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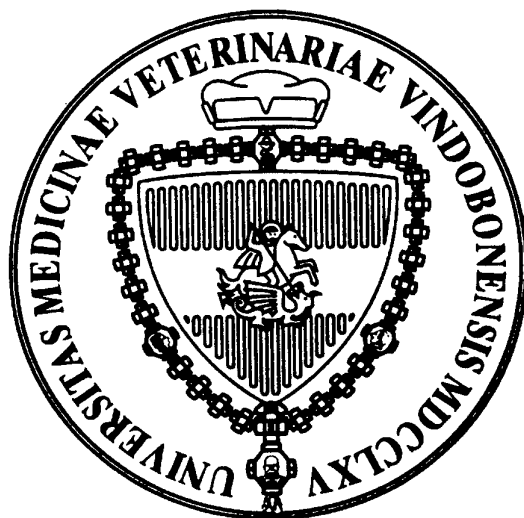
PROCEEDINGS

of the

**1st EUROPEAN CONFERENCE ON
VETERINARY VISUAL ELECTROPHYSIOLOGY**

(Organization: Ron OFRI, DVM, PhD, Israel)

May 30, 2000



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and the

COMBINED SCIENTIFIC MEETING

of the

**EUROPEAN COLLEGE OF VETERINARY
OPHTHALMOLOGISTS (ECVO)**

and the

**EUROPEAN SOCIETY OF VETERINARY
OPHTHALMOLOGY (ESVO)**

May 31, 2000

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May 31, 2000

at the

**University of Veterinary Medicine Vienna
AUSTRIA**

**Veterinärplatz 1, A-1210 Wien
Hörsaalzentrum**

Precongress of the IVth International Congress on Small Animal and
Horse Diseases

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(Organization: Ron OFRI, DVM, PhD, Israel)

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CREDITANSTALT

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um wachsen zu können.“



The 1st EUROPEAN CONFERENCE ON VETERINARY VISUAL ELECTROPHYSIOLOGY

(Organization: Ron OFRI, DVM, PhD, Israel)

**University of Veterinary Medicine Vienna
AUSTRIA**

**Veterinärplatz 1, A-1210 Wien
Hörsaalzentrum**

May 30, 2000

Precongress of the IVth International Congress on Small Animal and Horse
Diseases

THIS MEETING IS SUPPORTED BY:

- The European Office of Aerospace Research & Development
(ASVOR)
- The European Society of Veterinary Ophthalmology (ESVO)

PROGRAM AGENDA

THE FIRST EUROPEAN CONFERENCE ON VETERINARY VISUAL ELECTROPHYSIOLOGY

(May 30, 2000 - Veterinary University of Vienna, Austria)

- 13:00 **Welcome and announcements**
- Scientific Session - Ron Ofri R, chairman**
- 13:10 **Guest Lecture**
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- 14: 45 **Break**
- 15:15 NARFSTRÖM K, Wallin-Hakansen BK, Hertel E, Ekesten B
Familial retinopathy in Swedish Basenji dogs: Clinical & electrophysiological
findings
- 15:30 PERCICOT CL, Raz D, Lambrou GN, Ofri R
The effect of luminance changes on the multifocal ERG of non-human
primates
- 15:45 OFRI R, Raz D, Lambrou GN, Percicot CL
Changes in the multifocal ERG of glaucomatous *Cynomolgus* monkeys

- 16:00 EKESTEN B, Gouras P
UV-and M-cone signals at single ganglion cell level in the murine superior colliculus
- 16:15 **Guest Lecture**
Dr. Vittorio PORCATTI - Institute of Neurophysiology CNR, Pisa, Italy
Visual Electrophysiology in Mice
- 17:00 NARFSTRÖM K, Ekesten B, Rosolen SG, Spiess BM, Percicot CL, Ofri R
Recommondations for a harmonized European protocol for clinical ERG recording in the dog
- Presentation of committee report
 - Discussion

Current Developments in Retinal Electrodiagnostics

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Changes to retinal function are common features of genetically induced disorders of the CNS. Electroretinographical (ERG) methods are well suited to detect such changes non-invasively in vivo and can often reveal which parts and/or layers of the retina are involved.

In analogy to the use in human patients, ERG methods can also be applied in animal models to detect potential dysfunction of the retina. From a research point of view, this is particularly of interest in knock-out mice that carry mutations homologous to human diseases. Typical examples are the rhodopsin (no rod function, model for autosomal-recessive retinitis pigmentosa) and the CNG3 knock-out mouse (no cone function, model for achromatopsia). Besides the opportunity to study the pathophysiology in detail and to test the efficacy of potential new treatments, these models also allow to determine the contribution of the affected cell groups to the normal ERG separately.

In cooperation with the Max-Planck-Institute for developmental biology, ERG recordings have furthermore been demonstrated to be very informative in the functional screening of zebrafish mutants. These mutants are tested at an age of usually 5 dpf (days post fertilization), when practically all components of the retina are functional. A customized holding setup permits to perform the recordings with the same Ganzfeld equipment as in humans and mice. In addition to the detection of mutants, the zebrafish work is useful for the analysis of developmental changes to the ERG, and for the test of short-term drug effects.

An important question in many disorders is by what mechanism genetical defects cause malfunction of certain cell groups and/or functional systems of the retina.

The analysis of such mechanisms can be facilitated by the study of patients with pathophysiologically informative degenerative diseases of the retinitis pigmentosa group, but also more general disorders like the practically complete lack of serum retinol in a family with RBP deficiency.

Multifocal electroretinography (MF-ERG), a technique developed by Sutter & Tran, has become a widespread diagnostic tool since its emergence a decade ago.

In contrast to the Ganzfeld method, which yields one single ERG response produced by a light stimulus homogeneously distributed across the whole retina, the multifocal method utilizes a set of independent hexagonal stimulus patterns that allow for the calculation of the functional retinal topography, both for amplitude and implicit time, within a 30° visual field.

A newly developed multifocal ERG setup that uses a Scanning-Laser-Ophthalmoscope (SLO) does also allow for simultaneous fundus visualization and stimulation, which is important for non-fixating subjects such as anesthetized animals.

The possibility to detect local retinal damage is a major diagnostic advancement. This is particularly true in the area of inherited retinal degenerations, and many of them that involve predominantly the macula like Stargardt's disease can not be detected in Ganzfeld ERG. In animals, the applicability of this technique has been shown in cats with ARCD (Abyssinian rod-cone degeneration).

FUNCTIONAL EVALUATION OF 3 APPALOOSA HORSES WITH CONGENITAL STATIONARY NIGHT BLINDNESS (CSNB).

L Mertel, A de Gresti, E Boniperti, L Seguso

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Università di Milano, Italia.

Purpose: CSNB, a typical Appaloosa's retinopathy, has been detected in Italy two years ago. During a preliminary recent (January-March 2000) survey on the incidence of CSNB in the North of the country another affected horse was found. This work is intended to describe the phenotype of these first Italian cases and to draw the attention on an apparently "forgotten" disabling disease.

Methods: The ophthalmic examination and a rod and cone-mediated visual functional performance test, with an obstacle course, concerned 22 Appaloosas. Hay packs were positioned in a passage to force the horse to deviate. Horses suspected to be night blind, a 10 ms old filly and 2 adults affected by ERU, of 8 (n°1) and 14 (n°2) years of age respectively, underwent photopic and scotopic (after 10' of dark adaptation) clinical electroretinography in heavy sedation. As normal controls another age matched filly and a 12 years old horse underwent ERG.

Results: Mydriasis with slow and incomplete PLRs were observed in the nervous filly which manifested behavioural changes even in day light. Ophthalmoscopy was normal in all the horses. On contrary to the controls, the filly and horses n° 1 and 2 could not manage the scotopic obstacle course. In these horses the absence of scotopic b-wave with "negative" ERG morphology indicated the lack of night vision, confirming the diagnosis of CSNB. Hypovoltage of photopic ERG in the filly suggested a partial day blindness as well.

Conclusions: The diagnosis of CSNB is based on absence of night vision, normal fundus and a scotopic ERG dominated by a negative potential. The ERG may detect a concomitant cone disfunction confirming the suspect of partial day vision, a more serious phenotype of CSNB. The presence of CSNB in Italy, emerged after the examination of few horses, suggests the opportunity of a wide survey of the disease in the Italian Appaloosa population, raised to a significant number in the last decade, to protect the breed, in the light of the presumed autosomal recessive inheritance.

RETINAL DEGENERATION IN THE BERNESE MOUNTAIN DOG IN FRANCE : A PRELIMINARY REPORT

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This study describes clinical findings in five males and one female Bernese Mountain Dogs, 8 weeks to 3,5 year-old (Fig.1), after ophtalmoscopic examination, fluorescein angiography and electroretinography. Two adults were affected of mild impaired night visual capacity, the third adult was normal. The affected pups were night blind (dogs 2 and 5) or totally blind (dog 3). The fundus was normal in puppies (dogs 2,3,5) and in the examined adult female (dog 6). The fundus of the dogs 1 and 4 was characterised by an horizontal tapetal hyperreflective band near the tapetal-non tapetal junction; the fluorescein angiography performed in the dog 1 was showing in this zone a filling defect at the early phase, then peripheral neovascularization and diffusion beginning at the venous phase. In the dogs 1,2,3,5, ERG examination has been undertaken: The results are exhibiting an important hypovoltage with blue and white stimulations, even a flat ERG with blue stimulation in the dogs 1 and 5. According to the literature, functional signs are early visible so as in 3 of these dogs (pups only : dogs 2,3,5, with total blindness in the dog 3). The hyperreflective horizontal zone was noticed in two parents (3,5 year-old males : dogs 1 and 4), the visual abnormalities of which were slight and stationary. The functional and ERG signs can be compared to those observed in congenital stationary night blindness (retinal dystrophy) in the Briard Sheepdog. The ophtalmoscopic and angiographic appearance of the hyperreflective horizontal band in the tapetal lesions described in early evolving stages of progressive rod cone degeneration in the Labrador Retriever.

VARIABILITY OF ERG WAVE COMPONENTS ON NORMAL BEAGLE DOG.

