Multi-Electrode Electroretinography:  

Measuring Spatial Differences in Corneal Potentials

BY

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THESIS

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DEDICATION

I dedicate this work:

To my parents, whom I owe my life and every achievement that I had, and will have

To my loving husband, Masoud Hosseini, for always being there for me

To my late grandmother, if it wasn’t for her I might have not been here today
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<td>3DCAI</td>
<td>3 Dimensional Cardiac Activation Imaging</td>
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<tr>
<td>Aβ</td>
<td>Amyloid Beta</td>
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<tr>
<td>AMD</td>
<td>Age-related Macular Degeneration</td>
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<td>ARVO</td>
<td>Association for Research in Vision and Ophthalmology</td>
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<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
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<td>BSPM</td>
<td>Body Surface Potential Map</td>
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<td>CLEAr Lens</td>
<td>Contact Lens Electrode Array</td>
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<td>EEG</td>
<td>Electroencephalography</td>
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<td>ERG</td>
<td>Electretinogram</td>
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<td>FPC</td>
<td>Flexible Printed Circuit</td>
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<td>HVF</td>
<td>Humphrey Visual Field test</td>
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<td>IP</td>
<td>Intraperitoneally</td>
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<td>ISCEV</td>
<td>International Society for Clinical Electrophysiology of Vision</td>
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<tr>
<td>LED</td>
<td>Light Emitting Diode</td>
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<td>meERG</td>
<td>Multi-electrode Electretinogram</td>
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<td>mfERG</td>
<td>Multi-focal Electretinogram</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>NEP</td>
<td>Neprilysin</td>
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<td>NEVL</td>
<td>Neural Engineering Vision Laboratory</td>
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<td>OCT</td>
<td>Optical Coherence Tomography</td>
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LIST OF ABBREVIATIONS (Continued)

OD       Oculus Dexter
ONH      Optic Nerve Head
OS       Oculus Sinister
PCB      Printed Circuit Board
PERG     Pattern Electroretinogram
PhNR     Photopic Negative Response
ph cd m²  Photopic candela per meter squared
PMMA     Poly(methyl methacrylate)
PPERG    Peripheral Pattern Electroretinogram
RGC      Retinal Ganglion Cell
RMS      Root Mean Square
ROC      Receiver Operating Characteristic
RPE      Retinal Pigment Epithelium
sc cd s m² scotopic candela second per meter squared
SD       Standard Deviation
sNEP     Soluble Neprilysin
SNR      Signal to Noise Ratio
SS       Standard Score
Sub-Q    Subcutaneous
SVM      Support Vector Machine
LIST OF ABBREVIATIONS (Continued)

TTX  Tetrodotoxin
UIC  University of Illinois at Chicago
SUMMARY

In degenerative eye diseases like glaucoma, diabetic retinopathy and macular degeneration, it is important to detect and diagnose the disease in early stages so that treatment can be initiated to stop or slow down the progressive and irreversible vision loss. In early stages, damage to the retina due to disease or trauma is usually localized, hence assessment of local retinal function is important.

In this work a novel technique was developed and its ability in detecting localized retinal damage was evaluated. A Contact Lens Electrode Array (CLEAR Lens) was employed to record 25 ERG waveforms from 25 locations on the cornea of a rat simultaneously. This technique is referred to as multi-electrode electroretinography (meERG).

meERG responses were recorded from healthy rat eyes (26 animals) and eyes with central (4 animals) and peripheral (9 animals) experimental lesions. Data analysis methods were developed to extract the maximum amount of information from the recorded waveforms.

A cluster analysis was performed to separate healthy animals from lesioned animals. The sensitivity and specificity of meERG are compared with those of conventional ERG. The area under the receiver operative characteristic (ROC) curve for meERG was calculated to be 80%. This analysis looked only at relative spatial differences in corneal potentials, and was independent of absolute amplitude.
information. This represents an entirely novel approach to ERG recording and analysis.
1. INTRODUCTION

1.1. Why Measure Corneal Potential Maps?

The main objective of this work is to provide proof of concept for a new approach, using ERG potentials measured simultaneously at 25 locations to compute a potential map of the cornea. This method is called multi-electrode electroretinography (meERG). In this method a full-field stimulus is used to evoke responses from the entire retina. Analyzing a corneal potential map that reflects responses from the entire retina may have a potential use in detecting focal lesions anywhere in the retina. This is in contrast to the multi-focal ERG that probes only the central retina.

1.2. Technical Challenges to Measuring Corneal Potential Maps

Since invention of the ERG more than 100 years ago, different stimulus types have been devised to probe different cell types, functional pathways, and locations across the retina. However, the recording technology, which is a single electrode on the cornea, has been relatively unchanged. In this work, a novel Contact Lens Electrode Array (CLEAr Lens) is employed to record 25 ERG waveforms from 25 locations on the cornea of a rat simultaneously. This technique is referred to as multi-electrode electroretinography (meERG). All recent efforts in recording ERG from localized areas of retina involve carefully designed spatial stimuli (focal and multi-focal ERG) but the recording method is the same (single corneal electrode).
The meERG uses full-field flash stimuli that avoids limitations imposed by complex stimuli. There are some potential benefits of “multiple electrode + full field stimulus” compared to “single electrode + focal stimuli”. Full field stimuli evoke responses from the entire retina, whereas focal stimuli evoke a response from only a limited area of the retina. When using full field stimuli, there is no stringent requirement for ocular clarity of extended fixation on a visual target during the test. Therefore, full field stimulus tests are easier for patients with low acuity, cataracts, or low attention.

1.3. Specific Aims of Study

When this project began, there was a working meERG system (stimulus and recording hardware and software) and working photocoagulation system built by other students in the Neural Engineering Vision Laboratory (NEVL). The main goals of the present work were to fully develop the meERG approach, including establishing robust recording and analysis protocols, and to make an initial exploration of the utility of meERG recording for detection of local retinal defects. To meet those main goals, the following Specific Aims were defined:

Developmental Aims:

1. Develop a protocol for reliable measurement of meERG signals.
2. Develop protocols for analysis of meERG signals.
Experimental Aims:

3. Characterize meERG potentials in healthy rat eyes (first normative meERG data set).

4. Evaluate sensitivity and specificity of meERG to experimental lesions in rat eyes.

1.4. Brief Summary of the Results for each Aim

A fully functional experimental setup (uniform full-field stimulus, 25-channel recording lens, data acquisition system, photocoagulation system, cryocoagulation probe and well-designed recording protocol) was developed in the NEVL. Recording meERG waveforms in response to full-field stimuli has become routine in our lab.

A data analysis method has been designed to analyze meERG waveforms and detect focal damage. Recorded signals were evaluated for a-wave and b-wave amplitudes, and the amplitudes were analyzed using a ratio approach that removes the majority of inter-animal and repeated-measure variance associated with absolute amplitudes, resulting in a high signal to noise ratio even for small spatial differences in corneal potentials.

Recording meERG responses from healthy eyes (right and left) and evaluating them has provided a normative data set which makes it possible to compare any new experiments to this normal group.

This work has evaluated the sensitivity and specificity of meERG to central and peripheral retinal lesions with an experimental group size large enough to achieve
80% statistical power. This is the first rigorous evaluation of meERG efficacy using a model of retinal dysfunction. Sensitivity to the local experimental lesions was investigated. The area under the receiver operative characteristic curve was calculated to be above 80% for meERG.

1.5. Expected Impact of the meERG in Clinical and Research Settings

In degenerative eye diseases like glaucoma, diabetic retinopathy and macular degeneration, it is important to detect and diagnose the disease in early stages so that treatment can be initiated to stop or slow down the progressive and irreversible vision loss. In early stages, damage to the retina due to disease or trauma is usually localized, hence assessment of local retinal function is important. In clinics, structure and function of the retina are evaluated to detect these diseases. Optical coherence tomography (OCT) is an imaging technology used to measure cell loss (structure). Tests like the Humphrey visual field test (HVF) and multi-focal electroretinogram (mfERG) are used to evaluate the function of retina. All of these tests are limited in their application to the peripheral retina (beyond 30 degrees), where some prevalent diseases begin. meERG is sensitive to local functional changes in central and peripheral retina.
2. BACKGROUND

2.1. Brief Anatomy of the Eye

The retina consists of several layers of different cell types that forms the inner surface of the eye. The human retina is approximately 0.5 mm thick (Kolb, 2005) and contains approximately 55 different cell types, each with a different function (Masland, 2001). A cross section of the retina is shown in Figure 1. Photoreceptors (rods and cones), ganglion cells, amacrine cells, horizontal cells and bipolar cells are visible in this cross section. Nerve cell bodies in the retina form three layers which are called outer nuclear layer, inner nuclear layer and ganglion cell layer. Synapses occur in two layers which are called inner plexiform and outer plexiform layers (Kolb, 2005). The outer nuclear layer is furthest from the cornea and contains the rods and cones, the two types of photoreceptors. In most mammalian species, rods are approximately 20 times more prevalent than cones (Masland, 2001). The rods and cones form the outer most part of the retina, bipolar and horizontal cells and amacrine cells, which form the inner nuclear layer, come next. The inner nuclear layer is followed by the ganglion cells and their axons which form the optic nerve head. The nerve fiber layer forms the vitreous surface of the retina and is the first layer to receive light entering the eye.
Figure 1. (A) Cross section of a human eye (B) Schematic cross section of a mammalian retina. The outer most part of the retina contains the rods and cones followed by the outer nuclear layer, outer plexiform layer, inner nuclear layer and the inner plexiform layer. (Kolb, 2005)

When light enters the eye, it passes through cornea, aqueous humor, lens, vitreous humor and cell layers of the retina and reaches the photoreceptors. The central part of the retina has a high density of cones and peripheral part of the retina has high density of rods (Purves, 2001). The cones are functioning in higher levels of light and are responsible for color vision. The rods are capable of functioning in minimal light and are responsible for night vision (Kolb, 2005). When a photon is absorbed by a photoreceptor, a hyperpolarization of these cells occurs, which in turn results in a decrease in the release of glutamate neurotransmitter in the synaptic cleft. Glutamate causes the depolarization of the ON-bipolar cells and the hyperpolarization of the OFF bipolar cells (Masland, 2001). The signal then travels to the ganglion cells and propagates through the optic nerve to the lateral geniculate nucleus and then primary visual cortex in the brain for additional processing.
2.2. The Electroretinogram

The electroretinogram (ERG) is a bioelectric signal that originates in the retina and can be recorded from the cornea. When the cells in the retina respond to a visual stimulus, current sources and sinks form at predictable times and predictable locations within the retina (Xu & Karwoski, 1994; Karwoski & Xu, 1999). Because of impedance difference between the source and sink points, potential differences are created which result in electrical fields. These electrical fields are created in the conductor volume of the eye and their spatial sum decrease sharply with distance (Dolsak et al., 1981). If an electrode is placed on the cornea the spatial sum of the electrical fields can be recorded, which is the ERG waveform. Contribution of each of the cell types in the retina create separate features of ERG waveform. Figure 2 shows the typical ERG response when stimulating the dark-adapted retina with a brief flash of bright white light. The leading edge of a-wave is mostly due to the activity of photoreceptors, and the b-wave mostly comes from ON bipolar cells.
Figure 2. Typical ERG waveform and contribution of different cell types in creating the ERG waveform. The ERG is recorded from a right eye of a dark adapted Long-Evans rat. Flash strength of stimulus was 1842 sc cd s m\(^{-2}\).

### 2.2.1. Common ERG Protocols for Glaucoma Detection

Based on the stimulus type and assessment of the activity of different cell types, different types are ERG can be recorded. Some examples are multi-focal ERG (mfERG), pattern ERG (PERG) and photopic negative response (PhNR), which will be described below. Electrodes commonly used to record ERG are, Burien-Allen and DTL. Figure 3 shows common ERG electrodes. With appropriate stimulation and recording protocol, ERG allows selective monitoring of function of rods, cones, bipolar cells and ganglion cells (Bach & Poloschek, 2013). Here, mostly we have focused on effective tests in glaucoma and the tests that provide functional mapping of retina.
Figure 3. (A) Disposable RetMap ERG electrode made with eye safe electrode. This is a new technology under development. (B) Reusable Burien-Allen lens. (C) Disposable DTL electrode. (D) All three electrodes shown together. (Image Courtesy: Shresta Patangay)
2.2.1.1. Pattern ERG

In the Pattern ERG (PERG) a reversing high contrast pattern, usually a checker board or grating, is used as a stimulus and the response is recorded using a single electrode on the surface of the cornea. The stimulus source has a constant mean luminance at each point in time. PERG stimulation evokes a response dominated by retinal ganglion cells. Typically, PERG measures the function of central retina (centered on fovea and optic nerve head) targeting 20-30 degrees of visual field (Bach et al, 2013). The test requires clear ocular media and stringent fixation. PERG has relatively high efficacy in detecting glaucoma (Bach & Poloschek, 2013). Figure 4 shows a sample PERG waveform.

Figure 4. A sample PERG waveform. The waveform is the average response from 11 healthy human subjects. Check size is 11°.
2.2.1.2. Photopic Negative Response

The full-field single flash ERG is a common test for assessment of retinal function (McCulloch et al., 2014). The stimulus source is a full-field (Ganzfeld) white stimulus which covers the entire visual field, and the ERG is recorded using a single electrode on the surface of cornea. Based on the design of the stimulus, that is, the luminance, color and kinetics, and state of dark adaptation, the response of specific cell types or functional pathways can be measured (McCulloch et al., 2014).

