Comparative Ophthalmology - scientific discovery and innovation create synergies for veterinary and human medicine

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Comparative ophthalmology is the study of similarities and differences between human and various animal species’ vision.

- Evolutionary – mammals share many similarities
  - Developmental Biology
  - Genetics
  - Anatomical
  - Physiological
Human / Animal Relationship

- Our understanding of these comparisons has yielded many breakthroughs in eye and neurological research of diseases and other causes of vision loss.
  - Leber Congenital Amaurosis (LCA)
    - RPE65 mutation
    - Congenital Stationary Night Blindness (CSNB) in animals
  - Retinitis Pigmentosa
    - Spontaneous animal model (Progressive Retinal Atrophy)
  - Glaucoma
    - Spontaneous and induced animal models
Examples of Benefits

• Leber Congenital Amaurosis (LCA)
  – *Successful RPE65 Gene Replacement and Improved Visual Function in Humans*
    *Ophthalmic Genetics* 2008, Vol. 29, No. 3, Pages 89-91
  – *Gene therapy restores vision in a canine model of childhood blindness*
    *Nature Genetics* May 2001, Vol. 28, Pages 92-95
Examples of Benefits

- Retinitis Pigmentosa
- Understanding Progressive Retinal Atrophy helps our understanding of RP
- Treatment options include transplantation, gene, and stem cell therapy
- Potential cures for animals and humans
Examples of Benefits

Glaucoma
Glaucoma

Ongoing Results; Pilot Study (3 eyes)

Original Data Presented at ACVO 2008:
Roberts SW and Woods, CW. “The Use of a Novel Porous Implant for Refractory Canine Glaucoma”.
Abstract Presentation; Am. College of Vet. Ophthalmologists 2008; Boston, MA
Glaucoma

Implant and Surgical Approach

- Create a limbal based flap
- Introduce implant through a 3mm incision
- Place implant body adjacent to choroid
- Close sclera
Innovative Technologies

• Electrophysiology of Vision
  • Diagnostic Medical Device
  • Research began in 1930’s
  • Quantifiable analysis of retinal “function”
  • Diseases and drugs affect the function of the retina before “structural” changes occur.
Dr. Kristina Narfström, DVM, PhD, and Diplomate of the European College of Veterinary Ophthalmology (ECVO), is Professor Emeritus at the University of Missouri-Columbia. She was the Ruth M. Kraeuchi Missouri Endowed Professor of Veterinary Ophthalmology. She holds adjunct professorships at the Mason Eye Institute, Department of Ophthalmology, and at the Faculty of Biomedical Engineering at the University of Missouri-Columbia. Her research work concerns primarily hereditary retinal disease processes of animal models, including retinal functional testing, using electroretinography. She is also engaged in comparative research concerning treatment modalities for hereditary retinal blinding diseases such as the use of gene therapy, stem cell implantation and retinal prosthetics. Narfström is cofounder and scientific director of RetVetCorp.
HMsERG™

- Handheld Multi-species Electroretinogram
- Scotopic / Photopic
- Single / Dual Stimulator
- Flash ERG / VEP
- Battery operated
- Automated Protocols
- Truly portable
- Mini Ganzfeld dome
- ISCEV guidelines
- Analytical software
  - Amplitudes / Implicit Times
  - Oscillatory Potentials
Non-communicative Patient
Electroretinogram – Definition

- The electroretinogram (ERG) is a tracing of the summed electrical changes of a large number of retinal neurons and glia in response to a light stimulus.
Electrophysiology of Vision

- Flash Electroretinography (ERG)
- Focal ERG
- Vision Evoked Potential (VEP)
- Pattern ERG
- Multi-focal ERG
Cellular Structure and Electrophysiology of Vision

Selective Rod- and Cone-ERG Responses in Retinal Degenerations
<table>
<thead>
<tr>
<th>PROVISIONAL DIAGNOSIS</th>
<th>EOG</th>
<th>ERG</th>
<th>BRIGHT FLASH ERG</th>
<th>PATTERN ERG</th>
<th>FLASH VEP</th>
<th>PATTERN VEP</th>
<th>SPECIAL VEP</th>
</tr>
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<tbody>
<tr>
<td>Inherited retinal dystrophies</td>
<td>+</td>
<td>+</td>
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<td>Vascular diseases including diabetes</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Opaque media or trauma</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Retrobulbar neuritis*</td>
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<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>Unexplained visual loss</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>Infant with questionable vision</td>
<td>+</td>
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<tr>
<td>Albinism</td>
<td></td>
<td>+**</td>
<td></td>
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<td></td>
<td>+</td>
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<tr>
<td>Toxic and nutritional eye disease</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>Glaucoma</td>
<td>X</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Suspected intracranial lesion</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>