SG Rosolen¹, P. Lazard², P-F. Isard³, F. Rigaudière⁴.

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Purpose : To evaluate the variability of electroretinogram (ERG) waves components using a technique to record bilateral photopic and scotopic ERGs in normal Beagle dog.

Methods: ERGs were performed in 60 (120 eyes) young adults (30 males and 30 females) ophthalmologically healthy anesthetized Beagle dogs ranging from 18 months to 20 months using the VisioSystem (DIOPTRIX, Toulouse, France). All animals were previously adapted to a photopic background (30 cd/m²) for at least three hours. Normal cones-system functioning was checked by recording ERGs elicited by a 10 μ s standard achromatic flash (2.5 cds/m²) repeated three times under a photopic background at 1 Hz frequency in full-field like conditions. Normal rods-system functioning was checked by recording ERGs elicited by a 10 μ s short-wave-length flash (0.025 cds/m²) repeated three times under a scotopic background at 1 Hz frequency in full-field like conditions at 20 minutes of dark-adaptation. Bilateral ERGs were recorded using limbic active electrodes which enable globe fixity and eyelids opening. Axial length were previously measured using a B-scan ultrasonography. A-wave and b-wave amplitudes and implicit times were measured. A-wave amplitudes and implicit times were measured from baseline to peak. B-wave amplitude and implicit time were measured from a-wave trough to b-wave peak. Retinal surface was calculated according to axial length measurements.

Results: a-wave and b-wave ERG components values are presented in the table

	Photopic ERG				Scotopic ERG	
	a-wave		b-wave		b-wave	
	Amplitude (μ V)	Impl. Time (ms)	Amplitude (μ V)	Impl. Time (ms)	Amplitude (μ V)	Impl. Time (ms)
Mean	-28.3	10.9	145.8	30.3	170.5	60.0
S.D.	7.9	0.8	22.2	1.3	38.5	2.7
RE + LE	120	120	120	120	120	120
% variation	27.9	7.3	15.2	4.3	22.6	4.5

Axial length mean and standard deviation measurements for combined eye were 22.2 mm +/- 0.87 mm respectively. The calculated retinal surface ($CRS=2/3 \times 4 \pi R^2$) variation was 7.9%.

Conclusions: The b-wave amplitude variability is higher than that of retinal surface variability. Therefore the b-wave amplitude variability could not only be due to the retinal surface variability. ERG-wave amplitudes are linked to the number of stimulated photoreceptors. 1) interindividual variability of photoreceptor density (number per surface unit) could explain this amplitude variability. 2) the variability of tapetum lucidum surface could also be involved in ERG-wave amplitude variability. According to these results and globe size variability in dogs, each lab should establish its own standards for each breed.

Acknowledgement. Authors acknowledge the Société Française d'Etudes et de Recherches en Ophthalmologie Vétérinaire (S.F.E.R.O.V.) and DIOPTRIX (Toulouse, France)



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FAMILIAL RETINOPATHY IN SWEDISH BASENJI DOGS. CLINICAL AND ELECTROPHYSIOLOGICAL FINDINGS.

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Purpose: Since the last 10 years sporadic cases of a not previously reported retinopathy have been diagnosed in Swedish Basenji dogs. A clinical study was designed to clinically evaluate funduscopy and functional changes in affected animals observed during the last year.

Methods: Nine affected dogs were examined using routine ECVO procedures, i.e. indirect ophthalmoscopy and slitlamp biomicroscopy after pupillary dilation performed after visual behavioural testing and neuro-ophthalmologic examination. In three affected dogs electroretinography (ERG) was performed using previously described methods (1).

Results: A bilateral retinopathy was found in dogs of both sexes, at the age of 2 to 10 years. Two of the cases were siblings and most of the other cases were interrelated. None of the dogs were blind. Only one case (7 yrs. old) demonstrated visual problems in darkness. Funduscopy changes consisted of focal or splashed-out grayish color changes in the midperipheral and peripheral tapetal fundus in the vicinity of peripheral arterioles and venules. Partial or complete peripapillary arcus formation was found in all affected cases. ERG in two cases showed scotopic and photopic a- and b-wave responses of normal amplitudes. The implicit times of both the rod and cone systems were increased, however. In the visually impaired dog the rod system was severely reduced and there was no measurable dark adaptation of rods. The amplitudes of the cone system were within limits for normal dogs although the timing was again abnormal for all responses.

Conclusions: It appears that the Basenji breed is affected by a specific type of hereditary retinopathy. The clinical and electrophysiological findings performed in our laboratory up to now indicate that the retinal disease is not PRA of the classical canine type. In order to describe the pathophysiology of the disease further electrophysiological and specific morphological studies are needed.

Reference:

1. Narfström, K., Andersson, B.-E., Andreasson, S. and Gouras, P.: Clinical electroretinography in the dog using Ganzfeld stimulation: A practical method of examining rod and cone function. *Documenta Ophthalmologica* 90:279-290, 1995.

THE EFFECT OF LUMINANCE CHANGES ON THE MULTIFOCAAL ERG OF NON-HUMAN PRIMATES

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Purpose To study the effect of changes in stimulus luminance on the multifocal electroretinogram (MF-ERG) in Cynomolgus monkeys (*Macaca fascicularis*).

Methods Unilateral MF-ERG were conducted in 9 normal macaque eyes. Monkeys were paralyzed (to maintain fixation) and ventilated; nitrous oxide inhalation and IV propofol were used for analgesia. Eyes were dilated and refracted. Stimulus consisted of 103 non-scaled hexagons, each alternating randomly between black and white at 75 Hz, projected on the central 46° of the retina. Two recordings, using mean luminance settings of 14 cd/m² (93% contrast) and 100 cd/m² (97% contrast), were conducted in each eye. Signals were filtered at 1-300 Hz and amplified X10⁵.

Results The typical Cynomolgus MF-ERG response to a high luminance stimulus consists of an initial negative deflection ("a-wave") followed by two positive peaks. These peaks, with latencies of 21-26 msec (P₁) and 30-38 msec (P₂), are in contrast to the single positive peak (latency 22-32 msec) that characterizes the human MF-ERG response. Progressive reduction in mean stimulus luminance resulted in progressive decrease of P₁ amplitude. Using a high-luminance stimulus, P₁>P₂ (P₁/P₂ amplitude ratio = 2.27 ± 1.54), while P₁<P₂ using a low-luminance stimulus (P₁/P₂ = 0.77 ± 0.3). These differences were statistically significant (p<0.05).

Conclusions The origin of the 2 positive peaks in the macaque MF-ERG is unclear. However, the different response of P₁ and P₂ to luminance changes implies that the "double peak" may be the consequence of superimposition of a luminance-sensitive response component on the typical single-peak. Alternatively, the two peaks may originate with different retinal generators.

Acknowledgements Funded by a research grant from CIBA Vision (a Novartis company), Basel, Switzerland, to the Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Israel.

CHANGES IN THE MULTIFOCAL ELECTRORETINOGRAM OF GLAUCOMATOUS *CYNOMOLGUS* MONKEYS

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Purpose To detect changes in the multifocal electroretinogram (MF-ERG) of glaucomatous *Cynomolgus* monkeys (*Macaca fascicularis*)

Methods MF-ERG recordings were conducted in 9 macaques with unilateral experimentally-induced glaucoma. Monkeys were paralyzed (to maintain fixation) and ventilated; nitrous oxide inhalation and IV propofol were used for analgesia. Eyes were dilated and refracted. Stimulus consisted of 103 non-scaled hexagons, each alternating randomly between black and white (contrast 82%) at 75 Hz, projected on the central 46° of the retina. Signals were filtered at 1-300 Hz. First order analysis was used to determine latency and amplitude of the first negative peak (N_1 or "a-wave") in the central retina and in 4 peripheral regions.

Results Glaucomatous eyes were characterized by increased N_1 latency. In the central retina, mean ($\pm$ SD) N_1 latency was 12.60 ± 0.99 msec in normal eyes, and 13.98 ± 1.16 msec in glaucomatous eyes; this difference was significant ($p=0.01$). In the peripheral retina, the differences between normal and glaucomatous eyes were greater, with significant latency delays >2 msec in all quadrants of glaucomatous retinas. No significant differences in N_1 amplitude were noted between the groups.

Conclusions Our results suggest that the MF-ERG may be used in detection of glaucoma. Delays in N_1 could be the result of preferential damage to faster-conducting magnocellular Y-elements in glaucomatous eyes. Alternatively, it is possible that in normal eyes another response component, with faster latency, is superimposed on N_1 ; this component may be missing in glaucomatous eyes, resulting in the delayed appearance of N_1 .

Acknowledgements Funded by a research grant from CIBA Vision (a Novartis company), Basel, Switzerland, to the Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Israel.

UV- AND M-CONE SIGNALS AT SINGLE GANGLION CELL LEVEL IN THE MURINE SUPERIOR COLLICULUS.

B. Ekesten¹ and P. Gouras².

Unit for Ophthalmology, Faculty of Veterinary Medicine, SUAS, Uppsala, Sweden¹,
Edward S. Harkness Eye Institute, Columbia University, New York NY².

Purpose: We have recorded single ganglion cell responses at the level of the superior colliculus in order to study organization of cone signals in normal mice.

Methods: Experiments were performed on anesthetized, normal mice of both sexes. Recording from ganglion cells in the superior colliculus was performed with stereotactically positioned microelectrodes. Both full field chromatic flashes and punctate, moving, oriented stimuli were used. Spectral sensitivity functions for each cell was determined by varying the energy of the flash at each wavelength with neutral density filters in order to elicit a constant near threshold response.