Photopic negative response (PhNR) is a protocol of single flash ERG and is recorded under photopic conditions. The stimulation source is a full-filed Ganzfeld source with blue background, which saturates the rods, and red flash stimulus. The most likely origin of PhNR is retinal ganglion cells (RGC), because intravitreal injection of tetrodotoxin (TTX), which blocks sodium channels of RGC, eliminates PhNR (Viswanathan et al., 1999). PhNR has relatively high efficacy in detecting glaucoma (Bach & Poloschek, 2013). Figure 5 shows a sample photopic negative response. PhNR is the negative peak after b-wave in the ERG response. The i-wave is defined as a post-b-wave component in the human photopic ERG response, and it is claimed to originate in retinal ganglion cells (Roselen et al., 2004).
2.3. **Existing methods for Mapping Retinal Function**

2.3.1. **Humphrey Visual Field Test (HVF)**

In the Humphrey Visual Field Test (HVF), the patient looks into a dome and fixates on a small target. Spots of light then flash at defined locations in the visual field, and the patient presses a button if the spot is visible. The clinician measures the patient’s visual field using this test. Numerical display shows patient’s retinal sensitivity in dB. Gray scale is the graphical representation of numerical display. Areas that appear gray or black in the test shows lower sensitivity. This test is restricted to the central visual field because spatial acuity is limited in the periphery and perceptions are blurred, hence the test will not be reliable in periphery. HVF can test ~30 degrees of visual field, requires clear ocular media, reasonable acuity and stringent fixation. A sample HVF test is shown in Figure 6.
2.3.2. The Multi-Focal Electroretinogram (mfERG)

mfERG was first proposed by Sutter and Tran (1992). The mfERG stimulus is a field of contiguous hexagons which are scaled to elicit approximately equal amplitude responses from a normal retina. Each hexagon alternates between high and low luminance in a predefined, pseudo-random temporal sequence called an m-sequence. mfERG is recorded with a DTL electrode. mfERG mostly records cone and bipolar cell responses (Bach & Poloschek, 2013). mfERG covers 50 degrees of visual field, also requires clear ocular media, reasonable acuity and stringent fixation. This test has low efficacy in detecting glaucoma (Bach & Poloschek, 2013). A sample mfERG response is shown in Figure 7.
2.4. Measuring Corneal Potential Maps

2.4.1. History of Measuring Corneal Potential Maps

Multi-electrode electroretinography (meERG) was first performed by Sundmark (1958). He used a contact glass with nine electrodes in a linear array covering the cornea and sclera. He observed that the amplitude of b-wave, recorded at different locations on the cornea and sclera are not identical. The amplitude of b-wave was smaller on sclera compared to the cornea. See Figure 8, panel A.

The relation between the spatial distribution of retinal activity and the spatial distribution of corneal potentials has been demonstrated using photocoagulation lesions in rabbit eyes by Holland & Herr (1964). They used four electrodes to record the ERG from four positions on the cornea. Using ERG waveforms, they calculated the null position (electrical center) on the surface of cornea. In the healthy eye, the iso-potential region of the cornea happened to be the same as the physical center but in the case of eyes with a lesion, the iso-potential had shifted in the opposite direction relative to the area with the lesion. See Figure 8, panel B.
Cringle & Alder (1987) explored the distribution of ERG potentials on the sclera in normal dog eyes and eyes with laser damage lesions. They treated the eyes of the dogs with photocoagulation. The eyes were enucleated and flash ERG was recorded differentially between a corneal contact lens electrode (silver/silver chloride electrode embedded on a contact lens) and a movable electrode on the sclera. The ground electrode was immersed in the eye bath. The scleral ERG electrode was advanced in one or two mm increments from a location on the optic nerve (20mm) to the limbus (0mm) and ERG was recorded at each location. Measurements were done in normal and treated parts of the same eye. The distribution of the b-wave for control eyes was similar in all cases. The amplitude of the b-wave was increasing until almost 16mm from limbus toward optic disc and then started to decrease a little bit. There is an axial symmetry in the distribution of b-wave amplitudes of the normal eye. This symmetry is shown in panel C of Figure 8.

For treated eyes, in treated side the amplitude of the b-wave was significantly lower than untreated side. Distribution of b-wave amplitudes in untreated side was different than the normal eye. The b-wave amplitudes are larger in the untreated part. In the eyes with larger lesion, the amplitude of b-wave in the untreated side is larger compared to the eyes with smaller lesion.

Job et al., (1999) have created a three dimensional electromagnetic model of a human eye. In their model, they assumed the eye’s geometry is radially symmetrical sphere. They have included the inhomogeneities of the eye in their model. Three dimensional Laplacian equation was used to do the modeling. They have simulated
different types of stimulus (full-field, central focal and peripheral focal) on the retina and have created the corneal potential map based on activity of retina using their 3D model. They have also stimulated the focal scotoma on central or peripheral retina and created the corneal potential map. The results for full-filed and peripheral focal stimulus are shown in Figure 9.

Figure 8. (A) The result of experiment done by Sundmark (1958), vertical axis shows the value of b-waves recorded by an electrode on different location on the cornea. Each trace represents the response of different experiment. The experiment was performed 24 times. (B) The result of experiment done by Holland and Herr (1964). The circles in the right represents the appearance of the retina, the darker areas are the location of the lesion. The circles on the left represents the surface of the cornea, presence of lesion in the retina, shifts the electrical center of the cornea. This is shown by smaller circles and the arrow (C) The result of experiment done by Cringle and Alder (1987), the presence of lesion in the retina changes the distribution of the b-waves between cornea and sclera compared to the normal retina. The lines represent the isopotential contours on the surface of the eye.
2.4.2. Body Surface Potential Mapping for Brain and Heart

Reliably measuring corneal potentials and interpreting them can provide information about regional differences in the retinal response to the stimulus. Comparing the distribution of corneal potentials before and after creating a lesion in the retina is used to optimize the electrical model of the eye. The model is then used to solve an inverse bioelectric source problem.

Electrophysiological functional mapping has been extensively done for brain (He & Lian, 2002) and heart (Han et al., 2008) based on body surface potentials.

Functional neuroimaging using non-invasive methods can characterize and image electrical sources in the brain. High resolution neuroimaging can facilitate localization of epileptic discharges (He & Lian, 2002). He & Lian, (2002), have reviewed the research done to combine information from electroencephalography
(EEG) and magnetic resonance imaging (MRI) to create a model to solve the EEG inverse problem. EEG provides high temporal resolution while MRI provides high spatial resolution, thus integrating EEG inverse problem solutions with MRI provide high spatio-temporal mapping of brain activity (He & Lian, 2002).

In the work done by Han et al., (2008), they evaluated the performance of a 3-dimensional cardiac activation imaging (3DCAI) technique, which is a non-invasive tool that can capture important features of ventricular excitation and has the potential to be used as an imaging tool for cardiac diseases (Han et al., 2008). To evaluate this technique Han et al, (2008) recorded body surface potential maps (BSPMs) from 40-60 locations from chest of 4 New Zealand rabbits. They also did invasive recording by inserting 20-25 needle electrodes into the heart. The activation sequence obtained from 3DCAI using BSPM was in agreement with invasive intra-cardiac recording (Han et al., 2008).

To perform the same method on the eye, a reliable computational model of the eye is needed. There have been several attempts to solve inverse problem for the ERG (Davey et al., 1988; Van Schijndel et al., 1997; Job et al., 1999), but the efforts were hindered because of lack of reliable data to optimize and validate the electrical model of the eye. The measurement of spatially distinct corneal potentials can provide this data set.
3. meERG POTENTIALS IN HEALTHY RAT EYES

3.1. Overview

Multi-electrode Electroretinography (meERG) is a novel technology developed and performed in the Neural Engineering Vision Laboratory (NEVL). To perform reliable experiments and compare meERG to commercially available diagnostic tools, it is important to develop a fully functional system with repeatable experiments. A lot of time and effort has been devoted to developing protocols and troubleshooting of the recording system. These efforts include:

- Creating a uniform stimulus source.
- Developing protocol for assembling, filling and cleaning of the CLEAr Lens.
- Design of a printed circuit board (PCB) as an interface between the lens and the amplifier.
- Sealing the flexible printed circuit (FPC) connector between the electrodes and the PCB to avoid accumulating salt residue.

After repeatable experiments could be performed routinely, the next major step was developing a data analysis approach which could extract useful information from the data.
In this chapter, the performance of the recording system has been evaluated and normative data set for healthy rat eye has been created. Most of the information in this chapter is included in Krakova et al. (2014).

3.2. Methods

3.2.1. Multi-Electrode Electroretinogram (meERG)

The full-field ERG is the sum of contributions from all of the cells that respond to the stimulus. At a particular point on the cornea, the contribution of each of the cells is weighted by the orientation of its associated equivalent dipole, and by the impedance between that cell and the point of measurement. Figure 10 shows the schematic of this concept. The contact lens electrode array used in this study was designed to measure distinct ERG potentials at several locations on the cornea, and to exploit the weighted contribution phenomenon.
3.2.2. Animals

Long Evans rats (purchased from Charles River Laboratories, Wilmington, MA) in the age range of 6-7 weeks, when the radius of curvature of the cornea matches the recording lens, were used. Rats were dark adapted for at least 1 hour. 100 mg/Kg (body weight) of ketamine and 10 mg/Kg (body weight) of xylazine were injected intraperitoneally (IP) for general anesthesia. The cornea was anesthetized with 0.5% proparacaine. The pupil was dilated with 2.5% phenylephrine HCL and 1% tropicamide. A regulated heating pad was used to maintain body temperature at 37-39°C during the experiment. All experiments were in accordance
with the ARVO Statement for Use of Animals in Ophthalmic and Visual research and Approved by University of Illinois at Chicago protocol ACC 11-154 and 14-128.

3.2.3. **Contact Lens Electrode Array (CLEAr Lens)**

The Contact Lens Electrode Array (CLEAr Lens) is designed to record spatially distinct potentials at several locations on the cornea. The CLEAr Lens is a plano-concave lens made of poly(methyl methacrylate), PMMA (the material used to make early corrective hard contact lenses) and has 25 through-holes arranged in a radial pattern. The diameter of each hole is 300 µm. During use, these holes are filled with saline solution to provide a conductive pathway from the surface of cornea to the recording electrodes on a flexible cable. The cable is made with a water-clear Parylene substrate. The electrodes are made of platinum-coated gold using standard photolithography processes on the cable by the William’s lab, University of Wisconsin, Madison. Exposed electrodes and contact pads are coated with platinum. The lens and the cable are bonded with pre-formed medical grade tape in a custom assembly jig. The electrodes cover almost 20% of the surface of the lens, and transmission of the lens is measured to be 60-70% of the incident light. All materials comprising the CLEAr Lens are known to be biocompatible, and are safe for contact with the eye. The CLEAr Lens gives the ability to record 25 distinct ERG waveforms simultaneously from the surface of cornea at each presentation of a stimulus. Figure 11 shows the CLEAr Lens and the 25 waveform recorded using CLEAr Lens.
The through-holes of the CLEAr Lens were filled with “CVS Saline Solution for Sensitive Eyes” to provide a conductive pathway between the cornea and recording electrodes. Electrode impedance spectroscopy was performed on several prepared lenses. The impedance of each channel was typically within the range of 60-70 kΩ at 100 Hz, which is less than 0.1% of the input impedance of the recording amplifier. The functionality and consistency of all channels were tested with a sine wave with amplitude of 1 mV p-p and frequency of 200 Hz before and after each experiment. The lens was washed with “Walgreens Sterile Multi-Purpose Solution for Soft Contact Lenses” and distilled water after each experiment to avoid formation of salt crystals and to remove protein and lipids in the holes.

3.2.4. Stimulus and Recording Instrumentation

The CLEAr Lens cable was connected to a 60-channel commercial amplifier (MEA60, Multi-Channel Systems, Germany) via a custom printed circuit
board. The amplifier has been modified by the manufacturer to have the pass-band appropriate for ERG recording: 0.2-2500 Hz, 3 dB.

A custom protocol in the MC-Rack data acquisition program was used to record the data. Sampling frequency was set to 5 kHz. An additional band-pass filter was used in the recording system with frequency range of 0.2-500 Hz (ISCEV standard recommendation is 0.3-300 Hz (Marmor et al., 2004)).

For all experiments, a back-lit translucent acrylic dome with Xenon flash lamp (standard flash head; Novatron, Inc., Dallas, TX) is used as the stimulus source. Light passed through an adjustable aperture (to control luminance). Luminance measurement of the dome verifies that the dome has a uniform ± 12% luminance. Visual angle subtended is ~117 degrees.

### 3.2.5. CLEAr Lens Placement

The position of the lens relative to the eye is adjusted with the cable oriented along the superior-inferior axis of the eye. When recording from the right eye, the cable extends from the superior side; when recording from the left eye, the cable extends from the inferior side. The position is verified by the pictures taken before, during and after the experiments using an infra-red camera installed in the stimulus source. The position of the lens was quite stable during recording. The whole process of preparing the rat and fitting the lens was done under the dim red light to avoid light adaptation. Figure 12 shows the electrode map when the CLEAr Lens is used to record from the right (OD) and left (OS) eye. The electrodes labeled as A1-A12
are called A-ring electrodes and the ones labeled as B1-B8 are called B-ring electrodes. Platinum needle electrodes were used as the reference electrode, placed in the ipsilateral cheek and the ground electrode, placed in the nape of the neck.