* not helpful during active phase but rather to monitor the recovery
** to exclude other conditions associated with nystagmus
Changing Paradigms

• In the Lab
  – Basic Research
    • Transgenic
    • Safety / Efficacy
    • Animal Models of Human Diseases
    • Detection / Diagnosis
  – Clinical Research Organizations

• In Surgery
  – Retina function check

• By the Bedside
  – Pediatric
  – Trauma (Sudden Vision Loss)

• In the Clinic
  – Retina (RP, Glaucoma, CRVO, Diabetic Retinopathy, Uveitis)
  – Cataract/Refractive (Screening, Endophthalmitis)
ERG in Research

- Pharmaceutical Development
  - Safety
  - Efficacy
- Transgenic animal model phenotype
- Does it effect the Retina?
- What in the retina is being effected?
- Quantification
- Use in complementary diagnostic tools
  FA/SLO/OCT
Rod Dark Adaptation
Abnormal Rod Only B wave
Scientific literature

- **ARVO 2006**
  - Jeong et al.

- **ISCEV 2006**
  - Narfstrom et al.

- **ACVO 2007**
  - Jeong et al.: Comparison of 2 ERG systems used in dogs: the HMsERG and the RETIcom

- **Upcoming Publications:**
  - Katz et al.: Retinal pathology in a canine model of late infantile neuronal ceroid lipofuscinosis, Accepted IOVS, 2008
  - Jeong et al.: Comparison of 2 ERG systems used in canine electrophysiology: the HMsERG and the RETIcom. Submitted
Commercial opportunity

• Clinically compared to larger, more expensive systems
• Early Detection / Progression
• Making ERGs easier to obtain
• Use in a variety of environments
• Data collection simpler
• EMRs and Information Sharing
Comparison of two electroretinography systems used in dogs: the HMsERG and the RETIcom

MB Jeong, 1 WG Son, 1 YW Park, 1 SA Park, 1 KM Seo, 1 CP Moore 2 and K Narfström 2

1Dept of Veterinary Surgery and Ophthalmology, Seoul National University, Korea, 2Dept of Veterinary Medicine and Surgery, University of Missouri-Columbia, USA

Purpose. To compare two different electroretinography (ERG) instruments used on the same animal in a clinical practice.

Methods. Retinal function in both eyes of 12 healthy Miniature Schnauzers was evaluated under general anesthesia using medetomidine (50 μg/kg, IM) and Ketamine (5 mg/kg, IM). Scotopic and photopic ERGs were recorded by a compact, portable mini-Ganzfeld ERG unit (HMsERG, RetVet Corp Inc., MO, USA) and a contact lens electrode with a built-in light-emitting diode (LED) stimulator, part of a commercial ERG equipment (RETIcom, Roland Consult, Brandenburg, Germany), respectively, following published Guidelines for Clinical Electroretinography in the Dog. 1 Light intensity used with the two different ERG equipments is showed in Table 1. The ERG waveforms, a- and/or b-wave amplitudes and implicit times of recordings by the two ERG units were compared. For statistical analysis, a paired student t-test, and median, and 5th and 95th percentiles were employed. In addition, the results were then fitted into a graphical representation of reference ranges for both ERG systems.

Table 1

<table>
<thead>
<tr>
<th>Light stimulation intensity (cd·m⁻²)</th>
<th>HMsERG (signal averager)</th>
<th>RETIcom (single flash)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1·ms light intensity</td>
<td>S1</td>
<td>0.01</td>
</tr>
<tr>
<td>S2</td>
<td></td>
<td>0.025</td>
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<tr>
<td>S3</td>
<td></td>
<td></td>
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<tr>
<td>S4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard light intensity (S-ST)</td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>Higher light intensity (S-H)</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Light Adaptation (cd·m⁻²)</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Photopic ERG</td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

Results. The waveforms of the ERGs obtained by the two ERG units were identical to those of previous studies. 2 Except for the amplitude of the b-wave for scotopic low light intensity stimulation, the overall changes of ERG parameters recorded by the HMsERG unit in response to changes in the protocol as to light stimulation were very similar to those recorded by the RETIcom equipment. This was found most clearly for the photopic single flash and 31 Hz flicker responses (Fig. 3 A, B, C, and D). There was a greater increment in b-wave amplitude and implicit times recorded by the HMsERG than those of RETIcom during dark adaptation (Fig. 3 E). Mean b/a ratio for the HMsERG were higher than those for the RETIcom, but the differences were not significant (Fig. 3 F). The results demonstrate that both ERG systems are comparable. As illustrated in Fig. 4, some major differences between the two units were shown by using 95% confidence intervals.