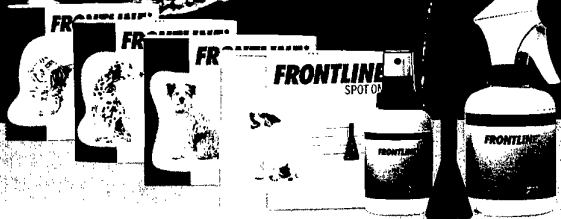
Results: In the superior colliculus, 65 ganglion cells were studied. Input from UV-cones and rods were found all over the visual field, whereas input from M-cones was predominantly seen along the horizontal meridian and in the lower half of the visual field. Small receptor fields from superficial collicular units were mainly seen along the horizontal meridian of the visual field. Most ganglion cells responded to both stimulus onset and the cessation of the stimulus light, that is they were on-off cells. Antagonistic inhibition between cone classes could not be proven in the superior colliculus.

Conclusions: M- and UV-cone signals are anisotopically represented at the level of the superior colliculus. However, retinotopic organization appears to be maintained. The presence of a visual stimulus, rather than its color (because the lack of antagonistic inhibition between cone classes) or a specific change in intensity of the stimulus (because most cells responded to both stimulus on and off), appears to be most important for triggering a collicular responses.

KEINE FLÖHE

KEINE ZECKEN

KEINE KOMPROMISSE



FRONTLINE SPOT ON 10% - Lösung zur kutanen Anwendung für kleine, mittelgroße und große Hunde. Zulassungsinhaber: Merial, Lyon, Frankreich. Hersteller: Merial, Lyon, Frankreich. Zusammensetzung: 1 Pipette zu 0,67 ml enthält: Fipronil 0,067 g (kleine Hunde), 1 Pipette zu 1,34 ml enthält: Fipronil 0,134 g (mittelgroße Hunde), 1 Pipette zu 2,68 ml enthält: Fipronil 0,268 g (große Hunde). Hilfsstoffe: Ethanol, Polysorbat 80, Polyvidon, Butylhydroxyanisol und -toluol, Diethylenglycolmonoethylether. Anwendungsgebiete: Zur Prophylaxe und Therapie des Flohbefalls (Ctenocephalides spp.) und Zeckenbefalls (Ixodes spp., Rhipicephalus spp., Dermacentor spp.) bei Hunden. Gegenanzeigen: Überempfindlichkeit gegen Bestandteile des Präparates. Weitere Angaben zu Nebenwirkungen, Wechselwirkungen und zu den besonderen Warnhinweisen zur sicheren Anwendung sind der „Austria Codex-Fachinformation“ zu entnehmen. Abgabe: Rezept- und apothekenpflichtig. Packungsgrößen: 3 Pipetten.

FRONTLINE SPOT ON 10% - Lösung zur kutanen Anwendung für Katzen. Zulassungsinhaber: Merial, Lyon, Frankreich. Hersteller: Merial, Lyon, Frankreich. Zusammensetzung: 1 Pipette zu 0,5 ml enthält: Fipronil 0,05 g. Hilfsstoffe: Ethanol, Polysorbat 80, Polyvidon, Butylhydroxyanisol und -toluol, Diethylenglycolmonoethylether. Anwendungsgebiete: Zur Prophylaxe und Therapie des Flohbefalls (Ctenocephalides spp.) bei Katzen. Gegenanzeigen: Überempfindlichkeit gegen Bestandteile des Präparates. Weitere Angaben zu Nebenwirkungen, Wechselwirkungen und zu den besonderen Warnhinweisen zur sicheren Anwendung sind der „Austria Codex-Fachinformation“ zu entnehmen. Abgabe: Rezept- und apothekenpflichtig. Packungsgrößen: 3 Pipetten.

FRONTLINE 0,5 ml und 1,5 ml - Pumpspray für Hunde und Katzen
Zulassungsinhaber: Merial, Lyon, Frankreich. Hersteller: Merial, Lyon, Frankreich. Zusammensetzung: 100 ml Lösung enthalten: Fipronil 0,25 g, Copolyvidon 2,0 g, Isopropanol 80,0 ml, Aqua purificata ad 100,0 ml. Anwendungsgebiete: Zur Parasitenprophylaxe und -therapie bei Hunden und Katzen, hochwirksam gegen Flöhe (Ctenocephalides spp.) und Zecken (Rhipicephalus spp., Ixodes spp.). Gegenanzeigen: Überempfindlichkeit gegen Bestandteile des Präparates. Weitere Angaben zu Nebenwirkungen, Wechselwirkungen und zu den besonderen Warnhinweisen zur sicheren Anwendung sind der „Austria Codex-Fachinformation“ zu entnehmen. Abgabe: Rezept- und apothekenpflichtig. Packungsgrößen: 100 und 250 ml.

Vertrieb:



Visual electrophysiology in mice
Vittorio Porciatti
Institute of Neurophysiology, CNR, Pisa, Italy

Introduction.

Mice with spontaneous mutations have been the traditional target for studies of retinal degeneration. Visual electrophysiology in these mutants has mainly dealt with the flash-electroretinogram to evaluate photoreceptor degeneration ¹. The introduction of genetic engineering techniques allowed generation of a wide variety of neurological mutant mice with specific molecular, anatomical and physiological alterations of the postreceptoral visual pathway. Unfortunately, basic knowledge of mouse visual physiology is scanty. This sets a severe limit for characterizing the visual phenotype of mutant mice and for the evaluation of the effects induced by experimental manipulation.

We have used the approach of pattern visual evoked potentials (P-VEPs) to address this issue ². P-VEPs have been used to characterize several aspects of visual physiology which have a counterpart in visual behavior (visual acuity, contrast sensitivity, motion sensitivity, response latency) . As compared to visual behavior, P-VEPs may have the advantage that different aspects of vision can be evaluated in the same animals, including those with poor behavior due to motor- or cognitive deficits. P-VEPs have been also used to obtain information on basic cortical layout (topography, laminar analysis) and ocularity.

Optical characteristics of the mouse eye.

The mouse eye is very small (about 3.38 mm anterior cornea- anterior choroid ³). This causes a retinoscopic artifact of about +10D. Yet, the mouse eye is emmetropic ⁴. In addition, the small pupil results in a very large depth of focus. This assures presentation of patterned visual stimuli with adequate retinal image quality independently of refraction. However, lens opacity may easily develop as a result of mechanical or chemical manipulation of the eye ⁵.

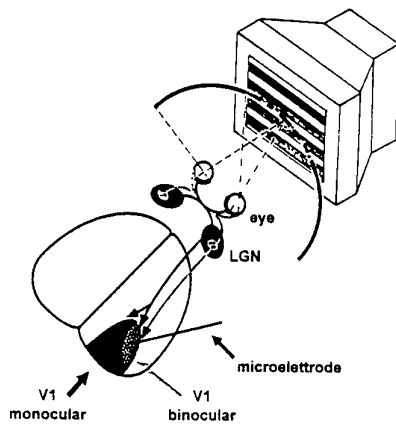


Fig. 1. Layout of the retino-cortical visual pathway (modified from ref. 7).

Layout of the retino-cortical visual pathway.

As shown in Figure 1, the central 30-40 deg of the upper portion of the visual hemifield is seen by the retinas of both eyes of the mouse. The temporal crescent (seen by one eye only) is about three times larger than the binocular field due to the lateral position of the eyes. Optic nerve axons relay to eye-specific portions of the Lateral Geniculate Nucleus and then project to the primary visual cortex (V1). In V1 the vertical meridian is represented laterally (about 3.2-3.4 mm from the lambdoid suture), while the temporal field of vision is represented medially. In V1 the monocular visual field has a larger representation than the binocular visual field. This is in agreement with the larger size of the temporal crescent as compared to the binocular hemifield. In the binocular portion of V1, the great majority of cortical neurons can be driven by stimulation of either eye. However, due to the stronger contribution of the contralateral, as compared to ipsilateral, input the ocular dominance distribution of visual cortical neurons in the binocular visual cortex is skewed towards the contralateral eye ^{6,7}.

Anesthesia and animal restraining.

Anesthesia requires a particular attention. Due to the small size of trachea and body, artificial ventilation is difficult and critical in the mouse. In our experience, intraperitoneal injection of 20% urethane (0.08 ml/10g) assured excellent anesthesia

while allowing several hours of stable VEP recording. Body temperature is monitored with a rectal probe and kept at 37.0 °C by means of a heating pad. Mice are mounted in a stereotaxic apparatus with a gentle pressure of ear bars and nose restrainer, allowing unobstructed viewing of the visual stimulus. Eyes are not restrained in a fixed position, nor are they kept artificially opened, since eyelids remain open and eyes do not diverge significantly during anesthesia. The pupil is not dilated, and eyes are not refracted. The presence of lens opacity is frequently checked and the cornea is kept moist with systematic application of physiological saline.

Electrophysiological recording.

A relatively large portion (3 x 3 mm) of the skull overlying the binocular visual cortex is carefully drilled and removed leaving the dura intact. A resin-coated microelectrode (WPI, Sarasota) with tip impedance of 0.5 M Ω is then inserted into the cortex perpendicularly to the stereotaxic plane. Common reference and ground electrode is a stainless steel needle inserted in the skin of the scalp. Electrical signals are amplified (50,000 fold), band-pass filtered (1-100 Hz), digitized (12 bit resolution) and averaged (at least 128 events).

Visual stimuli.

Typical visual stimuli are sinusoidal gratings of different spatial frequency and contrast alternating at 1 Hz, presented at 14 cm distance and centered on the vertical midline, covering 81 x 86 deg of the central visual field. Stimuli can be windowed to a vertical or horizontal stripe of 10 deg, which is presented at different eccentricities to determine the visual field azimuth and elevation yielding maximal VEP amplitude (VEP receptive field: see below).

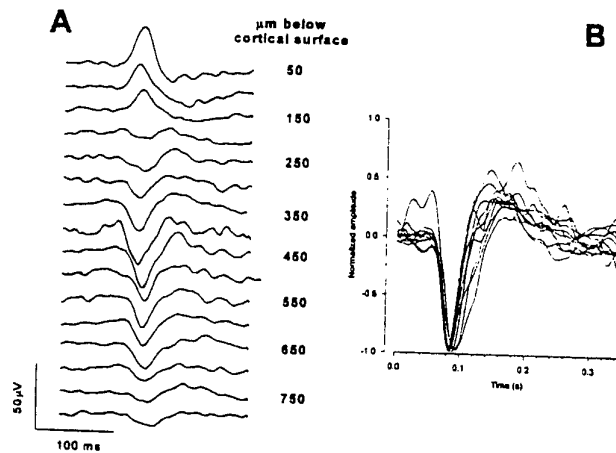


Fig.2. Depth profile of Pattern VEPs (modified from ref.2)

Depth profile of P-VEPs.