![Figure 12. Position of CLEAr Lens electrodes relative to cornea when recorded from right (OD) or left (OS) eye (Krakova et al., 2014).](image)

### 3.2.6. Recording Protocol

Different protocols for recording have been performed to answer specific research questions. Each will be described below. In all experiments, brief white flashes were presented as the stimulus. The time interval between the flashes was 90 seconds to give enough time for recovery of photoreceptors. Ten flashes for each luminance were delivered. Fundus photographs were taken from the retina after each meERG recording to verify the normal appearance of the retina in healthy eyes and the location of damage in the eyes with localized damage. Normal appearance of the cornea was evaluated using fluorescein (any damage and scratch on the cornea...
can change the recorded potential). This procedure was done after the experiment to avoid light-adaptation of animals before the experiment.

### 3.2.6.1. Protocol for Amplitude-Intensity (i.e. Sensitivity) Experiments

To evaluate whether meERG responses recorded using the CLEAr Lens are equivalent to responses recorded in conventional ERG (single electrode recording), both methods were used to record responses from one animal in the same day. Six different flash strengths were presented, each of them 4 times. The responses with the same flash strength were averaged for each data set. The responses were evaluated for the a-wave amplitude. All of the amplitudes were normalized to the amplitude of the brightest flash. The normalized amplitudes for each data set were plotted versus the flash strength and the following equation was fitted to the data to yield amplitude-intensity curves.

$$\frac{A}{A_m} = \frac{I}{I + I_0}$$

Eq. 1

In this equation, $I$ is the flash strength, $A$ is the amplitude of a-wave at each flash strength, $A_m$ is the a-wave amplitude of the brightest flash.

### 3.2.6.2. Right versus Left Eye

Because the distribution of the electrodes and electrode traces in the CLEAr Lens cable is different for right- and left-eye recording, it was important to examine the influence of this difference in the meERG responses. To do this,
meERG responses were recorded from the right and left eyes of several rats, and the spatial distributions of corneal potentials were compared using the meERG ratio method described below (section 3.2.7.2). For this set of experiments, a brief flash with luminance of 422 scotopic candela seconds per meter squared (sc cd s m\(^{-2}\)) was delivered. meERG responses were recorded from the right eye (n=7) and left eye (n=5) of healthy rats. Bright flashes were used to increase the likelihood of evoking a saturated response from the entire retina. Ten flashes were presented per experiment.

3.2.6.3. Normative Data Set

The development of a new ERG recording technology, the CLEAr Lens, led to a new type of ERG data, the spatial distribution of ERG potentials across the cornea. The next step in the development of the meERG technique was to establish a normative data set for healthy eyes that could be used for comparison in later experiments involving eyes with various types of retinal dysfunction. For this set of experiments, brief flashes with luminance of 21 and 1033 sc cd s m\(^{-2}\) were presented alternately. Each flash was presented 10 times. meERG responses were recorded from the right eye on day 1, another set of meERG was recorded from half of the animals on day 5-6 (the other half was used for experimental lesions). The first and, where available, second meERG data sets were all combined to form the normative data set (3 data sets out of 26 were repeated measure). The data were analyzed for each luminance separately to form the normative data set for each luminance.
3.2.7. Data Analysis

3.2.7.1. a-wave, b-wave Amplitude and Implicit Time for Each

a-wave amplitude: a-wave amplitude is considered to be the difference of the pre-stimulus baseline and the amplitude at 4ms following the stimulus for luminance of 1033 sc cd s m$^{-2}$ and 8ms for luminance of 21 sc cd s m$^{-2}$. b-wave amplitude: b-wave is calculated as the difference between b-wave peak and a-wave trough of ERG waveform. a-wave implicit time: the time between the stimulus and trough of ERG waveform. b-wave implicit time: the time between the stimulus and peak of the ERG waveform.

Figure 13 shows the features of rat ERG waveform. A Microsoft Excel Macro was created to plot the waveforms and extract the features above automatically for all the experiments.
3.2.7.2. **meERG Ratios**

meERG waveforms are evaluated for a-wave and b-wave amplitude and implicit times. To evaluate spatial symmetry in corneal ERG potentials, the values measured on each of the 12 peripheral electrodes in the A-ring (Figure 14) or the eight peripheral electrodes in B-ring were divided by the average amplitudes on the central five electrodes and multiplied by 100, which yielded 20 ratios for each eye. These meERG ratios are plotted by electrode position for both A-ring and B-ring separately to form radar plots. The meERG ratios are averaged (by electrode position) for all healthy-eye meERG responses to form a normative data set. Ratios are often used in ERG analysis to minimize effects of repeated-measure and inter-subject variance in absolute amplitudes.
Figure 14. (A) The method by which meERG ratios are calculated. For each animal, the meERG ratio is calculated by dividing the value of peripheral electrode to the average of 5 central electrodes, multiplied by 100. (B) The meERG ratios are calculated for all peripheral electrodes (A & B rings), and summarized in radar plots. (C) A sample radar plot created for a-wave amplitudes on A-ring electrode of a rat.

3.2.7.3. Inclusion/Exclusion Criteria

Before analyzing the data, all the raw data were checked to ensure that the data included in the analysis were of high quality. Inclusion/exclusion criteria have been applied to the data recorded with flash strength of 1033 sc cd s m\(^2\). Data sets excluded for this flash strength were excluded from the lower flash strength and sensitivity data sets as well. The following criteria were used to remove specific channels in an experiment or the entire experiment.

- While testing the filled CLEAr Lens with sine wave, the noisy channels, and channels with abnormal waveforms were eliminated.
- Outlier channel responses are removed. The response of a channel was taken as an outlier if the waveform recorded was larger or smaller than 1.5 times the average of the other electrodes’ signals.
• The runs with artifacts on the waveforms were eliminated.

• Experiments that had more than 5 non-working electrodes were eliminated.

• For all of the animals, a-wave values were calculated on all electrodes for each presentation of a flash. The values obtained for 10 flashes were averaged by electrode position to give 25 a-wave values for each animal. The meERG ratios (see section 3.2.7.2) for A-ring values were calculated. The standard deviations of meERG ratios for each animal were calculated. The animals with standard deviation of 11 or larger were removed. The criterion level of 11 resulted in inclusion of the most informative data sets (i.e. yielded the highest area under the ROC curves) when compared to other criterion levels between 5 and 20. The total number of data sets that were used in data analysis for normative data set were data sets obtained from 26 healthy eyes.

3.2.7.4. Smooth Corneal Potential Map

Interpolation was used to fill in the corneal potential values between the electrode positions to form smooth corneal potential color map for visualization and also to fill in for ERG values for electrode location that were missing or excluded. For interpolation the 3D spline method presented by Law et al. (1993) was used. Equation 2 shows the applied interpolation method.

\[
\phi(x, y, z) = \sum_{i=1}^{n} p_i K_{m-1}(x - x_i, y - y_i, z - z_i) + \sum_{d=0}^{m-1} \sum_{k=0}^{d} \sum_{g=0}^{k} q_{dgk} x^{d-k} y^{k-g} z^g
\]  

Eq. 2

Where:

\[
K_{m-1}(s, t, r) = (s^2 + t^2 + r^2)^{m-1} \log(s^2 + t^2 + r^2 + w^2)
\]
\[
s = x - x_i \quad t = y - y_i \quad r = z - z_i
\]

In equation 1, \( \varphi \) is the interpolated potential at the point \((x, y, z)\). \((x_i, y_i, z_i)\) is the electrode location, \( n \) is the number of electrodes, \( p_i \) is the coefficient of the basis function, \( q_i \) is the coefficient of the osculating function. \( m \) is the parameter that controls the order of spline. \( m \) is chosen to be 3 here. \( w \) is the diameter of the electrode. The coefficients \( p \) and \( q \) were initially solved by inserting the electrode positions and potentials on those electrodes.

### 3.2.7.5. Normalizing Corneal Potential Maps

In order to compare the spatial differences in corneal potentials across animals, the measured and interpolated values were converted to standard score (\( s_i \), number of standard deviations from the mean) using Equation 3.

\[
s_i = \frac{v_i - \bar{v}}{\sigma}
\]

Eq. 3

Where \( v_i \) is the interpolated potential at each interpolation point, \( \bar{v} \) is the average of measured potentials. \( \sigma \) is the standard deviation of the measured potentials. The result represents the number of standard deviations each interpolated potential is away from the average of measured potentials.

### 3.2.7.6. Sensitivity

The recorded data have been analyzed for sensitivity (stimulus strength required to reach half saturation). In each experiment two flash strengths
(saturating, 1033 sc cd s m\(^{-2}\) and sub-saturating, 21 sc cd s m\(^{-2}\)) were delivered. For each animal, as explained before, each flash strength was presented 10 times. The amplitude at the peak of the a-wave was determined for each flash strength separately and they are averaged by electrode position which results in 25 values of a-wave amplitude for sub-saturating stimulus, and 25 values of a-wave amplitude for saturating stimulus, for each animal. The common way to calculate sensitivity (I\(_{1/2}\)) is to fit sensitivity curve to the data. Because only two flash strengths were used, and any equation could be forced through two points, we chose to calculate the I\(_{1/2}\) value using Equation 4. This equation is the sensitivity curve solved for the I\(_{1/2}\) value.

\[
I_{1/2} = \frac{I(A_m - A)}{A}
\]  
Eq.4

In equation 4, I is the dim stimulus which is 21 sc cd s m\(^{-2}\), A\(_m\) is the amplitude of a-wave for saturating stimulus and A is the amplitude of a-wave for sub-saturating stimulus. To make sure the values calculated using equation 4 is similar to that of fitting method, for one animal, both methods were performed. The mean ± standard deviation for curve fitting method was calculated to be 8.96±0.1 and for solving method it was 8.98±0.1.

3.3. Results

3.3.1. meERG versus Conventional ERG

Figure 15 shows the amplitude-intensity plots for meERG and conventional ERG. I\(_{1/2}\) was determined to be 139 and 183 (sc cd s m\(^{-2}\)) for conventional ERG and
meERG respectively. 32% more light is needed to reach half saturation in the meERG. This result was consistent with the 60-70% light transmission of the lens.

Figure 15. Amplitude-Intensity plot comparing sensitivity between conventional ERG and meERG. The data is recorded in the same day from the same animal with same instruments (lens, amplifier, etc.). For meERG the 25 waveforms in each experiment are averaged and the a-wave is evaluated for average waveform. The inset plots the actual ERG waveform for conventional ERG (dashed line) and meERG (solid line). The flash strength is 1842 sc cd s m\(^{-2}\). In the Amplitude-Intensity plot the dashed line (conventional ERG) and solid line (meERG) shows the curve fitted to data using equation Eq.4. The symbols show ±1 SD for repeated values of same flash strength (Krakova et al., 2014).

3.3.2. Spatial Differences in Corneal Potentials

To investigate whether the cornea is isopotential, the difference plot (Figure 16) was created. The left-most panel of Figure 16 shows a representative set of meERG waveforms recorded in response to a single uniform saturating flash. The data presented were obtained directly from the recording system, and, for only this data set, neither interpolation of missing channels nor any kind of signal processing was
performed. The missing channel is shown as a straight line. The 24 waveforms on the left panel have been averaged and plotted as a single waveform in the middle panel. The waveform has been enlarged for better view. The right panel of the Figure 16 shows the difference plot (note different scale). The difference of these waveforms from a straight line, shows that the electrodes of the CLEAr Lens are not electrically shunted. The difference waveforms illustrate that the ERG responses on the superior-temporal quadrant of the cornea are larger than the average waveform but the ERG responses on nasal-inferior quadrant of the cornea are smaller than the average.

The typical baseline RMS value for raw meERG waveforms (left panel, Figure 16) was 11.2 µV, for the averaged waveform (middle panel, Figure 16) the RMS of baseline was 10.7 µV. The difference in squared RMS values represents the noise power that is not correlated across the channels, which was \(((11.2)^2 - (10.7)^2) = 9\%\) of total noise in raw waveforms. The RMS value of the baseline in the difference waveform (right panel, Figure 16) also represents the noise which is not correlated across the channels. For the baseline of the difference waveform the RMS value was calculated to be 1.7 µV, which is 2\% of the noise power in raw waveforms. Approximately 2-9\% of the noise is not correlated across the channels and is inherent in the recording system. The remainder of the noise originated from breathing, heartbeat or other sources that are sensed differently by CLEAr Lens and the reference electrode and is highly correlated across the channels. This noise can be removed by evaluating amplitude ratios rather than absolute amplitudes.
Figure 16. Using CLEAr Lens 25 distinct ERG waveforms can be recorded simultaneously across the cornea. Left panel shows 24 ERG waveforms recorded in single presentation of a flash. The waveforms are plotted in the position where they are recorded from the surface of cornea. The straight line indicates a non-working electrode in this experiment. The middle panel shows the average of 24 waveforms in the right panel. The waveform is enlarged for better view. The waveforms in right panel is the difference between original waveform of left panel and average waveform of the middle panel (Krakova et al., 2014).

