Module 1: Visual Function Assessment

Purpose of the module

To fully understand the mammalian visual system requires accurate assessment of its intricate functions. Many of these functions are compromised in diseases of the retina, cornea and lens. This module offers the following technical expertise and instrumentation for *in vivo* visual functional assessment procedures, especially in rodents:

- electroretinography (ERG)
- optokinetic response (OKR)
- *in vivo* ocular imaging including spectral domain-optical coherence tomography (SD-OCT)
- fundoscopy and fluorescein angiography (FA)
- slit-lamp biomicroscopy
- laser induction of vasculopathy
- assessment of intraocular pressure (IOP)

Location and contact information:

Carl Sanders Research and Education building, 2nd floor, Room: CB2908

Phone: 706-721-3449

Director: Dr. Sylvia B. Smith (SBSMITH@augusta.edu)
Co-director: Dr. Amany Tawfik (AMTAWFIK@augusta.edu)

Co-managers: Dr. Barbara Mysona (BMYSONA@augusta.edu) and Dr. Haiyan Xiao (HXIAO@augusta.edu)

Hours of operations: 9:00 a.m. – 5:00 p.m.

Instrumentation available to assess visual function

ASSESSMENT	INSTRUMENTATION	APPLICATION	TECHNICAL SUPPORT
ERG	Highly sophisticated rig custom-designed by Dr. Alan Saul, Department of Ophthal-mology	Assesses scotopic and photopic responses to light flashes as well as "natural" noise stimulus, which is a slowly varying luminance time series with amplitude inversely proportional to temporal frequency. This unit is best suited for interrogation of very low functioning retinas or subtle differences in inner retinal neuronal function. (Please see **details** at end of this page)	Investigators are asked to coordinate with Dr. Saul to perform these specialized analyses. His email address is: (ASAUL@augusta.edu)

ERG	Celeris Fully Integrated High Throughput ERG Testing system including a Celeris PERG (pattern ERG) stimulator from Diagnosys LLC USA	This is a fully-integrated ERG system enabling high throughput and reproducible results, best suited for routine ERGs – such as scotopic and photopic ERGs. It is also capable of performing PERG.	Investigators will be trained by Module 1 personnel and will then be able to conduct their own analyses. Support is available throughout the session if there are difficulties.
OKR	Cerebral Mechanics Inc. OptoMotry system	Virtual reality system for rapid quantification of optokinetic threshold response. It utilizes the OptoMotry© software allowing assessment of rodent visual acuity and contrast sensitivity. It is performed in un-anesthetized mice.	Investigators will be trained by Module 1 personnel and will then be able to conduct their own analyses. Support is available throughout the session if there are difficulties.
SD-OCT	Bioptigen Spectral Domain Ophthalmic Imaging System (SDOIS; Bioptigen Envisu-R2200), equipped with probes for rodent retina and cornea.	The system is operated using proprietary Bioptigen software and features the InVivoVue™ Diver 2.4 software for sophisticated measurements. It allows in vivo histologic analysis of cornea tissue or retinal layers. Note: Each PI will need to purchase a special "Porter" –an image storage unit for data that is compatible with the instrument. We can provide the necessary ordering information.	Investigators will be trained by Module 1 personnel to use SD-OCT and the software. They will be able to conduct their own analyses. Support is available throughout the session if there are difficulties.
OCT	Image-guided OCT attached to MICRON IV (Phoenix)	The OCT2 system includes a live, real-time fundus display with superimposed scan line to allow precise positioning of OCT imaging. The system supports 2D capture, and production of 3D volumes, and includes a set of powerful segmentation and visualization tools.	Investigators will be trained by Module 1 personnel for OCT and can then conduct their own analyses. Support is available throughout the session if there are difficulties.
Fundus Imaging & FA	Two MICRON IV *in vivo* high-resolution retinal imaging microscopes (Phoenix Technology)	Retinal Imaging microscope for in vivo retinal imaging of small laboratory animals. This allows capture of images of the fundus (back of the eye) and if used with fluorescein dye permits visualization of vessels (fluorescein angiography).	Investigators will be trained by Module 1 personnel for instru-mentation use as well as fluorescein injection. Investigators will be able to conduct their own analyses. Support is available throughout the session if there are difficulties.
Laser-Induced CNV	Phoenix MICRON Image-Guided Laser System	This device is attached to the MICRON IV system (described above). The laser produces precise, laser photocoagulation to generate retinal choroidal neovascularization (CNV). An OCT imaging system is also available to verify precisely the site of injury.	Investigators will be trained by Module 1 personnel to use the laser and will be able to conduct their own analyses. Support is available throughout the session if there are difficulties.

		Screens are provided to protect against laser-induced injury.	
Slit-Lamp Bio-microscopy	SL-DR slit-lamp (Topcon Medical Systems)	This slit lamp allows examination/high resolution imaging of the anterior segment of the eye (cornea, iris, lens). It can be used to visualize posterior ocular structures as an adjunct to OCT. It features a digital camera and magnification up to 40X.	Investigators will be trained by Module 1 personnel to use the slitlamp and will be able to conduct their own analyses. Support is available throughout the session if there are difficulties.
IOP	Tonolab tonometer (iCARE)	We have two hand-held tonometers available to measure IOP in lightly anesthetized animals. Data are not stored on a computer, rather pressures are recorded by the investigator.	Investigators will be trained by Module 1 personnel to use the tonometer and will be able to acquire pressure data on their own. Guidance is available if needed.