Figure 2A shows examples of local VEPs recorded at different depths of the binocular cortex (contralateral to the stimulated eye) in response to a pattern of horizontal sinusoidal bars of 0.06 c/deg and 90% contrast, reversed abruptly in contrast at 1 Hz (2 reversals/s). The microelectrode has been inserted 3.0 mm lateral to the lambdoid suture. The VEP waveform is very simple, with a major component peaking at 90-100 ms. In superficial layers, the VEP major component is positive, whereas in deeper layers is negative. Local VEPs have their maximal amplitude at intermediate depths (about 400 μ m electrode advancement), corresponding to the termination of geniculate afferents at the level of cortical layer IV. The depth profile of P-VEPs is very consistent across mice. In figure 2B, waveforms (normalized in amplitude) of VEPs recorded in 10 different mice at 400 μ m depth are shown superimposed to show good reproducibility of responses. We have maintained these experimental conditions for the evaluation of visual performance, cortical retinotopy and ocularity.

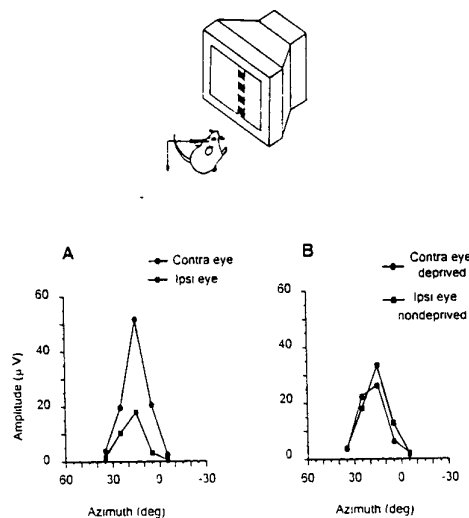


Fig.3. Retinotopy and ocularity of P-VEPs before (A) and after (B) monocular deprivation during the critical period (modified from ref. 11).

Retinotopy and ocularity of P-VEPs.

Local VEPs represent the integrated activity of a small population of neurons driven by a visual stimulus placed in an appropriate location of the visual field (VEP receptive field). For each position of the recording microelectrode over the visual cortex, the VEP receptive field can be evaluated by recording a series of responses to a small visual stimulus with different horizontal eccentricity from the vertical midline (azimuth) and elevation above the horizontal meridian. The VEP receptive field is defined as the visual field location at which local P-VEPs have their maximal amplitude. Systematic evaluation of VEP receptive fields for different microelectrode locations results in a topographic map of cortical representation of the contralateral hemifield. Figure 3 shows how this approach has been used to establish the receptive field of P-VEPs recorded from a microelectrode placed 3.0 mm lateral to the lambdoid suture. The visual stimulus has been windowed to vertical strip of 10 deg. The VEP amplitude is maximal for a specific position of the stimulus (about 20 deg eccentricity) and rapidly falls off for non optimal window positions. At 20 deg of eccentricity cortical neurons are binocular, and can be driven by stimulation of either eye. It can be noted in figure 3A that i) the VEP receptive fields of the two eyes coincide, and ii) VEPs driven by the contralateral eye are about three times larger in amplitude as compared to the ipsilateral eye. In the early postnatal life, from eye opening (P15) to about P35, a brief (4 days) period of monocular deprivation induces

a dramatic shift of ocular dominance of binocular cortical neurons (critical period for monocular deprivation^{6,7}). Figure 3B represents the ocularity of P-VEPs recorded in one adult mouse who was monocularly deprived from P24 to P28. As compared to nondeprived adult mice (figure 3A), VEPs recorded for the binocular cortex contralateral to the ex-deprived eye are much reduced in amplitude, whereas those of the ipsilateral, nondeprived eye are increased¹¹.

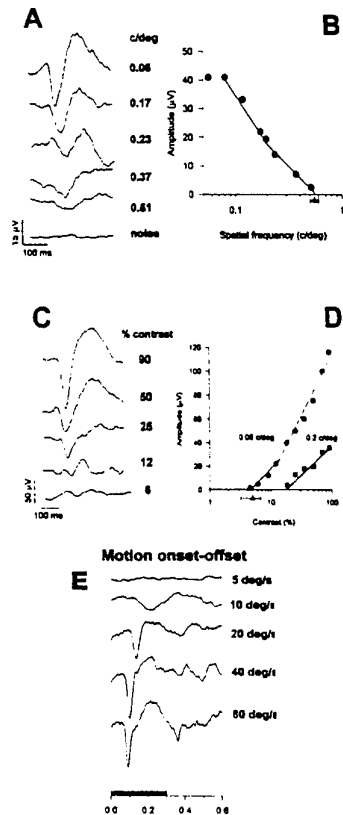


Fig.4. Evaluation of visual acuity (A,B), contrast threshold (C,D) and motion threshold (E) (modified from ref. 2).

P-VEP measures of visual capabilities.

Figure 4A-C shows how P-VEPs have been used to evaluate visual acuity, contrast threshold and motion threshold. All these measures have been obtained from the binocular cortex, at a location close to the representation of the vertical meridian. For visual acuity (Figure 4A), VEPs are recorded in response high contrast bar patterns of increasing spatial frequencies, and the VEP amplitude is extrapolated to 0 V (Figure 4B). The average VEP acuity of 10 wild type mice (triangle below abscissa in fig 4B) is 0.6 c/d, in excellent agreement with the mouse behavioral acuity⁸. For contrast threshold (Fig 4C and D), the amplitude of VEPs to stimuli with decreasing contrast is extrapolated to 0V. The average contrast threshold of 10 wild type mice for stimuli of low spatial frequency is about 5% (triangle below the abscissa in figure 4D). As shown in figure 4E, the onset of motion of grating stimuli elicits clear VEPs whose amplitude increases, and latency shortens, with increasing stimulus speed. Gratings of

0.06 c/g and 90% contrast, set in motion at 5 deg/s or lower speeds do not elicit recordable VEPs.

Pattern electroretinogram.

Patterned visual stimuli can also be used to elicit electroretinograms (P-ERGs). In mice, as in other mammals, retinal ganglion cells represent the main response generator of the P-ERG, since the response is abolished after retrograde ganglion cell degeneration⁹. The P-ERG can be recorded by means of a small silver loop leaned over the corneal surface by means of a microelectrode drive, paying attention to avoid occlusion of the undilated pupil. Overall, the characteristics of the mouse P-ERG are similar to those of the rat. However the P-ERG acuity is lower and corresponds to the P-VEP acuity. Anesthesia and restraining for P-ERG, as compared to P-VEP, recording are less demanding. In our experience, avertine (tribromoethanol in amylene hydrate, 20 µl/g body weight administered intraperitoneally) assures about one hour of stable recording and can be repeated many times without apparent damage to the animal.

Applications of the P-ERG /P-VEP technique in plasticity and degeneration of the CNS.

We have used the P-ERG / P-VEP technique to characterize the visual phenotype of different transgenic mice as well as to evaluate the effects of experimental manipulations. The results are summarized below.

- Adult mice overexpressing the human *bcl-2* gene have larger brains and optic nerves as a result of inhibition of apoptotic cell death during development. However their visual acuity, contrast threshold, and response latency are normal, while cortical topography is changed¹⁰. CNS neurons of adult *bcl-2* mice are little altered after lesions. The great majority of retinal ganglion cells survive, and their physiological response is not altered, long time after optic nerve section⁹.
- Adult mice overexpressing the BDNF neurotrophin have normal visual acuity, contrast threshold, response latency and cortical topography. However, the development of visual acuity is remarkably accelerated, as well as the critical period for ocular dominance plasticity¹¹.

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RECOMMENDATIONS FOR A HARMONIZED ERG PROTOCOL

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^bSecretary, Electoretinography Harmonization Committee, European College of Veterinary Ophthalmology

NOTE:

This protocol consists of 3 parts. The first part describes the basis for the test of rod function and of cone function in the dog. The second part is devoted to technical aspects of electoretinography, such as equipment, patient preparation, etc. The third part consists of two recommended recording protocols. The first protocol is an abridged protocol, designed for rapid evaluation of retinal function prior to cataract surgery. **This protocol should not be used to diagnose outer retinal diseases,** as it does not provide a comprehensive test of rod and cone function. Diagnosis of these diseases should be based on the second protocol, which provides exhaustive evaluation of outer retinal function.

TESTING OF ROD FUNCTION IN THE DOG

Dark adaptation

- The dog should be dark adapted for a minimum of 20 minutes
- The dynamic process of dark adaptation should be evaluated by periodic examination. This examination will be conducted by performing an ERG recording every 5 minutes (for a total of 4 such recordings during the 20-minute dark adaptation period) in response to low-intensity stimuli.

Stimulating light

- Stimulus Color-the committee recommends the use of white light. Chromatic stimuli may be used
- Stimulus Intensity-rods should be tested using a low intensity flash. The intensity of this flash should be 2 log units below that of the standard flash (SF) which is used to test cone function, or 0.02-0.03 cd/m²/sec. Note that the use of chromatic filters will lead to attenuation of the stimulus intensity.
- Number of test flashes-a single flash (repeated at 5 minute intervals) is sufficient to test rod function and to evaluate the process of dark adaptation. A clinician may decide to average the response to more than one flash. If the clinician elects to record, and average, the response to several flashes, these should be presented at a rate ≤ 0.5 Hz. The committee objects to averaging more than 4 flashes.

Flicker response

- Rod flicker response in the dog should be evaluated at a frequency of 10 Hz following the evaluation of the dark adaptation process and of the rod function.