### 3.3.3. Visualizing Spatial Differences in Corneal Potentials

For each animal, the meERG waveforms were evaluated for a-wave and b-wave amplitudes. These values were averaged over the number of repeated stimuli of within the same experiment (6-10 responses).

meERG ratios were calculated for 12 peripheral electrodes. Figure 17 shows the radar plots for two representative animals.
Figure 17. Comparison of right eye and left eye. The plots are for two representative animals (one right eye and one left eye). S, N, I and T shows, Superior, Nasal, Inferior and Temporal part of each eye. The numbers surrounding the plots indicate the number of electrodes in A-ring. Distance from the origin is the ratio presented as percentage. Upper panel is for a-waves and lower panel is for b-waves. (Krakova et al., 2014)

The 12 meERG ratios obtained from 7 right eyes and 5 left eyes were averaged separately across animals for each electrode position. Standard deviation (SD) was calculated for each electrode position (across animals) and was averaged across 12 electrodes. The mean meERG ratios are plotted in Figure 18. In these radar plots the solid line is the average value and dashed lines are ±1 SD.
Figure 18. Comparison of radial symmetry in left and right eyes. The plots for right eye are average of 7 animals, the plots for left eye are average of 5 animals. The orientation of the plots are the same as figure 13. The upper panel shows the a-wave radar plots; the lower panel shows b-wave radar plots. (Krakova et al., 2014)

There is a slight asymmetry along superior-inferior axis of the eye both in right eye and the left eye, even though the CLEAr Lens was rotated 180 degrees between the right and left eye, suggesting that the asymmetry not due to a property of the CLEAr Lens.
3.3.4. Evaluating Asymmetry of Right and Left Eye

Using Student’s t-test, the superior-inferior and temporal-nasal asymmetry was evaluated for the right eye and left eye experiments, and the results for each eye were compared. Table 1 summarizes the results. The values in Table 2, indicate a slight superior-inferior asymmetry in both right and left eye. Both the left and the right eyes show a slight superior-inferior asymmetry, where the meERG ratios are larger in the superior quadrant. This is the case whether the CLEAr Lens cable extends from the superior side of the eye (right eye recording) or from the inferior side of the eye (left eye recording), demonstrating that the asymmetry is not due to the optical properties of the CLEAr Lens or cable, but rather due to a natural asymmetry in the distribution of corneal potentials in the healthy rat eye.
<table>
<thead>
<tr>
<th></th>
<th>Superior (11,12,1)</th>
<th>Inferior (5,6,7)</th>
<th>Nasal (2,3,4)</th>
<th>Temporal (8,9,10)</th>
<th>P</th>
<th>A-wave</th>
<th>B-wave</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Eye Amplitudes (n = 7)</td>
<td>100.7 ± 1.7</td>
<td>98.1 ± 1.9</td>
<td>99.5 ± 1.7</td>
<td>99.1 ± 1.7</td>
<td></td>
<td>100.7 ± 1.8</td>
<td>97.6 ± 2</td>
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<tr>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td>&lt;0.0001</td>
<td>0.2</td>
</tr>
<tr>
<td>Left Eye Amplitudes (n = 5)</td>
<td>101.8 ± 1.7</td>
<td>99 ± 1.6</td>
<td>100.8 ± 2</td>
<td>100.2 ± 1.8</td>
<td></td>
<td>100.7 ± 2.9</td>
<td>99.4 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.2</td>
<td>0.4</td>
<td></td>
<td>0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 1. Asymmetry in meERG a-wave and b-wave amplitude ratios in left and right eyes. The numbers in parentheses in the second column indicate the A-ring electrode numbers that were used to represent each quadrant of the cornea. The numbers in a-wave and b-wave columns show the average of values in corresponding electrodes ±1 SD. The p values are obtained using Student’s unpaired t-test. (Data published in Krakova et al., 2014)

3.3.5. Asymmetry of Recording System

To check whether there is an asymmetry in the recording system, the sine wave amplitudes recorded before and after each experiment were analyzed. For each experiment, three sine wave sets were recorded before experiment and three after the experiment. Peak to peak values were calculated for each electrode, and the values were averaged by electrode position across the experiments. Here, the meERG ratios for pre and post experiment sine wave recordings were calculated the same way.
as for a-wave and b-wave. The results for sine waves are shown in Table 2. There is not a significant difference and asymmetry in the meERG ratios calculated for sine waves. The results suggest that the asymmetry observed in corneal potential map was not due to the recording system but instead originated in the retina.

<table>
<thead>
<tr>
<th>Sine wave Amplitudes</th>
<th>Pre Experiment</th>
<th>Post Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior (11,12,1)</td>
<td>100.5 ± 0.3</td>
<td>100.5 ± 0.3</td>
</tr>
<tr>
<td>Inferior (5,6,7)</td>
<td>100.2 ± 0.7</td>
<td>100.2 ± 0.7</td>
</tr>
<tr>
<td>p</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Nasal (2,3,4)</td>
<td>100.1 ± 0.5</td>
<td>100.1 ± 0.5</td>
</tr>
<tr>
<td>Temporal (8,9,10)</td>
<td>100.5 ± 0.6</td>
<td>100.5 ± 0.7</td>
</tr>
<tr>
<td>p</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 2. Asymmetry in sine wave amplitude ratios. The numbers in parentheses in the second column indicate the A-ring electrode numbers that were used to represent each quadrant of the cornea. The numbers in pre-experiment and post-experiment columns show the average of values in corresponding electrodes ±1 SD. The p values are obtained using Student’s unpaired t-test. (Data published in Krakova et al., 2014).

3.3.6. Normative Data Set

The meERG waveforms for 26 healthy eyes are averaged by electrode position to form the normative data set. meERG ratios are calculated for a-wave and b-wave amplitudes for A-ring and B-ring.
3.3.6.1. **Saturating Stimulus**

For a saturating stimulus (1033 sc cd s m\(^{-2}\)), radar plots for normative data set are plotted in Figure 19. The color plot is the potential map of the cornea which shows the standard score of values at each interpolation point. A slight nasal-temporal asymmetry in the healthy eye response can be seen. This was quantified using the meERG ratio approach described above, and plotted in the radar plots of Figure 19. The standard deviation of the meERG ratio values across animals was approximately 1.1% of the mean ratio value. This suggests that the meERG ratio values are consistent across animals with healthy eyes, and that even small deviations from a healthy eye response will be detectable with this analysis approach.
3.3.6.2. Sub-Saturating Stimulus

Sub-saturating stimuli are important to many ERG protocols, including amplitude-intensity series to evaluate sensitivity and the scotopic threshold response used to evaluate function of the proximal retina (i.e. ganglion cells). Because meERG data analysis assumes that the relative illumination of the retina is the same across flash strengths, it was important to analyze meERG responses to a sub-saturating flash and compare these to meERG response of a saturating flash (which
can “mask” non-uniformities in the stimulus). For sub-saturating stimulus (21 sc cd s m⁻²), radar plots for normative data base is plotted in Figure 20. The color map is the potential map of the cornea which shows the standard score of values at each interpolation point.

Figure 20. Normative data set for flash strength of 21 sc cd s m⁻². (A) Average corneal potential map evaluated at t = 8 ms (leading edge of a-wave), obtained after converting each individual potential map to standard scores (Eq. 2). Color scale is in units of standard deviations from the spatial mean. (B) Average meERG ratio values for A-ring electrodes evaluated at t = 8 ms; dashed lines plot +/- one standard deviation. (C) Average meERG ratio values for B-ring electrodes evaluated at t = 8 ms; dashed lines plot +/- one standard deviation. (D–F) Average corneal potentials evaluated at the peak of the b-wave; analysis as in A–C, respectively.

The asymmetry in the corneal potential map evoked by a sub-saturating stimulus was nearly identical to the potential map obtained with a saturating stimulus.
(compare panels A and D in Figures 19 and 20). This suggests that the custom-built meERG stimulus source used in this study is sufficiently uniform, and that meERG responses evoked by different stimulus strengths can be compared.

3.3.6.3. Sensitivity ($I_{1/2}$)

In unhealthy retina, sometimes retinal cells might be functioning but they might be less sensitive to light. The maximum response (i.e. the response to a saturating stimulus) might be the same for healthy and unhealthy eyes, but the amount of light required to reach half-saturation ($I_{1/2}$, a measure of retinal sensitivity) might be different. So $I_{1/2}$ maps might be more sensitive to early damage. For this purpose sensitivity analysis was performed to develop the required tools for this analysis and also to establish normative data set for this feature. The $I_{1/2}$ value (equation. 4) was calculated for all 25 electrodes of normative data set using flash strengths of 21 and 1033 sc cd s m$^{-2}$. meERG ratios are calculated for the $I_{1/2}$ values and radar plots are plotted in Figure 21.
3.4. Discussion

To our knowledge, this was the first attempt to record from multiple locations on the surface of cornea simultaneously. The motivation behind this work are: (1) to find the relation between the spatial distribution of corneal potentials and the spatial distribution of retinal activity. (2) The potential for using meERG data to validate the bioelectric model of the eye toward functional imaging of the retina.

The current electrodes (DTL & Burian-Allen) used to record full-field ERG signals, effectively average the potential over the area of the contact. The spatial differences in corneal potentials are largely ignored in conventional ERG. Ignoring the spatial differences in corneal ERG is okay because 1) the conventional electrodes average the potentials over a large contact area with the cornea, and 2) the absolute amplitude of the spatial differences is very small compared to the normative ranges.
for conventional ERG, from 35% below to 72% above the median value for maximum dark adapted a-wave amplitudes (Parvaresh et al., 2009).

3.4.1. Clinical Relevance

The obvious clinical application of meERG is analyzing of spatial differences in corneal potentials to evaluate the retinal health and focal deficits. A qualitative relationship between the focal deficit in retina and distribution of potentials in the surface of the eye has been demonstrated by Holland & Herr (1964) as well as Cringle & Alder (1987). Determining the quantitative relationship between deficit in the retina and distribution of corneal potentials, requires reliably recording corneal potentials from multiple location on the cornea simultaneously as it is done using CLEAr Lens. meERG provides repeatable recording with reasonable spatial sampling (Krakova et al, 2014).

Variance in absolute amplitudes between the repeated presentation of stimuli and across the subjects, results in a broad distribution of normative data base in conventional ERG (Parvaresh et al, 2009). For meERG, using the meERG ratio method has removed the variability in absolute amplitudes. Spatial differences in corneal potentials are a source of information derived from meERG that has not been previously available. The results show that the recordings are repeatable with standard deviation of 2-3% across the animals, and 1-2% when repeating a stimuli within an animal (Krakova et al, 2014).
3.4.2. **Source of Asymmetry in Corneal Potentials**

There was no prior expectation regarding uniformity or non-uniformity in the spatial distribution of meERG potentials. However, because of radial symmetry of ocular anatomy and cell distribution, a radial symmetry in responses was expected. The results indicate a slight and repeatable asymmetry in corneal potentials. The possible reasons for this asymmetry are:

- Inhomogeneous distribution of stimulus energy in the retina.
- Inhomogeneous responsivity of retina (due to cell density, photoreceptor orientation, length of outer segment (Williams et al, 1998).
- Asymmetry in conductivity or anatomy of ocular tissue.
- Asymmetry in the recording system (non-uniform gain on electrodes).

To evaluate the asymmetry in the recording system, a sine wave was introduced to all the channels simultaneously. There was a small gain difference between channels, due to small variations in electrode impedances. This explains 10-12% of the asymmetry (Krakova et al, 2014). The effect of the CLEAr Lens on the luminance of the retina has not been carefully evaluated. The lens was placed on the eye so that the cable was parallel to the superior-inferior axis of the eye. The rat was carefully examined during the experiment through an infrared camera attached to the center of the stimulus source. The rat was positioned in such a way that the center of the eye was aligned with the center of the camera. The metal traces on the cable attached to the CLEAr Lens are not radially symmetric in the superior inferior direction, however the asymmetry in the potentials were independent of cable
orientation. The asymmetry in the right and left eye responses were in superior-inferior direction but the cable was extended from superior side of right eye and inferior side of left eye.

The asymmetry present in the responses evoked by a saturating flash strength is the same as the responses to sub-saturating flashes. If the asymmetry was due to the inhomogeneity of stimulus source, it would be lower in the saturating responses because even areas with relatively low illumination would respond at near-maximum amplitudes.

The asymmetry in the responses could reflect the asymmetry in distribution of photoreceptors. The responses are evoked by a bright flash under dark adapted conditions. The a-wave reflects photoreceptor responses and the b-wave reflects the ON-bipolar cell responses. To our knowledge, the map of rod-photoreceptor density across the rat retina has not been reported. The map of retinal ganglion cells (RGC) across the retina shows asymmetry that is repeatable (Salinas-Navarro et al., 2009; Ortin-Martinez et al., 2010) or highly variable (Danias et al., 2002) between animals. The highest RGC density is reported to be in the superior-temporal quadrant. The complete cone maps created show that the L-cone is positively correlated with RGC but the S-cone is negatively correlated with RGC (Ortin-Martinez et al., 2010). The asymmetry in meERG responses matches with RGC density.
3.4.3. Multi-Electrode ERG vs. Multi-Focal ERG

At this early stage of meERG, it is not appropriate to quantitatively compare meERG with mfERG, so these two methods are compared qualitatively (Krakova et al, 2014).

In a typical mfERG recording the stimulus is presented to the eye for 4-8 mins via a computer monitor. The response is recorded through a single electrode (Hood et al., 2012). The single continuous waveform is analyzed to correlate the temporal changes in the recorded signal to the spatio-temporal changes in the stimulus. The correlated voltage changes are assigned to the area of the retina that corresponds to that location in the stimulus image (Sutter & Tran, 1992). mfERG covers 40-50 degrees of the visual angle and provides spatial resolution in the local luminance response that is unprecedented. Challenges in recording mfERG are:

- Test duration that is difficult for some subjects
- Good acuity and ability to fixate on a target during the test are required.
- Technical difficulty in recording from peripheral retina and isolating rod pathway.

meERG uses a full field stimulus (Ganzfeld), so it is compatible with any full-field stimulus protocol (scotopic, photopic, chromatic, flicker, paired-flash and step stimuli). meERG doesn’t need high acuity or stringent fixation. Because of the stability of CLEAr Lens during the test, there is a higher signal to noise ratio (SNR) which makes the test duration shorter than conventional ERG because less averaging is
required. Since a full-field stimulus illuminated the entire retina, the meERG reflects the response of the entire anatomical retina.

In meERG all the spatial information is obtained simultaneously unlike mfERG that it is obtained in time, so a single presentation of stimulus is enough to get the required information.

