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P Sanel
M.V.Sc. Scholar,
Department of Veterinary Surgery and Radiology, Bikaner, Rajasthan, India

SK Jhirwal
Assistant Professor and Advisor, College of Veterinary and Animal Science, Bikaner, Rajasthan, India

TK Gahlot
Professor and Head, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Corresponding Author:
P Sanel
M.V.Sc. Scholar, Department of Veterinary Surgery and Radiology, Bikaner, Rajasthan, India

Electro-diagnostic evaluation of retina using HMSERG system in dogs: A clinical study

P Sanel, SK Jhirwal and TK Gahlot

Abstract
ERG findings including rod, combined rod-cone, single-flash cone, and 30-Hz flicker responses were recorded with an LED-electrode in 40 eyes of 21 dogs divided into two groups using the HMsERG system. 20 eyes of 10 dogs had no vision abnormality (Group I) and rest of the 20 eyes of 11 dogs had various ocular abnormalities (Group II). Significant differences in relation to normal and affected eye were observed for the mean values of amplitude a-wave and b-wave for rod, combined rod-cone, cone and flicker responses between group I and group II ($p < 0.01$). Significant differences in b/a wave ratio of amplitude were observed for the mean values recorded for combined rod-cone and cone responses between group I and group II ($p < 0.05$). Thus, ERG is useful adjunct test for the diagnosis of retinal dystrophies and for pre-operative evaluation of retinal functions in conjunction with cataract surgery.

Keywords: Electroretinography, dogs, HMsERG machine, retina, electrodiagnosis

Introduction
Vision is a complex phenomenon in which light emanating from objects in the environment is captured by the eye and focused on to the retinal photoreceptors. Electrical signals originating from these cells pass through a number of cell types in retina and throughout the central nervous system (CNS) before arriving at visual cortex, where the sensation of vision occurs (Miller, 2008) [17].

Ocular electroretinography (ERG) is a valuable electrodiagnostic method that is used for evaluation of retinal function and detection of retinal disease in both humans and animals (Tzecov and Arden 1999; Safatle *et al.*, 2005) [24, 20]. It measures the retinal sensitivity, photoreceptors in outer retina and associated pathways in the middle layers of retina (Weinstein *et al.*, 1991) [25].

In dogs ERG is mostly used for the preoperative evaluation of patients with cataract (Jhirwal *et al.*, 2019) [10], for the characterization of disturbances that cause blindness such as glaucoma, retinal dysplasia, degenerative retinopathies, optic nerve hypoplasia, Sudden Acquired Retinal Degeneration (SARD), Progressive Retinal Atrophy (PRA) (Komaromy *et al.*, 1998) [11] and achromatopsia (Hurn *et al*., 2003) [7]. The use of ERG to assess retinal functions is also very important because many dog breeds are genetically predisposed to both cataracts and PRA (Clement *et al.*, 1996; Williams *et al.*, 2004) [4, 27].

Now a day's, veterinary ophthalmology is also developing with the same pace as other branches of veterinary in India. Keeping in the view of increase awareness of pet owners regarding diagnosis and treatment options the available techniques require more standardizations. In view of this the present investigation was undertaken to study the electroretinogram (ERG) of normal as well as dogs with various eye affections.

Materials and Methods
The present study was undertaken to evaluate the ERG of clinically healthy eyes and affected eyes of dogs using HMsERG system. The ERGs of normal dogs were studied to establish ERG system- specific limits of normality and were compared with that of dogs with various eye affections.

ERG was performed in 40 eyes of 21 dogs under general anaesthesia. All the eyes were divided into two groups (20 eyes each). Group-I included the normal eye dogs of both the sexes (10 dogs) and Group -II included the dogs with various eye affections of both the sexes (11 dogs). Prior to diagnosis all the dogs of group I were examined for any pre-existing ocular disorders that may lessen visual outcome and group II were examined to confirm the cause of

vision deficit before conducting ERGy by performing various ophthalmic examinations such as menace reflex test, corneal reflex test, pupillary light reflex test, dazzle reflex test, tapetal reflex test, maze test, Schirmer's tear test, non-contact tonometry, direct and indirect ophthalmoscopy, USG and fluorescein dye test.