TESTING OF MIXED ROD-CONE FUNCTION IN THE DOG

- This test consists of the response to a single high-intensity flash (2-3 cd/m²/sec) following the process of dark-adaptation or following the rod flicker test

TESTING OF CONE FUNCTION IN THE DOG

Light adaptation

- The dog should be light adapted for a minimum of 10 minutes using white background light.
- Intensity of the background light should be 30-40 cd/m². Calibration tables are now available on the Internet that would enable clinicians using commercial photography photometers to convert their readings into standard photometric units. The background light should be uniformly distributed across the retina using a Ganzfeld device or similar apparatus; ambient room light should not be regarded as uniform lighting for this purpose.

Stimulating light

- Stimulus Color-the committee recommends the use of white light. Chromatic stimuli may be used
- Stimulus Intensity-cones should be tested using a high intensity ($2-3 \text{ cd/m}^2/\text{sec}$) flash called a standard flash (SF). Note that the use of chromatic filters will lead to attenuation of the stimulus intensity.
- Number of test flashes-a single flash may be sufficient to test cone function. A clinician may decide to average the response to more than one flash in order to increase the signal/noise ratio. If the clinician elects to average the response, it is recommended that the stimulus shall consist of 16 or more flashes, presented at a rate of 4.9-5.1 Hz (taking care to avoid a frequency of precisely 5 Hz).

Flicker response

- Cone flicker response in the dog should be evaluated at a frequency of 50 Hz using SF intensity.

MISCELLANEOUS

- The committee recommends the use of full-field conditions, such as a Ganzfeld stimulator.
- The recommended flash duration is 10-20 μsec . Flashes lasting more than 5 msec should not be used.
- Bilateral stimulation may be used

Patient Preparation

- The preparation of the patient should be conducted in ambient light.
- Care should be taken to prevent the patient's pre-exposure to strong light. If fundus photography, or similar exposure is required, the protocol must be adjusted accordingly. A light adaptation period of one hour is recommended following fundus photography.
- Sedation is insufficient for ERG recording in the dog. Patients must be fully anesthetized. As the recording may be affected by the clinician's choice of anesthetic, the anesthesia protocol used in the recording should be reported. Normal values, from age-matched and breed-matched control dogs, must be recorded using the same anesthetic protocol.
- Proper oxygenation and ventilation must be maintained throughout the examination, as signals may be affected by oxygenation.
- Pupils must be fully dilated throughout the examination. Periodic evaluation of pupil size should be conducted.
- Eyelids must be open during the examination
- Proper positioning of the pupil (in relation to the stimulating light) must be maintained. The use of subconjunctival stay sutures, or other adequate means, is recommended.

Signal Acquisition

Electrodes

- The use of corneal contact lens electrodes with adequate curvature is recommended. Electrodes may be reused following visual quality inspection.

- Other types of electrodes may be used as the active electrode. Clinicians who do not use contact lens electrodes must take measures to prevent drying of the corneal surface.
- A reference electrode should be placed halfway between the temporal canthus and the ear. It should be at least 2 cm distant from the active electrode. Subcutaneous needles are recommended as reference electrodes.
- The ground electrode should be placed at an indifferent location, such as the pinna of the ear. Subcutaneous needles are recommended as ground electrodes.
- Electrode impedance should be evaluated and maintained below 5 k Ω .

Filters & Amplifiers

- Filter settings should be as wide as possible, subject to the local noise environment. It is recommended that the low filter should be no higher than 1 Hz, and that the high filter should be no lower than 300 Hz.
- The use of a notch filter should be avoided, subject to the local noise environment.
- Equipment should enable amplification of the signal so that recordings may be evaluated with high accuracy.
- Equipment should meet EU safety standards for (human) clinical ERG apparatus.

Reporting of Results

- Sweep time (duration of recorded response) should be 200 msec.
- A prestimulus baseline (background noise), as well as the onset of the light stimulus, should also be presented.
- Reports in literature should include a display of the patient's traces alongside the traces of a normal, age-matched dog of the same breed, anesthetized using the same anesthetic protocol. Calibration bars should be added.
- A report of an ERG recording should include the following parameters:
 - a-wave amplitude: measured from the baseline to the a-wave trough
 - b-wave amplitude-measured from the a-wave trough to the b-wave peak
 - a & b-wave implicit time: measured from the stimulus onset to the a-wave trough and b-wave peak, respectively.
 - An illustration of the dark adaptation curve

RECOMMENDED PROTOCOL FOR PRE-OP CATARACT PATIENTS

This protocol is intended to rapidly determine retinal function in patients that are about to undergo cataract surgery. It is NOT an adequate test of rod and cone function in patients that may be suffering from inherited photoreceptor degeneration or dystrophy. If any photoreceptor anomaly is suspected, the diagnostic protocol for photoreceptor evaluation (see below) should be used.

1. Dark adapt for 5 minutes
2. Test rod function using a single low-intensity flash
3. Test mixed cone-rod function using a single SF

RECOMMENDED PROTOCOL FOR DIAGNOSIS OF PHOTORECEPTOR DISEASES

1. Dark adapt for 20 minutes, while evaluating rod function and the dynamic process of dark adaptation every 5 minutes
 2. Perform the rod flicker test
 3. Test the mixed rod-cone response
 4. Light adapt for 10 minutes
 5. Test the cone function
 6. Perform the cone flicker test
- As this protocol is intended to test for inherited diseases that are bilateral, it is sufficient to conduct the test in one eye.

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and the
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May 31, 2000

University of Veterinary Medicine Vienna, Austria

Veterinärplatz 1, A-1210 Wien
Hörsaalzentrum

Combined Scientific Meeting of ESVO and ECVO

**May 31, 2000, University of Veterinary Medicine Vienna (VUW)
Veterinärplatz 1, A-1210 Wien**

PROGRAMME / PRESENTATION SCHEDULE

Wednesday, May 31, 2000

09.00 - 09.15 Welcome

I. Adnexal structures

Chair: K. Narfström

09.15 - 09.35 GIORDANO Christina, VERCELLI Antonella

Retrospective histological study on eyelid neoplasms of dogs and cats

09.35 - 09.40 Discussion

09.40 - 10.00 GRAUWELS Magda

A two-step technique for fixing a prolapsed nictitans gland in the dog

10.00 - 10.05 Discussion

10.05 - 10.25 ALLGOEWER Ingrid et al.

Cytology and magnetic resonance imaging in the diagnosis of orbital tumors

10.25 - 10.30 Discussion

10.30 - 11.00 Break

II. Anterior ocular segment / globe

Chair: F.C. Stades

11.00 - 11.20 LEBER Andrea

The use of topical interferon alpha-2b in feline corneal disease: a preliminary report

11.20 - 11.25 Discussion

11.25 - 11.45 HEIJN Albert

Porcine small intestinal submucosa, an alternative for conjunctival flaps?

11.45 - 11.50 Discussion

11.50 - 12.10 ROVESTI Gian Luca, BIASIBETTI M.

Retrograde penetration in the eyeball of a migrating grass awn: a case report.

12.10 - 12.15 Discussion

12.15 - 12.35 ROZE Maurice

Temporary blindness associated with dirofilariosis

12.35 - 12.40 Discussion

12.40 - 14.00 Lunch (VUW)

III. Posterior ocular segment

Chair: B.M. Spiess

14.00 - 14.20 ISARD PF et al.

A new foldable injectable 41 D intraocular lens designed for the canine eye:
The PFI/C-2000. Preliminary results of surgical technique

14.20 - 14.25 Discussion

14.25 - 14.45 OFRI Ron et al.

The development of the refractive error in the ostrich chick (*Struthio camelus*)

14.45 - 14.50 Discussion

14.50 - 15.10 GOEDDE Thomas, MEISSEL Hannes

Metabolic encephalopathy and retinopathy in a kitten - case report and clinical follow-up

15.10 - 15.15 Discussion

15.15 - 15.35 BJERKÅS Ellen et al.

Day blindness in two wirehaired dachshund siblings

15.35 - 15.40 Discussion

15.40 - 16.00 EKESTEN Berit, NARFSTRÖM Kristina

Rod-cone degeneration in the Abyssinian cat: differentiating heterozygotes from normals using ERG

16.00 - 16.05 Discussion

16.05 - 16.35 Break

Chair: B. Clerc

16.35 - 16.55 KORBEL Rüdiger et al.

Flourescein angiography in various raptor eyes and mammals

16.55 - 17.00 Discussion

17.00 - 17.20 CRISPIN Sheila

Multifocal Retinal Dysplasia (MRD) in the Golden Retriever in the UK

17.20 - 17.25 Discussion

17.25 - 17.45 WALLIN-HAKANSON Berit Kristina

The Collie Eye Anomaly: genetic conclusions from a population based study

17.45 - 17.50 Discussion

18.00 - 19.30 ECVO-AGM

20.00 get together at the „Old Danube“

RETROSPECTIVE HISTOLOGICAL STUDY ON EYELID NEOPLASMS OF DOGS AND CATS

C Giordano, A Vercelli.

Corso Traiano 99/d, I-10135 Torino, Italy

Purpose: A survey of 201 canine and 22 feline eyelid neoplasias was conducted to identify the prevalence of tumor types and the relation to breed, sex, age, and location.

Methods: In previous studies sebaceous neoplasias were classified in adenomas and carcinomas thus not considering the simple hyperplasia or the locally invasive form described as sebaceous epithelioma that has a malignant histologic aspect with a high mitosis rate due to its prominent basal cells component but has shown to have a good prognosis. In this study sebaceous eyelid tumors were classified according to the dermatologic classification that differentiate cutaneous sebaceous neoplasia in benign forms (hyperplasia and adenoma), locally invasive forms (sebaceous epithelioma) and highly malignant forms (carcinoma).

Results: In dogs sebaceous gland tumors were most frequently encountered, comprising 70,1% of all canine eyelid tumors. Compared to previous study (15,3% and 2%) the percentage of adenocarcinoma was 1,9 %.

Benign tumors (93%) were more prevalent than malignant forms (6,9%), the breeds more frequently represented were German Shepherd, Cocker Spaniel, Poodle and Boxer; the average age of dogs with eyelid tumors was 7,7 years.

Follow up in dogs showed no recurrence of sebaceous epitheliomas after surgical excision.