It is important to understand that the corneal electrode array doesn’t directly map the retinal activity, but it is coupled through a weighting matrix that relates every part of the retina to each electrode (Krakova et al, 2014).
4. meERG POTENTIALS IN RAT EYES WITH EXPERIMENTAL LESIONS

4.1. Overview

meERG is the only method available designed to record ERG waveforms from multiple locations on the surface of cornea simultaneously. Change in retinal activity can affect the potentials recorded from the surface of the cornea. In this chapter, experimental peripheral and central lesions have been created in the rat eye and meERG responses have been recorded before and after creation of these lesions. Sensitivity and specificity of meERG to these local lesions were compared with conventional single electrode ERG. Different classification methods (Cluster and support vector machine) have been evaluated to find the most useful method. The hypothesis being tested is: For localized retinal lesions in rat eyes, analysis of meERG responses will provide better sensitivity and specificity compared to conventional ERG.

4.2. Methods

4.2.1. Experimental lesions

The main goal of this study was to evaluate the effect of experimental lesions in central and peripheral retina on meERG responses, which would begin to assess diagnostic utility of meERG recording for local areas of dysfunction.
For both lesion types, the animal was anesthetized with 100 mg/Kg (body weight) of ketamine and 5 mg/kg (body weight) of acepromazine injected intraperitoneally (IP). Acepromazine was used instead of xylazine because it keeps the ocular media clear, with is necessary for photocoagulation.

Laser photocoagulation was used to create localized damage in the central retina. To produce the damage, a Laserglow Technologies, 532 nm LRS-0532-PF diode-pumped solid-state laser was focused through a Nikon SMZ1000 microscope to the central retina and energy of 8.15e-3 µJ/µm² per pulse was delivered. To determine the laser parameters, the energy delivered per unit area reported in literature to create laser damage in the retina, which is in the range of 1.36e-3 µJ/µm² to 1.52e-2 µJ/µm², was averaged (Cuilla, 2003; Chu et al., 1999; Humphrey et al., 1997; Hu, 2009). Clusters of non-overlapping burns were created to cover areas of 0.94 - 2.29 mm² (2.3 - 5.4% of retinal area). Lesions were created adjacent to the optic disc but restricted to one hemisphere. Location of lesion was selected to avoid major blood vessels on the vitreal surface of retina. The lesion was also further verified by flat mount photography and histology.

Cryocoagulation was used to create localized damage in peripheral retina because photocoagulation laser could not be focused in far-peripheral retina. A custom machined brass cryo-probe with tip diameter of 2 mm shown in Figure 22. The first prototype shown on the left was tested with help of Dr. Yannek Leiderman, an experienced eye surgeon at the Illinois Eye and Ear Infirmary. This design was difficult to use because the tip was short and it was too bulky, the refined design is
shown on the right. This probe was screwed to a plastic handle and was submerged into liquid nitrogen for at least 20 minutes prior to use.

The tip of the probe was applied to the sclera surface just posterior to the limbus with gentle pressure. A few trials were done to set the time needed to create lesion in retina without any damage to sclera. After several seconds, the retina turns white, indicating that cryo-damage has occurred. The area of the damage was confirmed by viewing the peripheral retina through a plano-concave lens and ophthalmic surgical microscope. The retina experiences thermal damage before the sclera (Suomalainen, 1993). Fundus photography was used to document the location of the damage and is verified with flat mount photography and histology. Twenty six seconds was found to be the optimal contact time.

Animals receiving experimental lesions also received 1mg/kg (bodyweight) sub-Q injection of Meloxicam for analgesia.

Figure 22. Custom built brass cryo probe. The probe on the left side of the ruler is the initial design. This probe was used to create lesions on two animals. The difficulties in the process of creation of lesion required the probe to be modified. The modified probe is on the right side of the ruler. The tip has a slight bend to make contact with the eye easier. The probe becomes thicker as it is further from the eye. This makes the probe to keep its low temperature when in contact with eye.
Figure 23 shows a sample fundus photograph for each type of lesion.

![Fundus photograph of the retina after creating focal lesions in the rat eye. (A) The picture was taken on the day central lesion was created using photocoagulation, the area of lesion is outlined with red. (B) The picture was taken on the day peripheral lesion was created using cryocoagulation, the area of lesion is outlined with blue.](image)

**Figure 23.** Fundus photograph of the retina after creating focal lesions in the rat eye. (A) The picture was taken on the day central lesion was created using photocoagulation, the area of lesion is outlined with red. (B) The picture was taken on the day peripheral lesion was created using cryocoagulation, the area of lesion is outlined with blue.

### 4.2.2. Recording

After recording the first meERG for normative data set on day 1, on some of the animals localized central damage (photocoagulation, n=5) or peripheral damage (cryocoagulation, n=11) were created on day 3-4, another set of meERG was recorded from lesion animals on day 5-6.

### 4.2.3. Lesion Verification

Immediately after recording the second meERG, the animal with lesion was sacrificed via CO₂ asphyxiation, the cornea of the right eye (the eye with the lesion) was marked with a cautery burn in the superior margin, the eye was removed and the cornea was slit with a razor blade. Fixative (2% paraformaldehyde/2.5%
glutaraldehyde) was injected into the vitreal cavity through the corneal slit, and the eye was immersed in fixative for 2-7 days, followed by immersion in 91% alcohol for storage until histological processing. After fixation and before histology, the cornea was dissected away and four slits were cut extending from the limbal region toward the posterior pole to create the flat-mount eye cup. This was photographed to document the area and location of the lesion, quantified using ImageJ software. To verify the extent of cellular damage, the flat-mount retina was sectioned through the lesion area and was stained with hematoxylin and eosin stain. Unfortunately, all but the two samples shown in Figure 26 were lost in tissue processing.

4.2.4. Lesion Detection – Cluster Analysis

To classify meERG responses as (healthy vs. lesion), a cluster analysis was performed. The parameters used to define the cluster space were the spatial distribution of corneal potentials, meERG ratios of each rat (healthy and damaged eyes) was compared to the mean meERG ratios of healthy eyes at each of the peripheral electrode positions. For healthy eyes, a leave-one-out cross-validation was used. Using n meERG ratios (n=12 for A-ring, n=8 for B-ring) as coordinated of n-dimensional space, the Euclidean distance was calculated between each data set and the normal mean using the following equation:

$$Euclidean\ Distance = \sqrt{\sum_{i=1}^{n} (R_n - R_{n,\text{HM}})^2}$$

Eq. 5
In equation 5, $R$ is the meERG ratio calculated for each animal, $n$ is the number of electrodes, which is 12 for A-ring and 8 for B-ring, $R_{HM}$ is the meERG ratios of healthy mean.

A histogram of Euclidean distances was plotted. These plots were used to create the receiver operating characteristic (ROC) curves. To create an ROC curve, a vertical line is defined as a decision boundary (threshold line). At each position of the threshold line on the x-axis, distances less than threshold is considered as healthy and above threshold is considered as lesioned eyes. The threshold line starts from zero of the histogram plot and moves toward the largest Euclidean distance. The step size of the threshold line is the same as bin sizes in the histogram plot. The true positive and negative rates as well as the false positive and negative rates are calculated. These rates are used to calculate the sensitivity and specificity of meERG and plot the ROC curve.

4.2.5. Lesion Detection – Support Vector Machine (SVM)

In addition to the cluster analysis, support vector machine (SVM) was used to further quantify differences in healthy and damaged retinas. SVMs, which demonstrate high accuracy in binary classification analysis, were separately trained using a-wave and b-wave amplitudes on 25 electrodes, a-wave and b-wave meERG ratios on 12 peripheral electrodes. For each feature, 39 animals (26 healthy and 13 lesioned eyes) were separated into 10 equally-sized groups. The SVM was trained using 9 of the groups and remaining 1 group was tested. Then what we consider the
training and testing groups was rotated so that all the rats were in the test group at least once.

The SVM training consisted of using the MATLAB™ function “svmtrain”, which builds a model of the training data based on the classifications of “diseased” or “healthy”. This model can be thought of as a dividing line between the diseased and healthy groups (a-wave amplitudes) for each channel. “svmclassify”, another MATLAB function was used, which takes input (e.g. a-wave amplitudes of a test rat) and classifies it according to the model (i.e. dividing line) created by “svmtrain”.

Classification was verified using 10-fold cross-validation for all input data types. To obtain reliable repeated measures of true positive and true negative rates despite modest sample sizes, the data was reshuffled and 10-fold cross-validated 20 times. The measures reported is the average of the 20 results.

4.2.6. Conventional ERG

In order to compare meERG with conventional ERG, the meERG waveforms on 12 peripheral electrodes were averaged to simulate the conventional ERG recorded using Burian-Allen lens in clinics, because this is close simulation of ring electrode that contacts cornea. Figure 24 shows a sample Burian-Allen lens and schematic of simulated conventional ERG.
4.2.7. Difference Normalized Potential Map

To interpret the difference between data of a single animal and normative response (Figure 19), the difference normalized potential map was created. To create the normalized difference potential map the standard score values of normative data base is subtracted from the standard score of a single animal by location. An example of difference potential map is presented in Figure 25.
Figure 25. Creating potential map illustrating difference from the normative data set. For each location on the normalized potential map, the response from the average normal response (converted to standard score, Eq. 2) is subtracted from single animal response (in this case an animal with central lesion). The resulting difference potential map plots the distance the individual response was from the normal mean, in units of standard deviations of the normative data.

4.3. Results

4.3.1. Representative Lesion

Representative lesion created using photocoagulation (central lesion) and cryocoagulation (peripheral lesion) are presented in Figure 26.
Figure 26. Representative experimental lesions in central and peripheral retina. (A) Flat mount eye cup prepared and photographed following fixation of tissue. Lesion created by laser photocoagulation is outlined in red (area = 1.01 mm², approximately 1.11 mm from ONH, circled in green). (B) Histological cross section of retina near margin of lesion shown in A, showing disruption of the RPE and near complete loss of all cell types. (C) Flat mount preparation showing lesion created by cryotherapy; lesion outlined in red (area = 6.56 mm², approximately 2.27 mm from ONH, circled in green). (D) Histological cross section of retina near margin of lesion shown in C, showing disruption of the RPE and near complete loss of all cell types.

Table 3 summarizes the experimental lesions created by area of the lesion and distance of lesion from optic nerve head (ONH) and location of the lesion.
4.3.2. Corneal Potential Distribution in Eyes with Lesions

4.3.2.1. Saturating Stimulus Response

For the data recorded with bright stimulus (1033 sc cd s m\(^{-2}\)) data from representative animals with central and peripheral lesion are shown in Figure 27. Panels A and H illustrate the flat mount picture of the eye with central laser damage (outlined in red) and peripheral cryo damage respectively. Normalized differential potential map based on a-wave amplitudes for laser animal and cryo animal are shown in panels B and I. Panels E and L illustrate the color map of b-wave
for laser damage and cryo damage respectively. Changes in corneal potentials that resulted from localized lesions are evident in potential maps. Presence of a lesion in the retina changes the corneal potential distribution compared to healthy eyes.

meERG ratios of a-wave and b-wave amplitudes for A-ring and B-ring electrodes have been calculated for all healthy animals and animals with central and peripheral lesions. meERG ratios of healthy animals were averaged by electrode position and has plotted as a black solid line in panels C, D, F, G, J, K, M and N of Figure 27. Dashed lines represent ±2 standard deviation of the normative data set. The red lines in panels C, D, F, and G plot the meERG ratios for the laser animal shown in panel A. The blue lines in panels J, K, M and N shows the meERG ratios for the cryo animal shown in panel H. The radar plots for animals with focal lesions is not in the range of typical healthy animal and is shifted outside of ±2 SD.
Figure 27. (A) Sample retina with a central lesion (outlined in red). (B-G) Saturating stimulus results for the retina shown in panel A. (B) Standard score difference between this animal and normative data set (a-wave) (C) Radar plots for the A-ring electrodes, a-wave values. (D) Radar plots for the B-ring electrodes, a-wave values. (E) Standard score plot at the peak of the b-wave. (F) Radar plots for the A-ring electrodes, b-wave values. (G) Radar plots for the B-ring electrodes, b-wave values. (H) Sample retina with a peripheral lesion (outlined in blue). (I-N) Results for the retina shown in panel H. (I) Standard score difference between this animal and normative data set (a-wave) (J) Radar plots for the A-ring electrodes, a-wave values. (K) Radar plots for the B-ring electrodes, a-wave values. (L) Standard score plot at the peak of the b-wave. (M) Radar plots for the A-ring electrode, b-wave values. (N) Radar plots for the B-ring electrodes, b-wave values.
For lesions in peripheral retina, the differences in corneal potentials were generally aligned with the radial direction of the lesion. Figure 28 shows four examples of peripheral lesion eyes, along with the corresponding potential difference maps. The amplitudes near the lesion area are smaller.