For ERGy, HMsERG[1] (Hand held multispecies Electroretinography) unit (clarity easy trace) included Koijman electrode (active) (Figure-1), reference electrode (Figure-2), ground electrode (Figure-3), patient cable, LED flash (Figure-4), Gaunzfeld stimulator (Figure-5), HMsERG Amplifier (Figure-6) and a display unit (Laptop and a Printer).

All dogs received topical 1% tropicamide[2] for maximal pupillary dilation as mydriatic (2 drops every 10 minutes) and 0.5% Povidine Iodine[3] for ocular antisepsis 2-3 times, 30 minutes prior to procedure.

All animals were fasted for at least 12 hrs before performing ERG. The electrodiagnosis of retina was performed under general anaesthesia using a combination of Ketamine HCl[4] (50mg/ml) and Xylazine[5] (23.32 mg/ml) for induction along with Atropine sulphate[6] @ 0.03mg/kg SC as premedicant and was maintained with Ketamine HCl till effect.

The dogs were placed in sternal recumbency in an exclusive dark room and head was positioned by placing a cotton pillow under the lower jaw, so that it provides stabilization and comfort. Physiologic body temperature of animals was maintained during procedure. All the electronic devices *viz.*, mobiles, batteries, etc. were kept off to avoid electrical interference with the ERG unit. Diagnosis was performed by the individual sitting on the front of the patient. Inadvertent entry of personnel's was avoided.

Impedance (electrical resistance from the electrodes to the ground) and biosignal (electrical potential of the electrodes without a stimulus) was checked every time before starting the measurement. As the ERG recordings are very small electrical potentials at the range of µv the electrical resistance should be below 10 KOhm. A normal bio signal should look approximately like a more or less spiky line and later the scotopic ERG was recorded for right eye. Before ERGs were recorded, impedance and baseline test were performed; the latter for evaluating the noise level in the environment.

Positioning of electrodes for ERG reading was done and electroretinography was performed as per the guidelines of Narfstrom *et al.*, (2002) [18] (Fig. 7 to 10).

Wave amplitude and implicit time were determined for each response. The a-wave amplitude was measured from the baseline to the a-wave trough, and b-wave amplitude was measured from a-wave trough to the b-wave peak. Then b/a ratio of amplitude were calculated. The a-wave and b-wave implicit time were measured from the stimulus onset to the a-wave trough and b-wave peak, respectively.

The data were subjected to a two way analysis of variance (ANOVA) followed by a critical difference test for the comparison of mean values. A probability level of $p<0.05$ was considered as statistically significant. The mean values and mean standard error (SEM) were presented in tabular form.

Results and Discussion

The ERGs of normal dogs were studied to establish ERG system- specific limits of normality and were compared with that of dogs with various eye affections. Lee *et al.* (2009) [14] studied the normal ERG in healthy Shih Tzu dogs using HMsERG in order to establish ERG system specific limits for normal eyes. Aguirre (1973)[1] studied electroretinogram of dogs with various eye affections, were remarkably similar.

Out of the 40 eyes of 21 dogs, 20 eyes of 10 dogs had no vision abnormality (Group I) and rest of the 20 eyes of 11 dogs had various ocular abnormalities (Group II) *viz.*, mature cataract, sudden acquired retinal degeneration (SARD), blindness of unknown origin, hyphema, posterior synechiae with mature cataract (unilateral) and lens luxation with mature cataract (unilateral). Saroglu and Ekici (2010) [22] and Jeong *et al.* (2013) [9] also performed pre-diagnostic test such as menace reflex test, corneal reflex test and pupillary light reflex test before recording of ERG to rule out any ophthalmic disorder.