In cats the most frequently encountered eyelid tumor was squamous cell carcinoma / SCC (54,5%). Average age was 8,8 years.

Conclusions: In cats, due to the high incidence of SCC, biopsy of the lesions before surgical excision is strongly recommended.

A TWO-STEP TECHNIQUE FOR FIXING A PROLAPSED NICTITANS GLAND IN THE DOG.

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Purpose: To describe a new surgical technique for fixing a prolapsed third eyelid lacrymal gland. This technique seems to be particularly useful in the brachycephalic and giant breed dogs and to correct prolaps recurrences.

Method: After standard prepping of the surgical site, a blepharostat is put into place. The first step will be placing a "purse-string" type suture around the nictitans gland, in the conjunctiva of the posterior face of the third eyelid using a 4/0 monofilament non-resorbable suture placed on a +/- 12mm cutting needle. The knot will be placed on the anterior face of the third eyelid.

The second step is transpalpebral. A 3 to 5 cm skin incision is made infero-nasally at the level of the orbital rim. The subcutaneous tissue is sharply dissected up to the level of the orbital fascia which is then incised for 3 to 5 cm, 0.5 cm above the orbital rim.

The fat pad is then seen.

With a Graefe forceps the base of the nictitans gland and the cartilage is grasped, a 3/0 monofilament non-resorbable suture is passed through this base and is then anchored at the periosteum of the orbital rim.

Excess tension should be avoided in order to avoid folding of the nictitans membrane.

The orbital fascia, the subcutaneous tissue and the skin are then sutured.

Post-operative medical treatment includes: systemic large spectrum antibiotics and NSAIDs for 4 days, topical antibiotics qid for 8 days.

The technique has been used on 26 eyes of 17 dogs of the following breeds: 6 Mastino Napolitanos, 3 St. Huberts, 5 English Bull Terriers, 2 Great Danes, 1 Beauceron.

The age range was 3 months to 2.5 years, 7 were males and 10 were females.

Most of the glands were severely inflamed and had received a panoply of different medical treatments.

Five nictitans glands had had some kind of fixation surgery before.

Results: Follow up has been from 6 months to 2 years. No recurrences of prolaps have been noted.

Disadvantages: restricted movement of the third eyelid, small granuloma possible on/around the knot of the "purse-string" suture.

Advantages: quick and easy access to the nictitans gland and the orbital rim, fixation possible of large, inflamed glands, no obstruction of the lacrimal ducts.

CYTOLOGY AND MAGNETIC RESONANCE IMAGING IN THE DIAGNOSIS OF ORBITAL NEOPLASIA

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Purpose: To determine the diagnostic value of cytology and magnetic resonance imaging (MRI) in orbital neoplasia.

Methods: 29 cases of feline (n=15) and canine (n=14) orbital neoplasia were evaluated. All cases underwent a complete ophthalmologic and general examination. Orbital fine needle aspiration biopsy (FNAB) was obtained without sonographic guidance. Cellular material was brought onto a glass slide and allowed to air dry. Slides were stained according to May-Grünwald-Giemsa and examined by light microscopy. 26/29 cytologic specimens were available for evaluation.

24/29 cases underwent MRI. In five cases MRI was either impossible due to metal implants (n=2) or not justified due the obviously infiltrative nature of the neoplasia (n=3).

Histologic specimens were obtained in 16/29 cases.

Results: The tumor diagnosis based on cytology only (n=10) and confirmed by histology (n=13) was adenocarcinoma (n=5), fibrosarcoma (n=5), squamous cell carcinoma (n=4), lymphoma (n=4), carcinoma (not subtyped) (n=2), osteosarcoma (n=2) and mastocytoma (n=1).

Correlation of cytology and histology was good for adenocarcinoma and squamous cell carcinoma. Contrary to discrete round cell tumors diagnosis of mesenchymal tumors was difficult in some cases. Due to lack of specific cytologic criteria exact subtyping of carcinoma and sarcoma was not possible in every case. Since paired cytologic and histologic samples were only present in 13/29 cases diagnostic accuracy of cytology could not be demonstrated in this study.

However cytology revealed the diagnosis "malignant neoplasia" in 26/29 cases. Three specimens contained only blood, inflammatory cells and/or necrotic tissue. In these cases diagnosis was based on MRI.

On MRI all 29 cases showed infiltration of a contrast enhancing mass into orbital bones and/or sinuses. According to these findings none of the patients was a good surgical candidate.

Conclusions: FNAB/cytology seems to be a good diagnostic tool of minimal invasive nature to establish the diagnosis of malignant orbital tumors. If surgical therapy is considered further imaging techniques seem to be mandatory.

THE USE OF TOPICAL INTERFERON ALPHA-2B IN FELINE CORNEAL DISEASE: PRELIMINARY REPORT.

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Objective: To evaluate the effect of topical interferon alpha-2b (Intron A, Essex R) in cats with corneal necrosis, chronic epithelial erosions, and herpetic (dendritic) keratitis.

Methods: Retrospective study comparing cats treated with interferon with those without interferon therapy. Statistical analysis by means of student's t-test.

Results: The mean healing time of patients with dendritic keratitis was 21.4 days and 87.5 days for those treated with or without interferon, respectively. The mean healing time of patients with chronic erosions was 32.7 days and 76.1 days for those treated with or without interferon, respectively. Cats with corneal necrosis, which weren't receiving topical interferon had to be treated by keratectomy in 71.4% of the cases. In contrast, in the majority of the cats receiving topical interferon, the sequestrum sloughed spontaneously and only 15.0% had to be treated surgically.

Irrespective of the clinical diagnosis, all cats included in this study had a prolonged history of unsuccessful therapy with various medications, including antiviral drugs, antibiotics, corticosteroids, and cyclosporin A.

Conclusion: Interferon alpha-2b appears to have a beneficial effect in cats suffering from herpes-induced corneal disease.

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PORCINE SMALL INTESTINAL SUBMUCOSA, AN ALTERNATIVE FOR CONJUNCTIVAL FLAPS?

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Purpose: Disinfected porcine small intestinal submucosa (PSIS) is a commercially available product. The manufacturer claims it contains collagen (Type I, III and V), fibronectin, decorin, hyaluronic acid, chondroitin sulphate A, heparan sulphate and growth factors (TGF β , bFGF). It has been used a.o. in the repair of large skin defects. It could be effective in the repair of corneal defects, but reports have been limited so far.

Methods: PSIS was used as a corneal graft in 8 eyes in 6 cats (unilateral) and 1 dog (bilateral, with a 4-month interval). Cases were presented with descemetocoeles (3), corneal necrosis (2), multiple deep ulcerations (1), melting cornea (2). In 4 cases there was aqueous leakage. The cats were Persians (4) and Domestic Shorthairs (2), ages 1.5 to 13 years, the dog was a LH-Dachshund, age 10 years. After debridement the defects were closed with a single or double layer of PSIS. In all cases 1-2 mm of healthy epithelium was removed before suturing the graft into the defect. 7 grafts were sutured with a simple interrupted pattern using 8-0 Vicryl, in 1 case 8-0 nylon was used. A temporary tarsoraphy was used in 3 cases, a third eyelid flap was used in both eyes of the dog. In all patients an elisabethan collar was applied. All cases were treated with topical antibiotics. Topical atropine sulphate and systemic NSAIDs were applied when necessary.

Results: In 1 cat, partial dehiscence of the graft was noted at 11 days PO. In 1 other cat, leakage through the graft occurred after rubbing of the eye by the cat, after removal of the elisabethan collar. This cat was then treated with a conjunctival flap, which was also leaking after another episode of eye-rubbing. The 4 other cats healed well with minimal scarring, related to the depth of the initial defects. Both eyes in the Dachshund healed well, with minimal scarring. All patients, except perhaps the cat rubbing, seemed to be comfortable shortly after surgery. The material can easily be cut to fit the defect. In contrast to conjunctival flaps, the material has no "stretch" and does not stop aqueous leakage immediately. In the successful cases the material was absorbed by corneal tissue, which became apparent within a week after surgery.

Conclusions: PSIS can be used as an alternative to conjunctival flaps. In the successful cases there was minimal corneal scarring, and corneal thickness seemed to be normal. It is readily available. As there is no stretch in the material, the cutting can be time consuming, especially if 2 layers are applied. However, preparing a conjunctival flap can be time-consuming too. In large perforations, leakage will not be stopped immediately. At this moment, it is not clear if 2 layers give a better result than 1 layer. To prevent dehiscence, it seems to be important to "oversize" the graft. The dehiscence in one case may have been due to the increasing size of the globe after surgery, as the initial aqueous leakage caused a low iop. Furthermore, additional protection of the cornea, e.g. by temporary tarsoraphy, seems to be appropriate. A further study in laboratory animals, including histopathology of the grafted sites, would be interesting.

Commercial interest: none. For the first 3 eyes the material was supplied for free by the manufacturer, for the other cases the material was bought.

RETROGRADE PENETRATION IN THE EYEBALL OF A MIGRATING GRASS AWN: A CASE REPORT.

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Clinical report

A 4-month old Bernese mountain dog, male, was presented at the Ambulatorio Veterinario Associato "M. E. Miller" in Cavriago (RE), Italy, for a painful right eye for some days, treated by the referring veterinarian with a topical antibiotic-corticosteroid ointment. The history of the dog was not significant for trauma or previous diseases. The clinical examination revealed blepharospasm, a severe uveitis, with 4+ flare, IOP 8 mm Hg (Tonopen XL, Mentor, CA), absent PLR, both direct and consensual, absent menace reflex. A mild exophthalmos was present, and fluorescein test was negative. The left eye was clinically normal. The exploration of the mouth evidenced the presence of a grass awn partially penetrated in the retromolar right space, and it was extracted. Ultrasonography of the right eyeball evidenced fibrin in the vitreous. The presumptive diagnosis was endophthalmitis secondary to severe orbital cellulitis.