For the a-wave values on each of the 24 electrodes (M is excluded), the standard score values were calculated using Equation 2. For each electrode a vector starting from electrode M and pointing toward the electrode location and amplitude of the standard score was assigned. These vectors were combined to form a “shift vector” for each animal. Shift vector summarized the magnitude and direction of the global shift in corneal potentials due to the lesion; these shift vectors are shown in red on the flat mount photographs in Figure 28. Seven of nine eyes with peripheral lesions showed good correlation between the direction of shift in corneal potentials and the location of the lesion ($R^2 = 0.81$). Magnitude of the global shift was weakly correlated with the area of the lesion ($R^2 = 0.22$). The global shifts in corneal potentials for the four eyes with central lesions did not appear to be correlated with the location or size of the lesion.
Figure 28. Correlation of shift in corneal potentials with location of the lesion. A. Left image is the flat mount eye cup photograph showing the cryocoagulation lesion area (outlined in blue). Superimposed in red is a vector indicating the magnitude and direction of the global shift in corneal potentials compared to the average healthy eye, vector origin at the ONH. Right image is the difference map of corneal potentials for the eye on the left. B-D, three additional eyes with peripheral lesions presented as in panel A.
The effect of experimental lesions on the absolute amplitude of corneal potentials was compared to the lesion area. The absolute amplitude of a-wave on 12 A-ring electrodes were averaged for lesioned animals before and after treatment. The percent reduction in amplitude was compared to lesion area, expressed as a percentage of retinal area, using linear regression. For peripheral lesions, the amplitude reduction was somewhat correlated with lesion area ($R^2 = 0.48$) and approximately 15% greater than expected based on lesion area (slope = 1.15). For central lesions, the reduction in ERG amplitude was highly correlated with lesion area ($R^2 = 0.98$), but approximately seven times greater than expected based on lesion area (slope = 7.35). This suggests that a reduced visual response was present for retina well beyond the area of obvious damage in the flat-mount preparations, especially for the photocoagulation model.

For all post-treatment meERG recording, in control animals as well as animals with experimental lesions, there was an additional reduction in meERG amplitudes of approximately 12% that was independent of lesion area ($y$-intercept) attributed to the general experimental procedures (repeated anesthesia, fundus photography and meERG recording within 6-7 days) and short recovery time between photocoagulation and cryocoagulation treatments and post-treatment meERG recording.
4.3.2.2. Sub-Saturating Stimulus Response

For the data recorded with a sub-saturating stimulus (flash strength = 21 sc cd s m⁻²) data from representative animals with central and peripheral lesions are shown in Figure 29. Panels A and H illustrate the flat mount picture of the eye with central laser damage (outlined in red) and peripheral cryo damage respectively. Normalized differential potential map based on a-wave amplitudes for laser animal and cryo animal are shown in panels B and I. Panels E and L illustrate the color map of b-wave for laser damage and cryo damage respectively. Changes in corneal potentials that resulted from localized lesions are evident in potential maps. Presence of a lesion in the retina changes the corneal potential distribution compared to healthy eyes.

meERG ratios of a-wave and b-wave amplitudes for A-ring and B-ring electrodes have been calculated for all healthy animals and animals with central and peripheral lesions. meERG ratios of healthy animals were averaged by electrode position and has plotted as a black solid line in panels C, D, F, G, J, K, M and N of Figure 29. Dashed lines represent ±1 standard deviation of the normative data set. The red lines in panels C, D, F, and G plot the meERG ratios for the laser animal shown in panel A. The blue lines in panels J, K, M and N shows the meERG ratios for the cryo animal shown in panel H.

The results presented in Figures 25 and 26, shows retinal lesions change the responses of saturating and sub-saturation stimulus in the same way.
Figure 29. (A) Sample retina with a central lesion (outlined in red). (B-G) Sub-saturating stimulus results for the retina shown in panel A. (B) Standard score difference between this animal and normative data set (a-wave) (C) Radar plots for the A-ring electrodes, a-wave values. (D) Radar plots for the B-ring electrodes, a-wave values. (E) Standard score plot at the peak of the b-wave. (F) Radar plots for the A-ring electrode, b-wave values. (G) Radar plots for the B-ring electrodes, b-wave values. (H) Sample retina with a peripheral lesion (outlined in blue). (I-N) Results for the retina shown in panel H. (I) Standard score difference between this animal and normative data set (a-wave) (J) Radar plots for the A-ring electrodes, a-wave values. (K) Radar plots for the B-ring electrodes, a-wave values. (L) Standard score plot at the peak of the b-wave. (M) Radar plots for the A-ring electrode, b-wave values. (N) Radar plots for the B-ring electrodes, b-wave values.
4.3.2.3. Corneal Maps of Sensitivity ($I_{1/2}$)

As mentioned in section 3.3.6.3, in unhealthy retina, sometimes retinal cells might be functioning but they might be less sensitive to light, so $I_{1/2}$ maps might be more sensitive to early damage. For this purpose sensitivity analysis was performed to develop the required tools for this analysis. Methods from section 3.2.7.6 are used to calculate sensitivity parameter ($I_{1/2}$). Representative data for central and peripheral lesion animals are shown in Figure 30. Panels A and E illustrate the flat mount picture of the eye with central photocooagulation damage and peripheral cryocoagulation damage, respectively. Normalized $I_{1/2}$ maps for animals with central and peripheral lesion are shown in panels B and F.

meERG ratios of the sensitivity parameter, $I_{1/2}$, for A-ring and B-ring electrodes were calculated for all healthy animals and animals with central and peripheral lesions. meERG ratios of healthy animals were averaged by electrode position and are plotted as a black solid line in panels C, D, G, and H. Dashed lines represent ±1 standard deviation. The red lines in panels C and D show the meERG ratios for the central lesion animal shown in panel A. The blue lines in panels G and H show the meERG ratios for the peripheral lesion animal shown in panel E. The $I_{1/2}$ maps have not been quantitatively compared to corneal potential maps yet. This is a part of the project was an initial effort. More work needs to be done to know what information $I_{1/2}$ maps are providing.
Figure 30. (A) Sample retina with a central lesion (outlined in red). (B-D) Sensitivity ($I_{1/2}$) results for the retina shown in panel A. (B) Standard score difference between this animal and normative data set (C) Radar plots for the A-ring electrodes, $I_{1/2}$ values. (D) Radar plots for the B-ring electrodes, $I_{1/2}$ values. (E) Sample retina with a peripheral lesion (outlined in blue). (F-H) Sensitivity ($I_{1/2}$) results for the retina shown in panel E. (F) Standard score difference between this animal and normative data set (G) Radar plots for the A-ring electrodes, $I_{1/2}$ values. (H) Radar plots for the B-ring electrodes, $I_{1/2}$ values.

4.3.3. meERG Sensitivity to Local Lesions – Cluster Analysis

4.3.3.1. Saturating Stimulus

The sensitivity and specificity of meERG to localized experimental lesions was evaluated using cluster analysis and receiver operator characteristic (ROC) curves, and the results were compared with conventional ERG. meERG ratios for a-wave amplitudes on A-ring electrodes were used as the cluster spare parameter. Cluster analysis (12D Euclidean distance) was used to create the histogram of animals
in panel A of Figure 31. Receiver operating characteristic (ROC) curve shown in panel B of Figure 31 subtends an area of 80%.

The a-wave amplitudes on the 12 peripheral electrodes were averaged to simulate the conventional ERG recorded using a Burian-Allen electrode. The absolute amplitude of a-wave values have been summarized in the histogram of panel C in Figure 31. The area under the ROC curve for conventional ERG is 84%. The results show that meERG, which is based solely on spatial differences in corneal potentials, has high sensitivity and specificity in detecting local lesions in central and peripheral retina.
Figure 31. (A) Cluster analysis based on a-wave amplitudes evoked by a saturating stimulus for 26 normal, 4 central lesion and 9 peripheral lesion eyes. a-wave amplitude meERG ratios were calculated for each of the 12 peripheral electrodes. These meERG ratios were taken to be coordinates in a 12-dimensional feature space. For each data set, the Euclidean distance from the mean was calculated. For healthy eyes a leave-one-out technique was used. Distances are summarized in a histogram. (B) The ROC curve created from the histogram in panel A. (C) Absolute amplitudes of a-wave on A-ring electrodes were averaged to simulate the conventional ERG. A histogram of simulated conventional ERG amplitudes was created. (D) The ROC curve created from the histogram in panel C.

Sensitivity and specificity for different meERG response features are compared in Table 4. The area under the ROC curve was calculated for a-wave amplitude, b-wave amplitude and a-wave/b-wave. a-wave/b-wave is analyzed because this ratio is suggested as useful tool for assessment of clinical ERG and retinal function. The a-
wave amplitude depends on the luminance of the stimulus and integrity of photoreceptors, while b-wave amplitude depends on the a-wave and integrity of retina and its signal transmission. Therefore, a-wave /b-wave is a measure of integrity of retina (Perlman, 1983). For meERG, analysis has been performed for A-ring, B-ring and A&B-ring together. Area under the curve for analysis based on a-wave amplitudes is larger than for other features. Also, asymmetry in meERG waveforms caused by lesion affects peripheral electrodes (A-ring) more than the B-ring electrodes, which are more centrally located.

<table>
<thead>
<tr>
<th>Spatial meERG Ratios</th>
<th>Area Under the ROC Curve (%)</th>
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<tbody>
<tr>
<td></td>
<td>a-wave</td>
<td>b-wave</td>
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<tr>
<td>A-ring</td>
<td>80</td>
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</tr>
<tr>
<td>B-ring</td>
<td>81.5</td>
<td>68.6</td>
</tr>
<tr>
<td>AB-ring</td>
<td>80.9</td>
<td>79.1</td>
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Table 4. Area under the ROC curve calculated for different features used in the cluster analysis.

4.3.3.1.1. meERG Sensitivity to Local Lesions – SVM

A support vector machine (SVM) approach implemented in MATLAB was used to evaluate the ability to automatically detect local lesions with meERG data.
Cluster analysis has been compared to SVM in Table 5. Sensitivity and specificity were calculated for a-wave and b-wave amplitudes for conventional ERG, cluster analysis and SVM classification. Using the histograms shown in Figure 30 the decision boundary is selected so that the sensitivity is larger than 80% (red arrows on ROC curve). The sensitivity and specificity of meERG and conventional ERG were calculated at this decision boundary and were compared with SVM. Cluster analysis has larger sensitivity and specificity when using meERG ratios but SVM has larger sensitivity and specificity when using absolute amplitudes.

<table>
<thead>
<tr>
<th></th>
<th>a-wave</th>
<th></th>
<th>b-wave</th>
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<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Sensitivity</td>
<td>Specificity</td>
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<tr>
<td>Conventional ERG</td>
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<td></td>
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<tr>
<td>A-ring average</td>
<td>85%</td>
<td>73%</td>
<td>92%</td>
<td>50%</td>
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<tr>
<td>Cluster Analysis</td>
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<tr>
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<td>84%</td>
<td>72%</td>
<td>92%</td>
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<tr>
<td>A-ring meERG Ratios</td>
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<td>74%</td>
<td>30%</td>
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Table 5. Sensitivity and specificity of different methods in separating the lesioned eye from healthy is presented.

From Table 5, the best detection (D) was obtained with cluster analysis based on the a-wave meERG ratios on A-ring electrodes (D=sensitivity + specificity=162).
and absolute amplitudes of b-wave using SVM (D=164). These are the most effective methods for separating normal from lesioned animals. For future analysis, cluster analysis will be performed on a-wave meERG ratios on A-ring electrodes because using meERG ratio method eliminated the inter experiment and inter subject variance and it provides higher sensitivity and specificity.

4.3.3.2. Sub-Saturating Stimulus

Cluster analysis using a-wave meERG ratios on A-ring electrodes for meERG and conventional ERG was performed for sub-saturating stimulus (flash strength = 21 sc cd s m^{-2}). The results are shown in Figure 32. The area under the ROC curve for meERG is calculated to be 81%. The area under the ROC curve for conventional ERG is 85%.
Figure 32. (A) Cluster analysis based on a-wave amplitudes evoked by a sub-saturating stimulus for 26 normal, 4 central lesion and 9 peripheral lesion eyes. a-wave amplitude meERG ratios were calculated for each of the 12 peripheral electrodes. These meERG ratios were taken to be coordinates in a 12-dimensional feature space. For each data set, the Euclidean distance from the mean was calculated. For healthy eyes leave-one-out technique was used. Distances are summarized in a histogram. (B) The ROC curve created from the histogram in panel A. (C) Absolute amplitude of a-wave values on A-ring was averaged to simulate the conventional ERG. Histogram of animals was created. (D) The ROC curve created from the histogram in panel C.

4.3.3.3. Sensitivity (I1/2)

Cluster analysis on I1/2 meERG ratios on A-ring electrodes for meERG and conventional ERG is performed. The results are shown in Figure 33. The
area under the ROC curve for meERG is calculated to be 73%. The area under the ROC curve for conventional ERG is 72%.

Figure 33. (A) Cluster analysis based on spatial differences in $I_{\frac{1}{2}}$ values for 26 normal, 4 central lesion and 9 peripheral lesion eyes. $I_{\frac{1}{2}}$ value meERG ratios were calculated for each of the 12 peripheral electrodes. These meERG ratios were taken to be coordinates in a 12-dimensional feature space. For each data set, the Euclidean distance from the mean was calculated. For healthy eyes leave-one-out technique was used. Distances are summarized in a histogram. (B) The ROC curve created from the histogram in panel A. (C) Absolute amplitude of a-wave values on A-ring was averaged to simulate the conventional ERG. $I_{\frac{1}{2}}$ values were calculated for simulated conventional ERG. Histogram of animals was created. (D) The ROC curve created from the histogram in panel C.

4.4. Discussion

In degenerative eye diseases like glaucoma, diabetic retinopathy and macular degeneration, it is important to detect and diagnose the disease in early stages so that
treatment can be initiated to stop or slow down the progressive vision loss. In early stages, damage to the retina due to disease or trauma is usually localized, hence assessment of local retinal function is important. In clinics, structure and function of the retina are evaluated to detect these diseases. Optical coherence tomography (OCT) is an imaging technology used to measure cell loss (structure). Tests like the Humphrey visual field test (HVF) and multi-focal electroretinogram (mfERG) are used to evaluate the function of retina. All of these tests are limited in their application to the peripheral retina (beyond 30 degrees), where some prevalent diseases begin. In meERG, a full field uniform flash is presented to the retina, and the response recorded is from the entire retina. In the meERG ratio approach developed here to analyze the meERG data, variations in absolute amplitudes between similar animals and experiments are eliminated.