In the present study, a standard ana esthetic protocol was applied to all patients to make them unaware of the sounds from environment in order to avoid the artifacts that can develop due to patient's movement. All the 21 dogs were anaesthetized using a combination of inj. ketamine hydrochloride (5.0 mg/kg) and inj. xylazine hydrochloride (1.0 mg/kg) after premedication with inj. Atropine sulphate (0.004 mg/ kg). During the recording of ERG imaging no complications due to anaesthesia was encountered. Kommonen and Raitta (1987) [12] studied Electroretinography in Labrador Retrievers and used a similar combination of inj. Ketamine and inj. xylazine anaesthesia as followed in present study. They reported that inj. Ketamine and inj. xylazine anaesthesia did not show any clear effect on a and b wave amplitudes of ERGs in dogs. Kommonen *et al.* (2007) [13] found that increasing the propofol infusion rate elevated the b-wave amplitude, while a decreased rate produced a parallel reduction in the b-wave amplitude in normal beagle dogs**.** Lin *et al.* (2009) [15] reported that sedation with a combination of inj. Tiletamine and inj. Zolazepam was able to provide larger ERG waves during the short ERG protocol in dogs, compared to sedation with inj. Medetomidine and general anaesthesia with isoflurane. Jeong *et al.* (2013) [9] also studied clinical and electroretinographic findings of progressive retinal atrophy in Miniature Schnauzer dogs of South Korea by using combination of xylazine (2.0 mg/kg IM) and ketamine (10.0 mg/kg IM). Taking in view of the above mentioned, ketamine and xylazine was selected as suitable anaesthetic combination in present study.

In the present study, significant differences in relation to normal and affected eye were observed for the mean values of amplitude a-wave and b-wave recorded for rod, combined rod-cone, cone and flicker responses between group I and group II ($p< 0.01$) (Table-1 and Table-2). It was also reported that the amplitude of group-I was higher than group-II. Aguirre (1973) [1] studied electroretinogram of dogs with various eye affections and reported a significant reduction in amplitude of a-wave and b-wave for rod and cone, combined rod-cone and flicker response and a slight increased in value of implicit time of a-wave and b-wave. Sandberg *et al.* (1986) [21] reported a reduction in amplitude for rod and cone responses in the affected dog as recorded in our study. Saroglu and Ekici (2010) [22] also recorded a slight decrease in amplitude of the a-wave and b-wave of rod and cone for

[1] Handheld multi species Electroretinography, Clarity Medical Pvt. Ltd. Mohali, India.
[2] Tropicacyl, SUNWAYS (INDIA) PVT. LTD.
[3] TROYDINE Microbial Solution, Troikaa, Pharmaceuticals Ltd. Gujrat, India.
[4] Anket, NEON, laboratories limited, Mumbai, India.
[5] XYLAXIN, Indian Immunologicals Limited, Hyderabad, India.
[6] Atropine Sulphate injection, MORVEL Laboratories (p) Ltd. Mehsana, India.

affected eyes of dogs. In case of affected dogs of group-II this reduction in amplitude might be due to affections of anterior segment of eye and ocular media alteration (pupil size) with decrease effective intensity of the stimulus (Birch and Anderson, 1992) [2], decrease photoreceptor density (Weleber, 1981)[28] and bipolar or Muller cell death (Dorey *et al.*, 1989; Curcio *et al.*, 1993) [6, 5] and reduction in photopigment sensitivity might be an influential factor in dogs.

Significant differences in b/a wave ratio of amplitude were observed for the mean values recorded for combined rod-cone and cone responses between group I and group II ($p< 0.05$) (Table-1 and Table-2). Maehara *et al*. (2007) [16] also reported a significant difference in b/a ratio in the ERG of dogs with various eye affections. No significant differences in implicit times were observed for the mean values of a-wave and b-wave of rod, combined rod-cone, cone and flicker responses between the group I and group II ($p > 0.05$). Itoh *et al*. (2013) [8] also observed a non-significant difference in the values of implicit times for the left and right eyes in the same subject.

In the present study in the cases of mature cataract (n=3) the amplitude for all the responses was reduced while the b/a ratio was increased than normal values. The implicit time for rod response was increased while for combined rod-cone, cone and flicker response was decreased. Maehara *et al*. (2007) [16] and Jhirwal *et al.* (2019) [10] also observed the similar findings for mature cataractous eyes for amplitude and implicit time in all the responses. However, no significant difference in b/a ratio was reported by them as opposite to our study. They also opined that reduction in amplitude was caused by lens opacity or presence of anterior uveitis. Chiu *et al.* (2009) [3] also stated that decreased amplitudes or/and prolonged implicit times might be due to presence of focal lesions or alteration in opacity of lens. (Table-3 and Table-4).