Therapy

The therapy included prednisone 1 mg/kg q24h PO, amoxicillin and clavulanic acid 25 mg/kg q12h PO, diclofenac sodium topically 1 drop q6h, prednisolone acetate + neomycin topically 1 drop q6h, tobramycin topically 1 drop q8h. A second clinical examination, 3 days after the first one, showed a less intense inflammatory reaction, and a yellowish, fibrin-like 4-mm-diameter lesion in the superolateral quadrant, suspected to be a corneal abscess. The local prednisolone was discontinued, and a recheck scheduled for the next day. On clinical examination, the small tip of a foreign body was evident in the middle of the corneal lesion. The dog was anesthetized, the cornea incised, and a 2-cm-long grass awn extracted from the eyeball. The corneal lesion was treated by means of a pedunculated conjunctival flap; the dog recovered uneventfully from the surgery. A week after the surgery, the eye was not visual, and an increase in IOP was evident (32 mm Hg); a therapy with dorzolamide chloride topically 1 drop q12h, and timolol maleate topically 1 drop q12h, was instituted. Tobramycin locally was withdrawn, while diclofenac and the systemic therapy were maintained. Twenty days after the surgery, the eye was slightly buphthalmic, and ultrasonography revealed fibrin and blood clots inside the eyeball. An intravitreal injection of gentamicin 20 mg + dexamethasone 0,4 mg was performed to control pressure. The previously recommended pharmacological therapy was maintained for a week more, and then withdrawn. Only the diclofenac sodium was kept as a maintenance therapy. Three weeks after the intravitreal injection, the pressure dropped to 10 mm Hg, and after two months the dog showed no signs of discomfort. Seven months after the foreign body removal, the eye was not painful and cosmetically acceptable, although the owner refused a new surgery for curettage of the pedunculated conjunctival flap.

Discussion

The differentials we considered in this case were anterograde penetrating foreign bodies, trauma, retrobulbar abscess, and bacterial panophthalmitis. The retrograde migration of a grass awn was considered unlikely, especially after the removal of another vegetative foreign body during the first examination. Furthermore, in our experience, it is an unusual event and, to our knowledge, not reported before in the veterinary literature. We couldn't precisely determine whether the awn migrating craniad was a part of the one extracted from the

retromolar space or a different one. Migrating foreign bodies are almost invariably associated with infection, and this is why in treating this disease is mandatory to keep orbital and/or eyeball sepsis under control. In this case ultrasonography couldn't reveal the presence of the foreign body at the time of the first examination, but this diagnostic imaging technique is so operator-dependent, that it can't be concluded if a more experienced and skilled operator would be able to detect the foreign body. Of course, the foreign body could have been in a position not detectable at that time. The discussion was whether leaving the eyeball or enucleating it. We tried to save the eyeball mainly for cosmetic reasons, and the owner agreed in this, after being informed of the risk of a later enucleation, in case an uncontrolled septic panophthalmitis would develop.

TEMPORARY BLINDNESS ASSOCIATED WITH DIROFILARIOSIS

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Purpose: To report a very uncommon case of temporary blindness with no recurrence. A six year old Bobtail male presented sudden blindness with no other clinical signs.

Methods: The ophthalmological examination included a standard ocular assessment and electroretinography. The fundus was normal and ERG revealed a good retinal function. Blood chemistry and haematological examinations were normal except for the protein profile showing an increase of serum proteins and gammaglobulins. An abnormal interstitial density of caudal lung Lobes and pulmonary arteries dilatation were noticed on thoracic radiographs. Because of the electrophoretic abnormalities, serology tests for Borreliosis, Ehrlichiosis, Leishmaniasis, Dirofilariosis were done. All were negative except for Dirofilariosis.

Results: After examination the clinical diagnosis, based on the blindness associated with a normal fundus, of amaurosis is obvious. On the other hand, radiology, the protein profile and serology test positivity suggest an infestation by *Dirofiluria immitis*. After a preparation period of aspirin administration, the dog is treated with Melarsamine (Immiticide ND). The filaricidal treatment is followed by a three weeks period of aspirin administration. The vision returns ten days after the Melarsamine injections.

Conclusion: Return of vision after therapy and the lack of recurrence during a very long term follow up of six years strongly suggest Dirofilariosis as the cause of temporary amaurosis.

A NEW FOLDABLE INJECTABLE 41D INTRAOCULAR LENS DESIGNED FOR THE CANINE EYE : THE PFI/C-2000. PRELIMINARY RESULTS OF SURGICAL TECHNIQUE.

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Purpose: To evaluate a single 41D foldable injectable posterior chamber acrylic intraocular lens(IOL) implanted in canine eyes through a 3.2 mm corneal incision following phacoemulsification cataract surgery.

Methods: The PFI/C-2000 (12 mm diameter with 5 mm optic; 1.47 refractive index) was placed on a specific cartridge and folded. 22 folded PFI/C-2000 were implanted in 20 canine patients following phacoemulsification to remove cataract; ophthalmic examination including biomicroscopy, intraocular pressure measurement were performed at 1, 8, 15, 30 and 90 days following surgery. Retinoscopy was performed at day 30.

Results: Surgery and implantation were uncomplicated; all patients demonstrated a mild ocular inflammatory reaction characterized by flare and mild corneal edema at day 1 which resolved completely by day 8. Three eyes developed anterior chamber fibrin postoperatively, which resolved completely by day 30. Postoperative intraocular pressure ranged from 9 to 22 mm Hg. Implantation resulted in 13 emetropic eyes, 6 myopic eyes (-0.5dt to -1dt) and 3 hyperopic eyes (+0.5dt to +1dt).

Conclusions: Advantages of this IOL design include ease of implantation through the same incision (3.2 mm) used to remove cataract and its autocentration. PFI/C-2000 is the first acrylic hydrophile foldable injectable IOL used for canine cataract surgery introduced through a 3.2 mm corneal incision. Resistance of the material, its low humid vitreous transition (T.V. +20°C) and its young module index allow an excellent foldability and slow and controlled IOL unfolding movement to avoid eccentric placement.

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THE DEVELOPMENT OF THE REFRACTIVE STATE IN THE YOUNG OSTRICH CHICK (*Struthio camelus*)

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Purpose: Few refractive studies have been conducted in species with large eyes. Furthermore, these studies have been limited to refraction of adults. There are no reports on the development of the refractive state in animals with large eyes. The adult ostrich (*Struthio camelus*) possesses the largest eye in the avian world, and the fifth largest in the animal kingdom (axial length 39 mm). The aim of this study was to follow the development of the refractive state in ostrich chicks.

Methods: Spot retinoscopy was used to measure the refractive state in 35 ostrich chicks, ranging in age from 0 to 37 days post-hatching. Birds were individually identified and followed over time, with at least 4 serial measurements taken from each eye during the experimental period. Ultrasonography was used to measure axial length.

Results: At hatching, ostriches are extremely myopic, with a mean (SD) refractive error of -4.5 ± 0.2 D. This error rapidly decreases, and by day 7 post-hatching, the eyes become slightly hyperopic, with a mean refractive error of $+0.4 \pm 0.1$ D. There were no significant changes in the mean refractive error from day 7 to day 37 post hatching ($+0.5 \pm 0.3$ D). Taking into account the small eye artifact, these values correspond to near-emmetropia from day 7 onwards. Inter-individual variability at any given age was minimal; the maximal range at any given age was only 0.5 D. Axial length increased from 15.98 ± 0.81 mm on hatching day to 18.55 ± 1.08 mm on day 19.

Conclusions: There are several interesting findings in this report, including the myopia on hatching day, the rapid onset of emmetropia, and the very low inter-individual variability. The myopia on hatching day casts doubts on whether the domestic chicken can be used as a representative bird model in the study of myopia in humans. Further keratometry and ultrasonography studies are needed to elucidate the mechanism of changes in the refractive error of the ostrich.

METABOLIC ENCEPHALOPATHY AND RETINOPATHY IN A KITTEN - CASE REPORT AND CLINICAL FOLLOW-UP

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Clinical report

A three month old unvaccinated female domestic short-hair kitten was presented with sudden loss of vision and ventroflexion as well as mild tremor of the head.

Physical examination demonstrated hypothermia, apathy, and widely dilated pupils on both eyes.

Abnormalities on neurological examination included generalized delayed proprioceptive testing as well as an absent menace reaction and absent direct and indirect pupillary light reflexes on both eyes. All other spinal and cranial reflexes were normal. On initial ophthalmoscopic examination no more alterations were found.

Further diagnostic work-up included complete blood count, serum chemistry profile, and urinalysis. All results were within normal limits.

ERG-testing on the day of presentation revealed no measurable response to stimulation with blue light.

A tentative diagnosis of metabolic-toxic encephalopathy and retinopathy was made.

Treatment

The cat was treated with thiamine at a dose of 75 mg/day and lactate Ringer solution at a dose of 50 ml/kg/day, both given intravenously. Canned cat food was given orally.

Follow-up

Once having started treatment the cat showed a better mental status and physical activity already on the second day. On the third day of hospitalization a mild response to pupillary light reflex testing was noted on both eyes. The ocular examination at that time revealed a bilateral retinal edema with massive mosaic-like retinal folding all over the tapetal fundus. No hemorrhages or retinal detachment were present. During the fourth day first signs of complex vision were noted and the cat returned to normal vision within the following four days.

Treatment with thiamine was continued for two more weeks at 25 mg/day orally.

Control examinations in the following weeks showed continuous resorption of retinal edema with disappearance of the folds from the periphery to the center. The retina blood vessels were attenuated and the colour of the tapetal fundus changed in several areas to blue and brown. These changes were documented by fundus photography.

ERG-testing six months after initial presentation revealed a normal response in both eyes and the cat was doing well.

Discussion

Similar retinal mosaic patterns were described in a case of ethylene glycol intoxication in a cat. Beside the ocular alterations this cat suffered from renal failure with formation of calcium oxalate crystals. Metabolic retinopathies commonly associated with disorders of the urinary system are described in dogs and humans as well.

The combination of systemic, neurological, and ophthalmologic deficits as well as the dramatic recovery following therapy with thiamine makes this case remarkable.