Fig. 2. Representative ERG waveforms recorded by the two ERG units. Scotopic ERGs (A and B; E and F) and photopic ERGs (C and D; G and H) are shown. Scotopic low intensity responses (A and E), which was subdivided into S1, S2, S3, S4, and S5 (from bottom to top in A; from top to bottom in E), scotopic standard intensity responses (blue line in B; upper line in F), and scotopic higher intensity responses (red line in B; upper line in F). Photopic single flash response (C and G) and 31 Hz flicker response (D and H). The light stimulus is shown by the vertical dashed line for the HMsERG, while the light stimulus is shown at the beginning of each recording for the RETIcom.

Fig. 3. The comparison of mean ±SEM amplitude (A, C, and E) and implicit times (B, D) of a- and b-waves recorded by the HMsERG (a solid line) and the RETIcom (a dotted line). The b/a ratio for the two ERG equipments are shown in F. The changes in a- and b-wave amplitudes and implicit time parameters obtained during a recording session using both ERG units are very similar in ERG responses except for amplitudes of the b-wave for scotopic low light intensity stimulation.

Conclusions. Both ERG units are appropriate for routine veterinary clinical use. It is recommended, however, to establish ERG system-specific reference ranges for each laboratory and clinic using a 95% confidence interval in order to obtain reliable results especially when evaluating hereditary retinal degenerative disease processes.

Fig. 4. Graphical illustration of the reference ranges depicting ERG differences between the HMsERG (a solid line) and the RETIcom (a dotted line) using the median set at 100% and 95% confidence intervals. Amplitude and implicit time values on the y-axis for the a- and b-waves, respectively, are shown as percentages of the values obtained by the two ERG units.

Reference.
The efficacy for functional evaluation of feline hereditary rod cone degeneration using a portable mini-Ganzfeld electroretinography unit

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1Dept of Veterinary Surgery and Ophthalmology, Seoul National University, Korea
2Retinal Diagnostics Research Group, Dept of Ophthalmology II, University of Tuebingen, Germany
3Dept of Veterinary Medicine and Surgery, University of Missouri-Columbia, USA
4School of Optometry, University of New South Wales and VisionTest Australia, 187 Macquarie St., Sydney, Australia

Background: Objective evaluation of retinal function is often needed in the clinical and research environment. We studied normal cats and cats affected with different stages of inherited rod cone degeneration to evaluate the efficacy of obtaining a diagnosis with a new portable mini-Ganzfeld ERG unit, using it in parallel with a conventional table-top Ganzfeld ERG. Previous studies, with large ERG equipment and extended protocols, have shown that a significant reduction in scotopic high intensity a-wave amplitude together with a corresponding increase in b-wave ratio in diagnostic for early stage feline rod cone degeneration.

Methods: Eleven affected cats in different stages of disease (S1 - S4; early (A), moderate (B) and advanced (C)), and four normal controls were anesthetized using a combination of medetomidine (0.09 mg/kg, IM) and ketamine (5 mg/kg, IM) and studied using the protocol recommended by ISCEV for diagnostic ERGs in humans. Cats were dark-adapted overnight and prepared under red lights. Scotopic ERGs were first obtained using a conventional tabletop unit (ERG System TOR, Global Eye Program, Rejmyre, Sweden). Before light adaptation, the units were switched and the small, portable ERG (Handheld multispecies ERG, HMsERG, RetVet Corp. Inc., Columbia, MO) was used, with the ISCEV protocol, followed by photopic recordings using the larger unit. A- and b-wave amplitude and implicit times were evaluated along with waveform shape. b/a-wave ratio and orbit potentials, respectively. The latter were obtained from responses to high intensity stimuli under scotopic conditions through digital filtering at 100 to 300 Hz. The table shows light stimulation parameters and mean ERG a- and b-wave amplitudes used for the HMsERG and for the ERG System TOR, respectively, in early stage of feline rod cone degeneration.