In the cases of SARD (n=3) the amplitude for all the responses was reduced while the b/a ratio was increased as compared with normal values. The implicit time for all the responses was increased except in case of cone where it was decreased. Parry *et al.* (1953) [19] studied electroretinogram during development of hereditary retinal degeneration and reported the reduction of amplitude of b-wave and later on a flat wave on electroretinogram as the condition of retinal degeneration progressed. Safatle *et al.* (2005) [20] reported non-detectable scotopic and photopic responses on ERG confirming the devastating effect of the retinal degeneration. Sussadee *et al*. (2015) [23] studied retinal degeneration and reported significant differences in the mean values of ERG amplitudes and implicit times and also reported similar findings as in our study for amplitude and implicit time (Table-3 and Table-4).

In the cases of blindness of unknown origin (n=2) the amplitude for all the responses was reduced while the b/a ratio was increased than normal values. The implicit time for all the responses was increased for all the responses. Aguirre (1973) [1] also reported a significant reduction in amplitude of a wave and b wave for rod, combined rod-cone, cone and flicker response while a slight increased in value of implicit time for a-wave and b-wave (Table-3 and Table-4).

In the case of hyphema (n=1) and posterior synechiae with mature cataract (n=1) the amplitude for all the responses was reduced while the b/a ratio was decreased in combined rod-cone response and increased in cone response than normal values. The implicit time for all the responses was decreased except in a-wave of rod responses (in hyphema) and except in both a- and b-wave of cone response (in case of posterior synechiae with mature cataract). Birch and Anderson (1992) [2] stated that reduction in amplitude might be due to ocular media alteration that resulted in decreased effective intensity of the stimulus (Table-3 and Table-4).

In the case of mature cataract with lens luxation (n=1) the amplitude for all the responses was reduced except for a wave of combined rod-cone and cone response. The b/a ratio was decreased in all the responses than normal values. The implicit time for all the responses was decreased except for a-wave of rod response and flicker response. Chiu *et al.* (2009) [3] also reported decreased amplitudes or/and prolonged implicit times in ERG and stated that focal lesions or alteration in opacity of lens might be the possible causes for this alteration than normal values (Table-3 and Table-4).

Significant differences in relation to normal and affected eyes were observed for the mean values of amplitude a-wave and b-wave recorded for rod (Table-5), combined rod-cone (Table-6), cone (Table-7) and flicker (Table-8) responses between group I and group II ($p<0.01$). Significant differences in b/a wave ratio of amplitude were observed for the mean values recorded for combined rod-cone (Table-6) and cone (Table-7) responses between group I and group II ($p<0.05$). No significant differences in implicit times were observed for the mean values of a-wave and b-wave of rod (Table-5), combined rod-cone (Table-6), cone (Table-7) and flicker (Table-8) responses between the group I and group II ($p>0.05$).

Table 1: Median and range of amplitude (µv) for group I and group II dogs

Group		Group I	Group II
ERG Responses		**Amplitude (µv)**	**Amplitude (µv)**
Rod	a	48.65 (23.4-115.8)	10.85 (0.1-66.3)
	b	90.05 (33.4-222.5)	16.3 (4.3-117.4)
Combined rod-cone	a	49.5 (20.4-137.3)	7.2 (0.1-64.9)
	b	93.7 (39.8-172.4)	17.7 (3.5-76.2)
	b/a	1.77 (1.25-3.01)	3.26 (0.32-102)
Cone	a	47.9 (28.2-176.4)	14.1 (0.1-67.4)
	b	89.55 (56.4-228.1)	23.15 (1.1-116.2)
	b/a	1.77 (1.19-2.15)	2.29 (0.43-44)
Flicker	a	20.05 (9.3-39.7)	7.65 (2.6-26.7)
	b	30.3 (17.3-70.1)	11.5 (3.3-48.1)