Training

This module affords investigators the opportunity to perform most of the analyses themselves, thus allowing the capacity to assess as many subjects as needed to obtain statistical power. For those who are not experienced in the use of the above-listed instruments, staff members are available for comprehensive training. In addition, they are present in the module throughout the day and are readily accessible should you encounter difficulties at any time during testing. Please coordinate directly with them if training is required. Additionally, please relay any problems to staff members immediately so that they can be addressed/corrected.

Module access and associated costs:

There are no charges associated with the use of equipment in Module 1. Access to the module is provided using a calendar sign-up system. The link for the scheduling mechanism is being established and will be inserted into this site as soon as available. If you need help with scheduling, either of the module managers (Dr. Mysona or Dr. Xiao) will be glad to assist.

Guidance for experimental design for studies of visual function

Investigators who would like to discuss appropriate methods for testing visual function are welcome to contact the module directors (Dr. Smith or Dr. Tawfik) for guidance.

Animal care and use considerations

Each PI must have their own IACUC-approved protocol to conduct specific visual function tests using this module including specification of the appropriate anesthetic for procedures. It is expected that all investigators will adhere strictly to the guidelines for animal care and use set forth by the Association for Research in Vision and Ophthalmology (ARVO).

Responsibilities associated with module use

DATA STORAGE: It is impractical and unwise to store data of a large number of investigators on the hard drives of computers that could fail. Therefore, investigators are expected to utilize a reliable system (BOX) to store data. Note: for the Biopitigen system a specialized "porter" is used for data storage. Module staff can provide the ordering information for the porter.

ANIMAL ANESTHESIA: Investigators must provide their own anesthesia. If any anesthesia includes DEA-controlled substances, it is the sole responsibility of the PI to obtain proper Federal/State DEA licenses and to ensure that proper records are maintained for substance use and disposal. Those records will reside within individual users laboratories (not in Module 1). Module 1 staff members cannot supply or administer any anesthesia to any experimental subjects.

Citation

Please cite the NEI Center Core Grant for Vision Research - **P30EY031631** in your publications if you use the instrumentation offered in this module.

VISUAL ELECTROPHYSIOLOGY
Details for more extensive analyses

Dr. Alan Saul tests animals, mostly rodents, with various methods. He developed means of testing zebrafish, and has experience with larger animals as well. Please contact him directly (ASAUL@augusta.edu) to discuss your specific experimental needs. There are four main strategies utilized to analyze visual electrophysiology:

Test	Details	Anesthesia	Approximate time
"Bright" full-field ERG	Animals are dark adapted overnight, and testing starts with a series of scotopic brief flashes of increasing intensities. A double-flash stimulus is then used to assess the level of dark-adaptation and recovery. The animal is then light adapted and tested with a range of photopic stimuli, including ON and OFF sawtooth patterns, brief flashes over several contrasts, a longer flash, and pseudorandom noise stimuli. These tests examine the function of outer retina and bipolar cells, primarily. They assess rod and cone function, sensitivity, timing, and certain other aspects of retinal function.	isoflurane anesthesia	Testing typically takes 60-80 minutes.
"Dim" full-field ERG	This test measures scotopic threshold responses (STRs). Animals are dark adapted overnight, anesthetized and tested with brief dim flashes. The intensities of the flashes start at or just below threshold for seeing an ERG response, and increase to levels still well below those used in the "Bright" tests, but enough to generate b-waves that arise from rod bipolar cells. STRs have two modes, an initial positive response (pSTR) followed by a negative response (nSTR). There are actually several less well-characterized modes that we measure as well. The pSTR arises from ganglion cells in mice, and the nSTR arises from an amacrine cell circuit. This test therefore provides an excellent view of inner retinal function. In many experiments, the pSTR and nSTR are dissociated in various ways.	Ketamine/ Xylazine	Testing takes about an hour.
Pattern ERG (PERG)	PERG is a photopic test done without pupil dilation. The animal is presented with two LED panels, with the two panels displaying counterphasing gratings that are out of phase with each other in order to separate the results from the two eyes.	Ketamine/ Xylazine	In the simplest case, it only takes 10-15

	Testing of temporal frequency resolution and contrast sensitivity can be done. The PERG is designed to look at ganglion cell function. The signals probably arise from action potentials in ganglion cell axons traveling toward the optic nerve head. It is therefore useful in traumatic optic neuropathy models as well as many other models of ganglion cell and optic nerve disorders.		minutes, but more extensive testing can be done in less than 40 minutes.
Visual evoked potentials (VEPs)	VEP provides a simple means to assess post-retinal processing. An electrode is placed over visual cortex, and grating, checkerboard, or flashed stimuli are presented to evoke responses from cortex. In demyelinating diseases, the latency of the cortical response is increased. The VEP is sensitive to disturbances in the optic nerve, the LGN, and cortex, and can be done simultaneously with PERG to differentiate between damage to the retina and damage behind the retina.	Ketamine/ Xylazine	Testing takes 10-20 minutes