include streak retinoscopy and photorefractometry.

Aqueous humor outflow

The effects of agents on aqueous humor outflow through the trabecular meshwork or through the uveoscleral pathways can be assessed in a number of ways. Total outflow facility (OF) determined by two-level constant pressure perfusion of the AC, gives a measure of the change in resistance to fluid outflow, mainly through the trabecular meshwork, in response to an agent. A noninvasive method to determine outflow facility, best suited to use with rhesus monkeys, is Schiotz tonography. More complicated procedures involving isotope dilution and accumulation techniques can be used to primarily assess effects on uveoscleral outflow. Measures of trabecular outflow and aqueous humor formation can also be obtained during the isotope studies.

Tonometry

Intraocular pressure determination performed with a minified Goldmann tonometer in monkeys, a Tono-pen calibrated for monkeys may be used when the slit lamp is unavailable or awkward for the experimental set up, the animal is uncooperative, or corneal changes distort the Goldmann mires. A Tono-pen will be used to monitor IOP in rats.

Aqueous and vitreous fluorophotometry

Agent effects on aqueous humor formation can be determined noninvasively using aqueous fluorophotometry following topical fluorescein administration to the cornea. Blood ocular barrier integrity/breakdown, which may be an indicator of agent toxicity, can be measured following intravenous fluorescein administration.

Drug/vector delivery

- Transcorneal - A 30 gauge needle is threaded through the cornea for several mm. The beveled tip is then pushed into the AC and vector is administered in a volume $\leq 20 \mu\text{l}$. The needle can then be withdrawn from the AC without loss of aqueous humor.
- Intravitreal - A 27-30 gauge needle is inserted directly into the vitreous through the pars plana at the 10:00 or 2:00 positions under microscopic visualization to deliver the desired material to the mid-vitreous or close to a specific area of the retina. The total volume injected is $\leq 50 \mu\text{l}$.
- Topical - test solutions are usually administered in 2-10 μl drops. Multiple drops can be administered up to a total volume of 50 μl . The animal is placed in a supine position and the drops are administered to the central cornea with the eyelid held open. Multiple drops of a given agent are usually separated by 30 seconds to 1 minute.
- Intracameral bolus or anterior chamber exchange - during outflow facility experiments, agents can be administered intracamerally by 10 μl bolus injection or exchange of the anterior chamber contents with 2ml of the test solution.

Ocular exams

Slit lamp stereobiomicroscopy of the anterior segment and fundus (Hruby, contact, +78 ir +90 diopter lens), gonioscopy and indirect ophthalmoscopy.

Experimental glaucoma

Experimental glaucoma can be produced in monkeys following trabecular laser destruction - A custom fabricated mirrored gonioscens (Ocular Instruments) filled with gonioscopic gel is placed on the eye following topical anesthesia. A standard clinical argon laser, portable red diode or green diode is used. Contiguous burns are placed in the mid-trabecular meshwork over 180-270° of its circumference in each session. Additional treatment sessions are usually necessary. A final IOP of within 10 mmHg of target can usually be obtained. If IOP exceeds the desired level or if the monkey displays any signs of discomfort, standard antiglaucoma medications can be administered once or twice a day.

Optic Nerve Transection (ONT)

ONT can be used to distinguish between elevated IOP-induced changes versus those due to the loss of axons that occurs following transection. An oculoplastic surgeon performs a lateral orbitotomy. The intraconal space is entered. At all times pressure on the globe is kept as light as possible and pressure is released periodically. Under visualization with an operating microscope, the optic nerve is exposed. Dural vessels and use of a cautery are avoided. The nerve is transected under direct visualization. The retina is then observed to ensure that no central retinal artery occlusion occurred.

For the rat optic nerve transection, animals are anesthetized as above. Under aseptic conditions, a lateral orbitotomy is made after lateral canthotomy (if necessary) and conjunctival incision. Blunt dissection is used to expose the course of the optic nerve on one side. The nerve is crushed or transected under direct

visualization. The wound is closed, bacitracin applied and animals observed until recovered from anesthesia. Each animal will have only one nerve crushed.

Rat photic injury

Male albino rats previously exposed to a normal 12 hour light/dark cycle will be exposed to 1200-1550 lux through green plexiglass. Briefly, fluorescent tubes surround a green plexiglass chamber that contains the rat. A fan will keep the chamber cool. Timing of light exposure will depend on the individual protocol.

Specular microscopy

Toxicity to the cornea can be evaluated by determining the number and shape of corneal endothelial cells.

Photography and fluorescence detection *in vivo*

GFP cotransduced with viral vectors can be detected *in vivo*. Fluorescence will be assessed under both the white light and the cobalt blue exciter filter of a standard clinical Zeiss slit lamp and, for the chamber angle, with either standard clinical mirrored gonioscopy lenses, a mirrored gonioscopy lens specially fabricated for the small monkey eye, or a Swan-Jacob gonioprism. The presence or absence of fluorescence will be documented photographically at selected times. Anterior chamber angle images will be obtained with a Topcon 50EX variable-angle fundus camera equipped with a digital imaging system. The monochromatic green filter provides a black-and-white reference image that reveals the tonality of ocular structures in a manner similar to what can be observed clinically. Corneal photographs are obtained with a Zeiss 40 SL-P photo slit lamp modified for simultaneous stereo photography using a cobalt blue filter and Kodak EliteChrome ISO 100 slide film.

Fluorescein angiography

Fluorescein angiography in nonhuman primates is performed in order to evaluate the retinal circulation. A butterfly needle attached to a syringe filled with 10% fluorescein dye is inserted into the saphenous vein. 0.1 ml/kg is injected and photographs of the retina are taken at desired intervals, usually prior to and during the injection of fluorescein and up to 5 minutes post-injection. If present, the pupils of both eyes are dilated with 1-2 drops of topical 2.5% phenylephrine and 1% tropicamide prior to the injection to facilitate visualization of the retina.