Table 2: Median and range of implicit time (ms) for group I and group II dogs

Group		Group I	Group II
ERG Responses		**Implicit time (ms)**	**Implicit time (ms)**
Rod	a	11.15 (4.3-20)	13.1 (2.4-30.3)
	b	37.35 (21.5-66.7)	31.55 (4.3-68.2)
Combined rod-cone	a	13.6 (5.1-25.9)	12.25 (0.1-22.5)
	b	35.2 (21.4-53.7)	37.3 (10.2-55.8)
Cone	a	10.7 (7.2-23.9)	12 (0.1-37.6)
	b	32.4 (19.2-60.9)	33.3 (1.1-82.4)
Flicker	a	11.4 (8.1-14.8)	11.5 (3.7-15.8)
	b	25.45 (22.1-30.8)	26.4 (6.8-39.7)

Table 3: Mean/ Value of amplitude (µv) for group I (normal eye) and various eye affections of group II dogs

Group / ERG Responses		Group I (n=10)	Group II (n=11)					
			Mature Cataract (n=3)	SARD (n=3)	Blindness of Unknown origin (n=2)	Hyphema (n=1)	Posterior Synechiae with mature Cataract (Lt. eye) (n=1)	Mature cataract with Lens luxation (Lt. eye) (n=1)
Rod	a	48.53	26.35	3.38	5.68	43.95	19.8	14.6
	b	93.21	47.85	10.18	15.8	72.15	52.3	13.7
Combined rod-cone	a	57.18	20.45	1.25	1.6	27.7	24.9	64.9
	b	100.06	42.58	9.97	12.28	47.2	36.7	21.4
	b/a	1.84	2.16	34.40	26.76	1.74	1.47	0.32
Cone	a	61.24	23.02	2.55	4.78	17.55	54.2	67.4
	b	102.95	47.35	5.43	12.4	52.05	116.2	29.3
	b/a	1.74	2.07	18.13	4.35	2.90	2.14	0.43
Flicker	a	21.56	9.45	5.47	8.03	24.05	3.4	17.2
	b	34.66	21.13	6.6	9.8	38.1	14	42.1

Table 4: Mean/Value of implicit time (ms) for group I (normal eye) and various eye affections of group II dogs

Group / ERG Responses		Group I (n=10)	Group II (n=11)					
			Mature Cataract (n=3)	SARD (n=3)	Blindness of Unknown origin (n=2)	Hyphema (n=1)	Posterior Synechiae with mature Cataract (Lt. eye) (n=1)	Mature cataract with Lens luxation (Lt. eye) (n=1)
Rod	a	11.15	18.22	9.55	18.43	21.05	10.3	15.2
	b	37.53	45.02	35.03	38.88	4.05	22.4	31
Combined rod-cone	a	14.12	10.7	12.83	16.08	11.7	8.3	11
	b	35.14	31.38	37.53	45.28	32	19.3	19.3
Cone	a	12.08	9.67	13.47	21.23	12.15	28.6	11.6
	b	33.88	33.47	32.45	47.15	33.25	82.4	19.2
Flicker	a	11.55	8.2	10.45	13.4	13.1	7.5	105
	b	26.1	22.77	23.95	29.3	25.2	23.1	39.7

Table 5: Mean and standard error (SE) of amplitude and implicit time for rod response

Group	Amplitude (µv)		Implicit time (ms)	
	a**	b**	a	B
I	48.53 ± 4.71	93.21 ± 11.57	11.15 ± 0.91	37.53 ± 3.30
II	16.17 ± 4.32	31.09 ± 6.78	15.40 ± 2.14	38.57 ± 4.56

** = significant difference (p<0.01)

Table 6: Mean and standard error (SE) of amplitude and implicit time for combined rod-cone response

Group	Amplitude(µv)			Implicit time (ms)	
	a**	b**	b/a*	A	B
I	57.18 ± 6.38	100.06 ± 8.39	1.84 ± 0.09	14.12 ± 1.33	35.14 ± 2.39
II	14.09 ± 4.02	25.85 ± 4.98	16.59 ± 6.83	12.41 ± 1.05	34.86 ± 2.93

** = significant difference (p<0.01)
* = Significant difference (p<0.05)

Table 7: Mean and standard error (SE) of amplitude and implicit time for cone response