When there is a focal damage in the retina, the contribution of the cells in that area is eliminated or reduced which results in asymmetry in the distribution of corneal potentials compared to a normative data set. The ability of meERG in detecting focal peripheral lesions gives this technology a potential use of detecting degenerative diseases starting in peripheral retina.

Earlier efforts to record and analyze ocular surface potential maps concluded that the spatial distribution of corneal potentials is influenced by the spatial distribution of the current sources, i.e. the distribution of activity in the retina; this was demonstrated empirically with experimental photocoagulation lesions (Holland
& Herr, 1964; Cringle & Alder, 1987) and theoretically predicted using models of local areas of deficit (Davey et al. 1988; Job et al. 1999).

It is difficult to compare the present results to earlier empirical work because of the use of different species (rabbit, dog), experimental setups (isolated perfused eye), incomplete description of the experimental lesions, and different recording locations (limbus, sclera). However, the earlier empirical work, and the ERG electric field models developed by Job et al. (1999) all agree that ocular surface potentials decrease in locations near the site of experimental lesions in the retina. The present study confirms this finding using comprehensive corneal potential maps, each derived from the response to a single full-field flash, representing a technical solution with the potential for clinical application.

The value of corneal potential maps depends largely on the ability to interpret them with regard to spatial differences in retinal function. The data presented in this work demonstrate that the normalized potential maps for healthy eyes are quite consistent, and that measurable differences from the normal spatial distribution are created by experimental lesions. This is true for lesions in the central retina and lesions in the far-peripheral retina, where very few clinical tests are effective.

4.4.1. Saturating Stimulus versus Sub-Saturating Stimulus

The stimulus source was built and calibrated in the NEVL. The source was built to be uniform and subtend the visual field of a rat. In the early experiments,
to avoid the possible asymmetry in illuminating the retina, which the CLEAr Lens could cause because of absorption and refraction of the light, saturating flashes were used. In the final set of experiments which were performed to evaluate the sensitivity and specificity of meERG in detecting focal lesions, sub-saturating and saturating stimuli were presented alternatively. Comparing the results between these data sets made it clear that even in the lower flashes the asymmetry in the responses are because of the anatomy of the retina and the stimulus system is not introducing asymmetry.

4.4.2. meERG versus Pattern ERG and Photopic Negative Response

The effectiveness of available ERG methods in detecting glaucoma has been reviewed by Bach & Poloschek (2013). Glaucoma is defined as loss of retinal ganglion cells. The two methods that directly measure the function of retinal ganglion cells are pattern ERG (PERG) and photopic negative response (PhNR).

Since 1983 when Bobak et al. (1983) first reported the reduction in PERG in glaucomatous eyes, there has been reports every year showing this reduction. Reports also show that the PhNR is significantly reduced in the eyes with glaucoma (Viswanathan et al. 1999). Machida el al. (2008) reported reduction of PhNR as well as PhNR/a-wave and PhNR/b-wave in eyes with glaucoma.

Preiser et al. (2013) have studied the sensitivity and specificity of PERG and PhNR in detecting glaucoma. They have recorded PhNR and PERG (0.8° & 16° checks) from preperimetric and manifest glaucoma patients. For preperimetric/manifest glaucoma
respectively they report area under the curve of (61%/78%) for PhNR amplitude, (80%/80%) for PhNR/b-wave ratio, (59%/76%) for 0.8˚PERG amplitude and (73%/79%) for 0.8˚/16˚ ratio of PERG.

A potential use for meERG is detecting glaucoma. The results of this work shows the ability of meERG in detection focal experimental lesions, with area under the curve of 80%. Recording meERG from healthy human eye and glaucoma patients will make comparison of meERG with PERG and conventional PhNR possible.

4.4.3. Future work

There are still questions that need to be answered about meERG and which are not in the scope of current work.

One of the main challenges in meERG is filling the CLEAr Lens before each experiment and cleaning it after each experiment. This process currently takes about 120 minutes and requires constant care and attention; it also has low channel yield. If the lens is not filled properly it results in loosing channels or changing the impedance of the channel. If the lens is not cleaned properly the residue of salt, lipids and proteins can change the impedance of the electrodes. It is preferred to fill the lens once with high channel yield and be able to sterilize it after each experiment.

The change in the tear film between the cornea and the concave part of the CLEAr Lens with time can change the amplitude of the ERG waveforms. Also any change in the shape of the cornea caused by the CLEAr Lens can affect the responses.
The future study needs to be done to answer the questions whether the tear film or the cornea is affected by the lens.

There is not a complete understanding of correspondence between the asymmetry in retinal function and asymmetry in corneal potential map. A possible experiment to give us information in this matter is focal stimulation of retina and recording meERG. When using CLEAr Lens, a conventional focal stimulus could not be used, because of the distortion of the light by the lens. The initial idea is stimulating the retina using a fiber optic from the back side of the eye. This will be one of the future experiments to give us more information about the relationship between retinal activity and the corneal potential map.

It should be noted that meERG recording technology is amenable to all full-field flash ERG protocols, and provides all of the information of conventional single-electrode ERG recording (amplitude and kinetics) in addition to the unique spatial information. Analysis based on a-wave amplitudes was effective for detecting the experimental lesions employed in this work; other flash ERG response parameters (e.g. photopic negative response amplitude or oscillatory potential amplitude) may be more appropriate for other types of retinal insult (glaucoma or vascular disease, respectively).
5. RELATED ERG WORK

5.1. ERG in a murine model of Alzheimer’s disease

This research has been done in Dr. Pepperberg’s laboratory. I have recorded the ERG waveforms for their team. Here the hypothesis tested and only the ERG results are presented. This work has been published in the journal of Experimental Eye Research in 2015. The permission to use parts of this work here, is included in the appendix.

5.1.1. Introduction

Amyloid-beta (Aβ) is a group of 38- to 43-amino acid peptides generated in the eye and other organs, which is aggregation-prone. Recent studies raise the hypothesis that excessive Aβ levels may contribute to certain retinal degenerative diseases such as age-related macular degeneration (AMD) (Dentchev et al., 2003; Liu et al., 2015; Ning et al., 2008; Isas et al., 2010). Since a major portion of Aβ is released as a monomer into extracellular space, a hypothesis being tested is whether a technology enabling the enzymatic break-down of Aβ in the living eye under physiological conditions could have therapeutic application. One of the endopeptidases known to break Aβ monomer into inactive products is Neprilysin (NEP) which is a membrane-associated protein. However, sNEP, which is a recombinant form of the NEP catalytic domain, is soluble in aqueous medium.
In order to test the ability of sNEP in breaking down Aβ in the microenvironment of the intact eye, the sNEP was intravitreally delivered on ocular Aβ levels in mice that exhibit readily measurable, aqueous buffer-extractable Aβ$_{40}$ and Aβ$_{42}$ (wildtype (C57BL/6J) and 5XFAD transgenic mice). Since intravitreally delivered sNEP decreases ocular Aβ levels \textit{in vivo} it is interesting to know how such treatment affects the status of the eye tissues. As a test of tissue status, ERG responses were recorded from sNEP-treated eyes as a measure of retinal function (Parthasarathy et al., 2015).

5.1.2. Methods

5.1.2.1. Animals

The ERG was recorded from six 10-month old C57BL/6J mice and six 2-3-month 5XFAD mice. The eyes were treated either with low dose (0.004 μg) or high dose (10 μg) of sNEP or PBS (control) (Parthasarathy et al., 2015). The mice were dark adapted for 3 hours and were anesthetized by intraperitoneal injection of a saline solution containing 0.15 mg/g (body weight) of ketamine and 0.01 mg/g (body weight) of xylazine. The cornea was anesthetized with 0.5% proparacaine HCL and the pupil was dilated with 2.5% phenylephrine and 1% tropicamide. A regulated heating pad was used to maintain the mice at 37-39°C during experiment.
5.1.2.2. Electrodes

Platinum needle electrodes were used as the reference electrode placed in the ipsilateral cheek and the ground was placed in the nape of the neck. ERG was recorded using a single stainless steel wire electrode in gentle contact with the surface of the cornea.

5.1.2.3. Recording

The same stimulus source used to record meERG responses was been used. A heat filter (Schott KG-3; Phillips Safety Products, Middlesex, NJ) was place between the xenon lamp and the translucent acrylic dome. The presented flash strengths ranged from 0.04 to 8.33 sc cd s m\(^{-2}\). Each flash strength was presented 3 times and the waveforms of same flash were averaged for each animal.

5.1.3. Results

Figure 34 shows the ERG waveforms recorded from C57BL/6J mice (A-C) and six 2-3-month 5XFAD mice (D-F). The relative Aβ values reported below the ERG waveforms, indicates the Aβ reduction on the day of ERG recording. The results show that treatment with both sNEP doses preserved overall robust ERG responsiveness (Parthasarathy et al., 2015).
Figure 34. (A-C) ERG responses recorded from the eyes of 10-month C57BL/6J mice. (D-F) ERG responses recorded from the eyes of 2-3-month 5XFAD mice. ERG data were obtained at 9 days following intravitreal treatment with PBS alone (A & D), 4 ng sNEP (B & E), or 10 μg sNEP (C & F). Mice were euthanized 2-6 hr after ERG recording. The values below the ERG waveforms in the figure indicate results obtained for relative $A\beta_{40}$ and $A\beta_{42}$ levels in the treated eyes. Each waveform is the average of 6 responses (3 responses are recorded from each of two identically treated mice). The flash strength of stimulus for each ERG waveform is reported on the left side of the figure.(Data published in Parthasarathy et al., 2015)
5.2. Peripheral pattern ERG system

5.2.1. Technical development goals

A novel stimulus source was developed that records pattern ERG (PPERG) from peripheral retina. For this purpose a reversing checkerboard is assembled on a hemispherical surface which fills the visual field. Each check is illuminated by a white LED (Bridgelux BXRA-C0402) behind the translucent surface of the hemisphere. The central part of the dome is black, and corresponds to the area that is subtended by a conventional PERG stimulus. There are 4 rows and 30 columns of checks that are used for stimulation. Figure 35 shows this stimulus source (PeriStim™).

Figure 35. Peri-Stim while it is used to record from a human subject (Photo courtesy: Shresta Patangay).
The system has an adjustable luminance, the On-luminance is measured to cover the range of 90-1670 photopic candela per meter squared (ph cd m\(^{-2}\)). The standard deviation of luminance across the checks for a given mean ON-luminance is approximately 16%.

5.2.2. Technical development contributions

The power circuitry was built to control the LEDs with constant current. A 10 amperes power supply is connected to the collector of power transistors, and the emitter is connected to the positive pin of the LEDs. A pulse generator is connected to the base of the transistor with inverting or non-inverting amplifier which has a gain of 1. To be able to add or remove any columns from the stimulus source, the negative pins of the LEDs are connected to ground through solid states relays which are controlled by Arduino.

An early version of the PPERG data acquisition system was developed. A continuous waveform was recorded using lab-view. The data was given to MATLAB to window the wave form and match it to the pulses controlling the checkerboard. The windows extracted were averaged to get the peripheral pattern ERG (PPERG).

Signals were recorded from human subjects to test the validity and repeatability of the recorded data. Figure 36 shows a sample waveform recorded using Peristim\textsuperscript{TM}.
Figure 36. Sample peripheral pattern ERG recorded using Peristim™. This signal is the average response from 6 healthy human subjects. The check size was 10°, the luminance was 1670 ph cd m⁻² and reversal rate was 4.6 reversals per second.
6. REFERENCES


7. APPENDIX

Lesioned Retina and Corneal Potential Maps

The flat mount photograph of retina with central or peripheral lesion (on the left), and the corresponding difference color plot (on the right) is shown for all animals. In the figures below the area of the lesion is outlined with red line.

The plots below show the animals with central lesion.
Figure 37. (A-D) flat mount eyecup showing central lesion outlined in red. Green circle shows the optic nerve head. (E-H) Difference map of corneal potentials plotting number of standard deviations from the average normal eye response at each location for the eye on the left side of the color map.

The plots below show the animals with peripheral lesion.
Figure 38. (A-I) flat mount eyecup showing central lesion outlined in red. Green circle shows the optic nerve head. (J-R) Difference map of corneal potentials plotting number of standard deviations from the average normal eye response at each location for the eye on the left side of the color map.

**Printed Circuit Board (PCB)**

Figure 39 shows the printed circuit board (PCB) designed as an interface between the lens and the amplifier. The EAGLE software was used to create this BCP.

![Printed Circuit Board](image)

*Figure 39. Schematic of PCB designed as an interface between CLEAr Lens and the amplifier.*

**Light Transmission of CLEAr Lens**

The light transmission of the CLEAr Lens and the electrodes were measured. Figure 40 shows the schematic of this measurement. A black cylinder made of plastic was placed around the light sensor. Diameter of the cylinder was the same as diameter of the concave pat of the CLEAr Lens. Inside the cylinder and the through holes of the CLEAr Lens was filled with saline. The first set of measurements were performed...
without the clear lens. A flash of light was presented from the stimulus source and the amount of light was measured. The process was measured three times and the average of three light measurements was 1013 sc cd s m⁻². In the second set of recording, CLEAr Lens was placed on top of the cylinder as shown in right panel of Figure 40. Using the same settings of the stimulus source, three sets of light measurement was performed. The average of three light measurements was 543 sc cd s m⁻². The light transmission of the CLEAr Lens was calculated to be 53.6%.