Results: Figure (left) shows results of ERG tracings using the HMsERG and the TOR units, respectively, in a case of early stage hereditary retinal degeneration. In affected animals, the mean amplitude of the scotopic a-wave using 3 (HMsERG) and 1 cd.s/m² (TOR) respectively, of light stimulation was significantly lower already in early disease: 197 ± 82 µV using the HMsERG (p<0.004) and 116 ± 44 µV for the TOR unit, when compared to results of controls: 559 ± 115 µV and 307 ± 65 µV, respectively. Similarly, significant differences between affected early stage cats and controls were found for the b-wave amplitudes, although not as marked (p=0.014 for the HMsERG) when using this level of light stimulation. For the b/a-wave ratio of affected cats, these were also significantly increased (p=0.037 for HMsERG) in early disease compared to those of normal cats using both units. A- and b-wave implicit times were not found to be diagnostic when comparing early stage affected and normal cats using either equipment, and 3 and 1 cd.s/m², respectively, of light intensity stimulation. OPs were reduced in affected cats in comparison to those of normal cats (data not shown) using both instruments. The ERG waveform shapes obtained using the portable unit were comparable to those of the conventional tabletop unit (Figure, left).

Conclusion: The portable mini-Ganzfeld HMsERG provided results that were remarkably similar to the conventional tabletop full-field ERG System TOR in normal and affected animals. Although subject to further evaluation, this study shows the efficacy of the portable unit in the diagnosis of generalized photoreceptor disorders. Additional work is underway to establish reference ranges using the portable ERG for research and in the clinical practice.

Methods:

<table>
<thead>
<tr>
<th>Light Intensity / Stimulation (white LEDs)</th>
<th>Mean amplitude</th>
<th>HMsERG</th>
<th>ERG System TOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>scalp a-wave: 76.5  (32.1)</td>
<td>30 Hz at 3.00 cd.s/m²</td>
<td>8.9  (5.1)</td>
<td>69.3  (36.8)</td>
</tr>
<tr>
<td>a-wave: 129.6  (56.3) b-wave: 576.5  (213.4) Photopic 3.00 cd.s/m²</td>
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<td></td>
</tr>
<tr>
<td>b-wave: 774.8  (341.8) Scotopic 0.01 cd.s/m²</td>
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</tbody>
</table>

TOR System

<table>
<thead>
<tr>
<th>Light Intensity / Stimulation (white LEDs)</th>
<th>Mean amplitude</th>
<th>HMsERG</th>
<th>ERG System TOR</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

Commercial relationships: Narfstrom K; Provisional patent application for the HMsERG through RetVet Corporation, Inc.

ARVO 2006

References:
Structure and Function

• Imaging, Histology provide data of retina cellular organization, interactions, and structural integrity

• Electrophysiology assesses the function of the retina/visual pathway
Analysis of topographic features in macular pucker. A, Grayscale SLO image of the fundus in a subject’s left eye. B, Overlay of the coronal OCT image (color photo C) superimposed on the SLO fundus image (grayscale photo A) with point-to-point registration. C, Coronal plane OCT image, in color. The overlay image (B) was used to characterize the macular hole and/or macular pucker (as in this case) for topographic analysis.

MACULAR HOLES AND MACULAR PUCKER: THE ROLE OF VITREOSCHISIS AS IMAGED BY OPTICAL COHERENCE TOMOGRAPHY/SCANNING LASER OPHTHALMOSCOPY
BY Jerry Sebag MD FRCOphth, Priya Gupta, Richard R. Rosen MD, Patricia Garcia MD, AND Alfredo A. Sadun MD PhD
Combining Structure and Function

Ocular findings in a Korean XLRS patient (case 16). **A:** Fundus photograph of the both eyes showed typical stellate pattern of schisis cavities in the macula. The inset presents an image of the macula magnified twofold. **B:** Fluorescein angiogram showed no definite leakage from the cystic cavities. **C:** Optical coherence tomography showed the schisis in the nerve fiber layer. **D:** Electroretinogram showed markedly decreased amplitude of b-wave and relative preservation of a-wave, which are key features of XLRS.

**Multi-modal Imaging**

- **Integration of Structure and Function in Real-time**
  
  “Synergising optical coherence tomography and ERG enhances retinal phenotyping in rodents”
  M Seeliger

  “Determination of optimal recording parameters for multimodal imaging using combined OCT/SLO and micro-mfERG”
  S Walker

  “Diabetic macular edema: correlations between retinal thickness, micro-perimetry and mfERG”
  S Coupland
Thank You

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