DAY BLINDNESS IN TWO YOUNG WIREHAISED DACHSHUND SIBLINGS

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Case report

Congenital day blindness was diagnosed in two of four wirehaired dachshund littermates, one male and one female. The affected dogs had shown clinical signs of reduced vision in daylight from early age. The male was suspected to be completely blind in daylight, while the bitch seemed to have some day vision. The two other littermates, as well as the mother, were normal. No information could be obtained about the father. The pregnancy had been normal. All four littermates were examined at 5 ½ months of age. The seven-year old mother was examined at the same time. Apart from the described visual disturbance in the two affected puppies, all the dogs were healthy, clinical and neurologic examination revealing no abnormal findings. Under darkened conditions, the affected dogs showed normal behaviour and avoided obstacles, while both bumped into objects when the procedure was repeated in full illumination.

Eye examination including ophthalmoscopy of all four littermates and the mother showed no abnormal findings, except for a possible slight attenuation of the retinal vessels in the affected dogs compared to the unaffected.

Electroretinography showed poor cone response in the two day-blind dogs. Responses to stimulation with blue light during dark adaptation were normal, while the response to red light stimulation was slow and poor. Flicker response was abnormal. ERG in the two normal littermates and the mother showed normal responses.

The affected female was euthanised and microscopic postmortem examination of the eyes was performed. Cone outer segments were decreased in number and those present were short and irregular. Cone nuclei showed degenerative changes of karyorrhexis and lysis and the outer segment layer was vacuolated. No other abnormalities than the ocular changes were found on necropsy.

Conclusions: Day blindness in the wirehaired dachshund has formerly not been reported in the literature. The described findings may be an accidental occurrence in just one litter of dogs, but it may also be that this represents a new disease in this breed.

ROD CONE DEGENERATION IN THE ABYSSINIAN CAT: DIFFERENTIATING HETEROZYGOTES FROM NORMALS USING ERG.

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Purpose: A recessively inherited rod cone degeneration has previously been described in the Abyssinian cat with similarities to human RP. ERGs during and after a long period of dark adaptation was evaluated in order to study rod function in homozygotes and heterozygotes in comparison to normal cats.

Methods: Twelve homozygous (affected) cats, 3 heterozygous (ophthalmoscopically normal) and 6 clinically normal, non-related cats were used. ERGs were performed during 90 minutes of dark adaptation and after 2 hours of dark adaptation using a bipolar contact lens, a Ganzfeld with a built in stimulus light source and an amplifier connected to a computer. Averaged responses were evoked by blue stimuli (KW no. 98), presented at a frequency of 0.1 Hz. In the latter experiment, scotopic ERGs covered a range of 4 log units.

Results: Maximum dark adapted b-wave amplitudes were reached for all cats after 60 to 90 minutes. At 90 minutes, b-wave amplitudes were significantly ($p < 0.05$) higher in normal cats compared to affected and heterozygous animals. However, b-wave amplitudes in heterozygotes were not significantly different from cats with early stages of disease. Amplitude/intensity studies at maximum dark adaptation showed that differences in amplitudes between the groups of cats were most obvious when stimulating with moderate light intensity.

Conclusions: These results show that heterozygotes have an altered outer retinal function although they are ophthalmoscopically normal throughout life. Moreover, we want to emphasize the difficulties in electrophysiologically differentiating heterozygotes from the early stage of retinal degeneration in this animal model.

Support: Foundation Fighting Blindness, Swedish Council for Forestry and Agricultural Research and Swedish Medical Research Council (19X-00938).

THE COLLIE EYE ANOMALY. GENETIC CONCLUSIONS FROM A POPULATION BASED STUDY.

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Purpose: To study the prevalence of chorioretinal dysplasia and coloboma in the Swedish population of rough collies during 1989-1997 and to test a hypothesis of simple autosomal recessive inheritance for the entire collie eye anomaly (CEA) complex.

Methods: 8.204 rough collies were examined for collie eye anomaly between 1989 and 1997 and the results were filed in the Swedish Kennel Club open computerised genetic health scheme register. All dogs were permanently identified.

Results: Breeders' policy during the study period was to select against coloboma but to allow breeding of CRD affected animals. The prevalence of CRD increased significantly from 54.2 per cent to 68.1 per cent ($p < 0.001$) while the prevalence of coloboma did not (8.3 per cent to 8.5 per cent, $p = 0.4$).

The prevalences of CRD in pups from normal x normal matings and CRD x CRD matings were significantly different from those expected with simple autosomal recessive inheritance (43 per cent versus 25 per cent; 85 per cent versus 100 per cent).

Conclusions: The results are compatible with polygenic inheritance but not with simple autosomal recessive. Breeding programs for genetic control of CEA may be influenced by these results.

MULTIFOCAL RETINAL DYSPLASIA (MRD) IN THE GOLDEN RETRIEVER IN THE UK

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Purpose: This study was conducted in order to establish the ophthalmoscopic appearance of dysplastic lesions in the ocular fundi of golden retrievers examined under the British Veterinary Association/Kennel Club/International Sheep Dog Society (BVA/KC/ISDS) Eye Scheme in the United Kingdom and to assess the incidence of dysplastic lesions in golden retrievers examined under the Eye Scheme from January 1998 to April 1999.

Methods: Between January 1998 and April 1999 a total of 4,091 certificates from golden retrievers certified under the BVA/KC/ISDS Eye Scheme were received by the British Veterinary Association. For each dog examination of the ocular fundus using direct and indirect ophthalmoscopy had been performed following the use of 1 % tropicamide (Mydracyl[®]; Alcon) and the results were recorded in writing and by means of annotated diagrams on the BVA/KC/ISDS Certificate of Eye Examination.

Results: Of the 4,091 dogs examined, 128 were certified as 'affected' for multifocal retinal dysplasia, an apparent incidence of 3.13%.

The morphology of the dysplastic lesions in the 128 golden retrievers certified as 'affected' for MRD under the Eye Scheme were single or multiple linear, single to multiple oval to round and large single irregular or horseshoe shaped 'geographic'. Most of the tapetal lesions were greyish in colour and hyporeflective, but in some dogs focal dysplastic lesions were hyper reflective and it was not uncommon to find diffuse hyper reflectivity in the central zone of geographic lesions. Linear and ovoid lesions were most commonly located in the tapetal fundus, less commonly in the non tapetal fundus where they were usually linear and white to grey in colour. Geographic lesions were predominantly unilateral, were located in the tapetal fundus dorsal to the optic disc and involved blond vessels in this area. In four of the 128 dogs there was insufficient information to determine the precise appearance and distribution of the lesions; in the remaining 124 dogs, 73 had unilateral lesions and 51 had bilateral lesions, it is also of note that 42 dogs had single dysplastic lesions affecting one eye only. In addition to retinal dysplasia, one dog had unilateral posterior polar subcapsular cataract and another had bilateral posterior polar subcapsular cataracts, two dogs, which were litter mates, had bilateral congenital nuclear cataract.

Conclusions: Multifocal retinal dysplasia might be considered a misnomer when a number of affected animals had single unilateral lesions and focal/multifocal retinal dysplasia might be considered a more accurate description. The true incidence of retinal dysplasia in the UK population of golden retrievers cannot be determined from this study for a number of reasons, which are concerned both with the complexities of diagnosis and logistical factors such as the number of dogs which are presented for eye examination under the scheme. The number of dogs examined under the Eye Scheme is a small proportion of the total UK golden retriever population. In 1998, for example, 14,803 golden retrievers were registered with the Kennel Club and 3,309 golden retrievers were examined under the Eye Scheme, thus 22.35% of golden retrievers registered with the Kennel Club were examined. However, the total population will also include an unknown number of unregistered golden retrievers which are seldom, if ever, examined under the Eye Scheme as well as a number of dogs examined previously and not presented for re examination.

FLUORESCCEIN ANGIOGRAPHY IN VARIOUS RAPTOR EYES AND MAMMALS.

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Purpose: The aim of the present study was to adapt fluorescein angiography (FAG) special needs of the avian patient and to compare the distribution of the dye within the eye of birds and mammals.

Methods: The fundi of 43 ophthalmologically healthy raptors (nine different species) were documented. In one group of unsedated and in another group of intubated raptors under isofluran anaesthesia 40 mg/kg BW fluorescein-sodium (10% solution) were administered via the superficial ulnar or jugular vein ($t = 0$ sec). In comparison FAG of the fundus was documented in 2 cats, 4 dogs, 2 sheeps, 2 goats and 7 horses as well with and without sedation via the jugular vein (ruminants, horse) or the cephalic vein (dog, cat). Additionally for the first time not only in birds but also in various mammals we were able to demonstrate video fluorescein angiography using a specially designed fluorescein head band ophthalmoscope in combination with a miniaturised video camera.

Results: Based on the specific morphology of the avian fundus a filling period (duration 4 ± 2 sec) was followed by a choroidal (duration in owls 10 ± 3 sec, other raptors 5 ± 2 sec) and a pecten period (duration in owls 6 ± 2 sec, other raptor species 3 ± 1 sec), which started approximately two seconds after the choroidal phase. Surprisingly it could be demonstrated that fluorescein is almost "shot" into the vitreous of birds ("calamary effect") with a frequency of up to 10 times/sec. After a maximum of 28 hours no dye could be demonstrated ophthalmoscopically. The distribution of the dye in the cat and dog was like documented before. In the small ruminant there is no phase of full fluorescence and in the horse it is not possible to separate the arterial from the venous retinal phase.

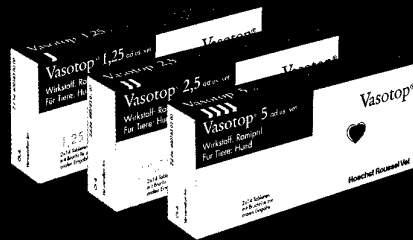
Conclusion: The escape of dye into the vitreous in birds may be seen as a proof of the nutritional function of the pecten. Beside the importance for further physiological studies clinical use of FAG in birds will be the diagnosis of subtle haemorrhages of the pecten and choroid, atrophy of vessels and the retinal pigment epithelium as well as retinal detachments and other diseases of the fundus.

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