Group	Amplitude(µv)			Implicit time (ms)	
	a**	b**	b/a*	A	B
I	61.24 ± 7.60	102.95 ± 10.42	1.74 ± 0.06	12.08 ± 1.20	33.88 ± 2.47
II	16.46 ± 4.12	30.80 ± 6.63	7.35 ± 2.53	14.41 ± 2.17	37.61 ± 4.04

** = significant difference (p<0.01)
* = Significant difference (p<0.05)

Table 8: Mean and standard error (SE) of amplitude and implicit time for flicker response

Group	Amplitude(µv)		Implicit time (ms)	
	a**	b**	a	B
I	21.56 ± 1.77	34.66 ± 2.84	11.55 ± 0.45	26.1 ± 0.63
II	9.52 ± 1.41	16.90 ± 2.78	10.49 ± 0.72	25.54 ± 1.40

** = significant difference (p<0.01)

Fig 1: Active electrode

Fig 2: Reference electrode

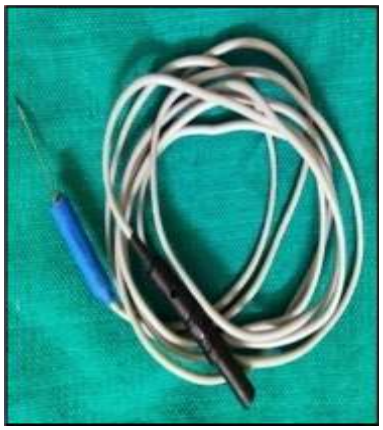


Fig 4: LED Flash

Fig 5: Gaunzfeld Stimulator

Fig 6: Amplifier

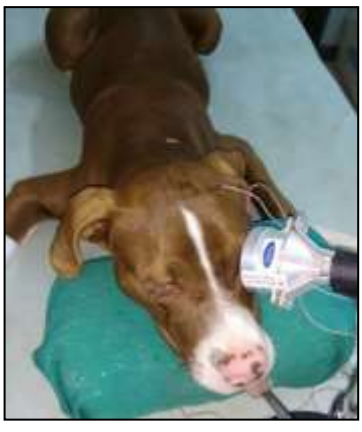
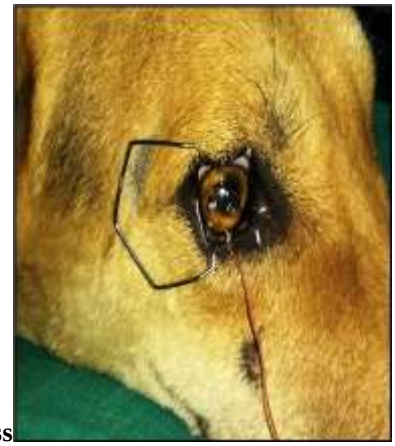


Fig 7: Positioning of the patient

Fig 8: Positioning of ground electrode

Fig 9: Positioning of reference electrode



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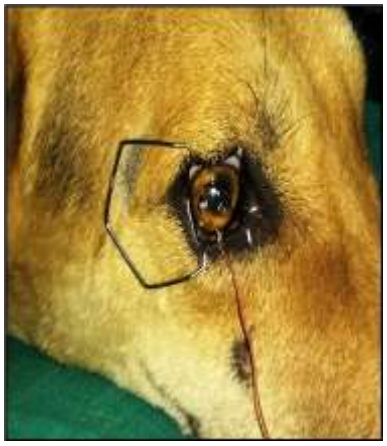

Fig 10: Positioning of active electrode

Conclusion

The results obtained by HMsERG system with an automated protocol were found reliable and reproducible. In order to obtain ideal ERG trace, it was necessary to sedate dogs chemically. The placement of recording electrode and position of the reference electrode must be standardized. ERG enables accurate and selective diagnostics of inherited retinal diseases. It provides both qualitative and quantitative information on retina functioning. Further, if abnormal results are obtained, it is possible to identify the cellular elements involved and characterise the visual deficit, its source, evolution and prognosis. Thus, ERG is useful adjunct test for the diagnosis of retinal dystrophies and for pre-operative evaluation of retinal functions in conjunction with cataract surgery.

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