Fundus photography

As glaucoma damage progresses, the optic disc usually becomes more cupped. Fundus photography can provide images of the fundus for assessment of cup/disc ratio and to document other changes that may be attributed to the test agent.

Heidelberg Engineering Confocal scanning laser retinal tomography (HRT)

Used for optic disc and peripapillary retinal contour analysis to track glaucoma progression.

GDx

Scanning laser polarimetry is used for retinal nerve fiber layer thickness assessment to monitor glaucoma damage. Adaptations have been made for more accurate and quantitative use in the monkey and even rodents.

Outflow facility in organ-cultured eyes

Paired normal rhesus (*Macaca mulatta*) or cynomolgus (*Macaca fascicularis*) monkey eyes will be obtained fresh at the time of euthanasia from monkeys at the Wisconsin National Primate Research Center or from the Animal Care unit at the University of Wisconsin-Madison campus. These monkeys are euthanized as part of other protocols or due to illness. No monkeys will be sacrificed specifically for these studies.

The eyes are bisected at the equator and the lens, iris and uvea will be removed. The remaining anterior segment shell containing the TM will be mounted in a special dish and placed in an incubator at 37° C. Media (DMEM) will be infused at 2.5 μ m/min via one port while pressure is monitored via another port. Following overnight equilibration, the OF will be measured by two-level constant pressure perfusion for 45-60 minutes via an external reservoir attached to the inflow tubing. At the completion of OF measurements, a constant infusion rate will be resumed until the next OF measurement.

Visual function electrophysiology

Noninvasive assessment of retinal and cortical function in anesthetized animal preparations. Corneal responses are recorded with the use of ocular surface or fiber electrodes to light stimuli (electroretinography). Subdermal needle electrodes are situated over the occipital cortices to measure the primary visual cortical response (visual evoked response) to a temporally modulated (flickering) light stimulus.

- Full-field electroretinography: evaluates the function of the whole retina with the use of the LKC UTAS-E 2000 Visual Electrodiagnostic Test System. The flashed electroretinogram (ERG) is a measure of

retinal function, thought to depend upon the massed response of rod and cone photoreceptors in the distal retina and upon radially aligned cell types in the proximal retina, such as bipolar and supporting glial cells. Amplitude and implicit time of the a-wave trough and b-wave peak are typical response measurements.

- Visual evoked response (VER): measures primary visual cortical activity in response to flashed stimulation of the retina with the use of the LKC UTAS-E 2000 Visual Electrodiagnostic Test System. Its peak latency and amplitude depend upon the integrity of the optical pathway from the retina to the brain in area 17 [V1]. The VER response is presumed to represent the synchronized mass response of perhaps millions of cortical elements.
- Multifocal electroretinography: permits analysis of localized retinal function with the use of the VERIS Science™ 4.9 electrophysiologic apparatus. Retinal responses from potentially hundreds of focal locations that cover the central 40 deg of retina are simultaneously recorded within approximately 7 minutes. Amplitude, implicit time, and root mean square measures of the first-order (K1) and the first slice of the second-order (K2.1) kernel responses of the multifocal electroretinogram are derived from trace arrays. The effects of retinal eccentricity are ascertained with 'ring' analyses. Quadrant, hemiretinal, and optic nerve head waveform analyses assess nasotemporal asymmetry.
- Consultation also is available for additional or specialized electrophysiologic test paradigms.

Models of ocular infection

This module can provide expertise and guidance for studies involving infections in the eye. Currently, most of the work supported by the module has involved viral infections in mice and rabbits. However, bacterial and fungal infections can be supported. Infections of the cornea or retina are also supported by the Animal Models module.

Ultrasound biomicroscopy

Used to image intraocular structures (B-scan) or measure intraocular distances (A-scan) in nonhuman primates. For B-scan either a fluid filled well will be created on the eye, using sterile BSS, or sterile surgical lubricating gel will be used as the transmitting medium.

Goniovideography, Infrared Videography and Scheimpflug Photography

Infrared videography (IR), goniovideography and Scheimpflug photography/videography of the ciliary body and lens/capsule with or without pharmacologic stimulation to determine the extent of ciliary muscle contraction and lens movement as related to accommodation.

Intraocular fluids and tissue sampling

Vitreous sample: This is performed under deep pentobarbital or isoflurane anesthesia. The monkey's pupils are dilated with 1 drop each of phenylephrine 2.5% and tropicamide 1%. A 23G needle attached to a tuberculin syringe is inserted through the pars plana 12-14 mm toward the papillo-macular nerve fiber bundle under direct visualization with an operating microscope, and 0.1-0.2mL of vitreous is aspirated from each eye.

Aqueous humor sample: Performed on monkeys sedated with ketamine plus medetomidine, acepromazine or diazepam, or under pentobarbital or isoflurane anesthesia. A 30-34-gauge needle is threaded through the cornea for several mm using a needle driver. The beveled tip of the needle is then pushed into the anterior chamber and aqueous humor is aspirated. The tunneling method of tapping the chamber allows self-sealing of the cornea upon withdrawal of the needle. The volume of aqueous extracted will be 50-80µl. This will not deplete the total volume and completely collapse the anterior chamber. The aqueous humor is restored within several hours by normal production in the eye.

Enucleation - will be performed to remove the eyes for morphological evaluation and biochemical studies. Generally this procedure is done bilaterally and the animal euthanized immediately before or thereafter without recovery from anesthesia. Animal can be perfused with fixative through the heart if desired, immediately prior to enucleation.

Other organs – any remaining organs can be harvested as required by the study. Fixation can be done prior to or after harvesting.