Figure 40. Schematic of measuring light transmission of CLEAr Lens. Left panel shows the measurement of stimulus luminance without CLEAr Lens. Right panel shows the measurement of luminance with CLEAr Lens. In this figure, the relative scale of the stimulus source and the CLEAr Lens is changed for better view.
Filling Protocol of Rat CLEAr Lens

Through holes of the CLEAr Lens was filled with “CVS Saline Solution for Sensitive Eyes” before each experiment to provide the conductive pathway between the cornea and the recording electrodes. The protocol of filling the lens is given here.

1. Ensure the rat lens is free of foreign debris and is securely attached to the cable and the PCB.
2. Turn on the heating of the vacuum chamber and set it to 39, the same as rat’s body temperature (this prevents the saline to freeze)
3. Position the lens so that the concave side is facing up.
4. Using a 1 ml syringe place 3 drops of “CVS Saline Solution for Sensitive Eyes” onto the cup of the lens.
5. Place the PCB and the lens attached to it, in the vacuum chamber.
6. Close chamber door. Ensure the depressurizing valve is in the open position and the pressurizing valve is in the closed position. Turn on the vacuum until the vacuum pressure gauge reaches 28.5 inHg.
7. Once 28.5 inHg is achieved wait until all the bubbles are exploded.
8. If saline freezes, procedure has failed. Dispose of used saline and restart process from step #4.
9. When there is no bubble left, opening the pressurizing valve and allowing the air back into the vacuum chamber.
10. Repeat the process from step #6 until no bubbles are formed in the pressure of 28.5 inHg.

11. Using a microscope; inspect the lens. If bubbles are present in the lens it will be necessary to repeat the procedure.

**Cleaning Protocol of Rat CLEAr Lens**

After each experiment, CLEAr Lens was washed thoroughly to prevent formation of salt and lipid residue on the electrodes.

1. Fill a large beaker with distilled water.

2. Immediately after removing the CLEAr Lens from rat, soak it in distilled water for 10 minutes (make sure the FPC connector is not inside water).

3. Remove the lens from distilled water and wick the water from the surface of the lens with Kim wipe.

4. Position the lens so that the concave side is facing up.

5. Using a 1 ml syringe place 3 drops of “Walgreens Sterile Multi-Purpose Solution for Soft Contact Lenses” onto the cup of the lens.

6. Place the PCB and the lens attached to it, in the vacuum chamber.

7. Close chamber door. Ensure the depressurizing valve is in the open position and the pressurizing valve is in the closed position. Turn on the vacuum until the vacuum pressure gauge reaches 28.5 inHg.

8. Once 28.5 inHg is achieved wait until all the bubbles are exploded.
9. When there is no bubble left, opening the pressurizing valve and allowing the air back into the vacuum chamber.

10. Remove the PCB and CLEAr Lens from vacuum chamber and add two more drops of “Walgreens Sterile Multi-Purpose Solution for Soft Contact Lenses” onto the cup of the lens.

11. Let the lens sit for 10 minutes.

12. Soak it in clean distilled water for 10 minutes (make sure the FPC connector is not inside water).

13. Wick the “Walgreens Sterile Multi-Purpose Solution for Soft Contact Lenses” with Kim wipe.

14. Using a 1 ml syringe place 3 drops of distilled onto the cup of the lens.

15. Place the PCB and the lens attached to it, in the vacuum chamber.

16. Close chamber door. Ensure the depressurizing valve is in the open position and the pressurizing valve is in the closed position. Turn on the vacuum until the vacuum pressure gauge reaches 28.5 inHg.

17. Once 28.5 inHg is achieved wait until all the bubbles are exploded.

18. When there is no bubble left, opening the pressurizing valve and allowing the air back into the vacuum chamber.

19. Wick the distilled water from the cup of the lens using Kim wipe.

20. Repeat the steps 14 to 19 two more times.

21. Store the CLEAr Lens in a container away from dust.
Approved Animal Protocols

10/18/2012

John R. Hetling
Bioengineering
M/C 063

Dear Dr. Hetling:

The protocol indicated below was reviewed in accordance with the Animal Care Policies and Procedures of the University of Illinois at Chicago and renewed on 10/18/2012.

Title of Application: ERG Recording After Laser Damage of Retina in Rat
ACC NO: 11-154
Original Protocol Approval: 10/24/2011 (3 year approval with annual continuation required).
Current Approval Period: 10/18/2012 to 10/18/2013

Funding: Portions of this protocol are supported by the funding sources indicated in the table below:

Number of funding sources: 1

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Sincerely,

Bradley Merrill, PhD
Chair, Animal Care Committee

BM/kg
cc: BRL, ACC File
March 3, 2014

John R. Hetling
Bioengineering
M/C 063

Dear Dr. Hetling:

The modifications requested in modification indicated below pertaining to your approved protocol indicated below have been reviewed and approved in accordance with the Animal Care Policies of the University of Illinois at Chicago on 3/3/14

Title of Application: ERG Recording After Local Damage of Retina in Rat

ACC Number: 11-154

Modification Number: 2

Nature of Modification:
1) Addition of 48 rats for new cryo injury model and for comparison with baseline and laser injury model.
2) Change in title to current title.
3) Addition of UIC COE/COM-Bridge funding

Protocol Approved: 10/24/2011

Current Approval Period: 10/18/2013 to 10/18/2014.

Current Funding: Portions of this protocol are supported by the funding sources indicated in the table below.

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| Funding Number
R21 EY018200 (A2 version) | Funded | 200802552 | UIC | John Hetling |
| College (COE/COM) | Bridge Funding to Respond to Manuscript and Proposal Critiques | N/A |
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This institution has Animal Welfare Assurance Number A3460.01 on file with the Office of Laboratory Animal Welfare, NIH. This letter may only be provided as proof of IACUC approval for those specific funding sources listed above in which all portions of the grant are matched to this ACC protocol.

Thank you for complying with the Animal Care Policies and Procedures of UIC.

Sincerely yours,

Bradley Merrill, PhD
Chair, Animal Care Committee

BM\mmbb
cc: BRL, ACC File
9/16/2015

John R. Hetling
Bioengineering
M/C 063

Dear Dr. Hetling:

The protocol indicated below was reviewed in accordance with the Animal Care Policies and Procedures of the University of Illinois at Chicago and renewed on 9/16/2015.

Title of Application: ERG Recording After Local Damage of Retina in Rat
ACC NO: 14-128
Original Protocol Approval: 10/16/2014 (3 year approval with annual continuation required).
Current Approval Period: 9/16/2015 to 9/16/2016

Funding: Portions of this protocol are supported by the funding sources indicated in the table below.
Number of funding sources: 1

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This institution has Animal Welfare Assurance Number A3460.01 on file with the Office of Laboratory Animal Welfare, NIH. This letter may only be provided as proof of IACUC approval for those specific funding sources listed above in which all portions of the grant are matched to this ACC protocol.

Thank you for complying with the Animal Care Policies and Procedures of the UIC.

Sincerely,

John P. O’Bryan, PhD
Chair, Animal Care Committee

JPO/kg
cc: BRL, ACC File

Office of Animal Care and Institutional Biosafety Committee (OACIB) (M/C 672)
Office of the Vice Chancellor for Research
206 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612
9/16/2016

John R. Hetling
Bioengineering
M/C 063

Dear Dr. Hetling:

The protocol indicated below was reviewed in accordance with the Animal Care Policies and Procedures of the University of Illinois at Chicago and renewed on 9/16/2016.

Title of Application: ERG Recording After Local Damage of Retina in Rat
ACC NO: 14-128
Original Protocol Approval: 10/16/2014 (3 year approval with annual continuation required).
Current Approval Period: 9/16/2016 to 9/16/2017

Funding: Portions of this protocol are supported by the funding sources indicated in the table below:

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Number of funding sources: 1

This institution has Animal Welfare Assurance Number A3460.01 on file with the Office of Laboratory Animal Welfare, NIH. This letter may only be provided as proof of IACUC approval for those specific funding sources listed above in which all portions of the grant are matched to this ACC protocol.

Thank you for complying with the Animal Care Policies and Procedures of the UIC.

Sincerely,

John P. O’Bryan, PhD
Chair, Animal Care Committee
JPO/kg
cc: BRL, ACC File
October 16, 2014

John R. Hetling
Bioengineering
M/C 063

Dear Dr. Hetling:

The protocol indicated below was reviewed at a convened ACC meeting in accordance with the Animal Care Policies of the University of Illinois at Chicago on 9/16/2014. The protocol was not initiated until final clarifications were reviewed and approved on 10/16/2014. The protocol is approved for a period of 3 years with annual continuation.

Title of Application: ERG Recording After Local Damage of Retina in Rat

ACC Number: 14-128

Initial Approval Period: 10/16/2014 to 9/16/2015

Current Funding: Portions of this protocol are supported by the funding sources indicated in the table below.

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Chair, Animal Care Committee

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Adam Mohamed <adam.mohamed@hindawi.com>  Wed, Aug 17, 2016 at 7:31 AM

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Adam Mohamed

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Sincerely,

Zahra Derafshi
851 S. Morgan St., #218
Chicago, IL, 60607

The above request is approved.

Approved by:

Date: 8/15/16

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8. VITA

Education

- **PhD Candidate**: Bioengineering, Neural Engineering, University of Illinois at Chicago (Spring 2012- Present) GPA: 4.

Research Interests

- Neural Engineering
- Vision Research
- Bioengineering
- Analog Integrated Circuit Design
- Mixed-Signal Circuit Design
- Programming with FPGA
- Micro Electro Mechanical Systems (MEMS)

Teaching Experiences

- Spring 2012- Present, TA of Bioengineering Courses, Department of Bioengineering, University of Illinois at Chicago.
- 2010, Electronics (level 3), Electrical Engineering Department, Tabriz University.
- 2009, Pulse Technique Lab., Electrical Engineering Department, Tabriz University.
- 2009, Electronics (Level 3) Lab., Electrical Engineering Department, Tabriz University.
- 2009, Digital Design Lab., Electrical Engineering Department, Tabriz University.
- 2009, Engineering Mathematics, Electrical Engineering Department, Tabriz University.
- 2009, Electronics (level 2), Electrical Engineering Department, Tabriz University.
- 2009, TA of VHDL programming language, Electrical Engineering Department, Tabriz University.
Research Background

- Design of printed circuit board as an interface between amplifier and contact lens electrode array, NEVL, Fall 2013.
- Design and fabrication of a custom laboratory instrumentation (control circuit for a pulsed laser photocoagulation system, LED-based stimulus source for a rat electroretinogram system), NEVL, Summer 2012.

Professional Background

- Graduate Assistant, Department of Bioengineering, University of Illinois at Chicago, Fall 2012.
- Lecturer at Islamic Azad University, Ilkhchi Branch, Iran, Spring 2011.
- Lecturer at Roshdiye University, Tabriz, Iran, Fall 2011.

Research Activities

- Peripheral Pattern Stimulus for Visual Function Testing. Funded by UIC-OTM (Fall 2013-Present, collaborate on this project)
- Recording ERG to evaluate the effect of intravitreally delivered neprilysin in mouse eye tissues (Summer 2014-Winter 2015).

Technical Skills

- Expert in programming microcontrollers, VHDL coding and FPGA, digital circuit design, analog circuit design, integrated circuit design, creating IC layout and creating PCBs.
- Proficient in Programming and Computing with AVR Studio, MATLAB, MaxPlus, Modelsim, Bascom, Eagle, Orcad, Hspice, Active HDL.
- Design of electrical circuits and systems and problem solving.
- Design of systems and circuits for medical use.
- Design and Development of experiment setup, data analysis, working with optics.
- IC designing
Affiliation

- Student Member, IEEE (Institute of Electrical & Electronic Engineers), 2009-2013.
- Student Member, IEEE Women In Engineering, 2009-2013.
- Student Member, The Association for Research in Vision and Ophthalmology (ARVO), 2014-Present.

Review & Judging:

- Reviewer of 16th IEEE International Conference on Electronics Circuits & Systems (ICECS), 2009, Tunisia
- Reviewer of 18th Iranian Conference on Electrical Engineering (ICEE), 2010, Iran.
- Reviewer of UIC Bioengineering Student Journal, 2014, Chicago, U.S.

Patents


Journal Publications

- Patangay S, Derafshi Z, Vajaranant T, Moss H, McAnany J, Hetling JR. “Pattern ERG responses in healthy and glaucomatous eyes elicited with a high-luminance pattern presented to peripheral retina.” In Final Preparation.

Conference Proceedings and Abstracts

- S Patangay, Z Derafshi, J Park, T Vajaranant, J McAnany, JR Hetling, “Pattern
electroretinogram (pERG) responses evoked by a novel high-luminance three-dimensional stimulus source that targets the peripheral retina”, ARVO Abstract, May 2015.


Presentations

- Seminar on "Floating-Point ADCs", University of Tabriz, Iran, Winter 2010.
- Seminar on "Gm-C Filters", University of Tabriz, Iran, Summer 2009.
- Seminar on "Organic Electronic", University of Tabriz, Iran, Fall 2008.
- Seminar on Multi-electrode Electroretinogram (meERG) for Bioengineering Graduate Society, Department of Bioengineering, University of Illinois at Chicago.

Awards
- 2nd place in research image contest, Department of Bioengineering, University of Illinois at Chicago, 2013.
- Finalist in image of research contest, University of Illinois at Chicago, 2014.

Invitations

- Invitation to be a speaker at 4th International Conference and Exhibition on Biosensors and Bioelectronics.
- Invitation to be a member of the editorial board at the Journal of Management and Production.