Electrophysiologic differentiation of homozygous and heterozygous Abyssinian-crossbred cats with late-onset hereditary retinal degeneration

Jennifer A. Hyman, DVM, MA; Vaegan, PhD; Bo Lei, MD, PhD; Kristina L. Narfström, DVM, PhD

Objective—To develop a method to electrophysiologically differentiate heterozygous-carrier Abyssinian-crossbred cats from homozygous-affected Abyssinian-crossbred cats before clinical onset of inherited rod-cone retinal degeneration.

Animals—14 back-crossed Abyssinian-crossbred cats of unknown genotype (homozygous or heterozygous) for inherited rod-cone retinal degeneration, 24 age-matched mixed-breed control cats, 6 age-matched heterozygous Abyssinian-crossbred cats, and 6 homozygous Abyssinian cats.

Procedure—Electroretinography (ERG) of heterozygous and homozygous cats revealed differences, especially for scotopic recordings. Frequent ophthalmoscopy and ERG (2 to 5 times; at intervals of 3 to 6 months) of back-crossed cats were performed. Amplitudes and implicit times were analyzed by use of a graphic representation of results. Ratios for amplitudes of the b-waves to amplitudes of the a-waves (b-wave:a-wave) were compared.

Results—8 back-crossed cats had decreased a-wave amplitudes, increased b-wave implicit times, and abnormal ERG waveforms. Values for the b-wave:a-wave for the highest scotopic light intensity were significantly higher for those same 8 cats.

Conclusions and Clinical Relevance—The 8 back-crossed Abyssinian-crossbred cats with abnormal results developed fundus changes over time consistent with disease. A graphic representation of ERG results can be used to differentiate between genotypes prior to funduscopic changes. Values for the b-wave:a-wave ratio provide confirmation. These ERG analyses may be applied clinically in the diagnosis of retinal degenerations in various species.

Impact for Human Medicine—Cats with hereditary rod-cone degeneration may be a useful model for comparative studies in relation to retinitis pigmentosa in humans. Similar evaluations of ERG results could possibly be used for humans with suspected generalized retinal degeneration. (*Am J Vet Res* 2005;66:1914–1921)

Recessively inherited rod-cone degeneration was first reported in Abyssinian cats in Sweden in 1983.[1,2] This disease is distinct from the autosomal dominant form of rod-cone dysplasia described in a group of cats in the United Kingdom.[3] Clinically affected cats with rod-cone degeneration have a normal-appearing fundus until they are approximately 1.5 to 2 years old, reduced **electroretinography (ERG)** responses after the age of 8 months,[4–6] and morphologic changes in photoreceptors as early as 5 months of age.[7] Heterozygous cats are not clinically affected but have ERG changes indicative of a reduction in the number of photoreceptor cells.[8] The disease in homozygous cats is progressive, whereas no definitive changes have been observed in cats that are heterozygous for the defect.[8,9] On the other hand, cats with dominantly inherited rod-cone dysplasia have an early-onset blindness, often accompanied by nystagmus and a rapid reduction in ERG amplitudes. The ERG is nonrecordable by a few months after birth, and affected cats typically are blind by 4 months after birth.[10] Recessively inherited rod-cone degeneration in cats has similarities to retinitis pigmentosa in humans[11–13] and progressive rod-cone degeneration in dogs.[14]

Clinically, recessively inherited rod-cone degeneration has a late onset; a bilateral, slowly progressive course then ensues, which ends in blindness at 3 to 5 years of age. There are no systemic effects.[11] Electrophysiologically, there is slightly reduced rod function early in the disease process (ie, before the development of signs detected by use of ophthalmoscopy).[6] Only minor electrophysiologic changes during early stages of disease have been observed for the cone system.[15] In young affected cats, dark-adapted b-wave amplitudes are significantly reduced, although retinal sensitivity is only slightly reduced early in the degenerative process.[6]

Reduced b-wave amplitudes during ERG have been described[8] in heterozygous carrier cats, compared with results for clinically normal control cats. The magnitude of the difference between carrier cats and

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From Eye Care for Animals, 11950 W 110th St, Ste A, Overland Park, KS 66210 (Hyman); the Optometry Department, School of Optometry and Vision Science, University of South Wales, Sydney, Australia 2052 (Vaegan); VisionTest, 187 Macquarie St, Sydney, NSW, Australia 2000 (Vaegan); the Department of Veterinary Medicine and Surgery, College of Veterinary Medicine (Lei, Narfström), and the Department of Ophthalmology, Mason Eye Institute, School of Medicine (Lei, Narfström), University of Missouri, Columbia, MO 65211.
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Address correspondence to Dr. Narfström.

some early-affected cats is small, thus causing problems in the identification of the genotype for specific cats. It would be useful to identify young heterozygous cats to facilitate the process of delineating the disease by the use of these cats in breeding programs and also in ongoing molecular genetic studies.

The objective of the study reported here was to develop a clinically useful method to electrophysiologically differentiate heterozygous-carrier from homozygous-affected Abyssinian-crossbred cats before the onset of fundic signs of retinal degeneration. A long-duration ERG protocol was used, which allowed us to study the effects of increases in stimulus intensity on the dark-adapted responses of a- and b-waves until high-intensity responses were obtained; it also enabled us to determine the effects after light adaptation with varying frequencies of light stimuli. The protocol was designed to detect changes in sensitivity and maximum amplitudes of the rod and cone visual systems in our group of unknown (ie, genotype not identified) back-crossed Abyssinian-crossbred cats. The results were then evaluated by use of a method proposed for use in dogs[16,17] to differentiate homozygous-affected animals with hereditary retinal degenerative disease from clinically normal heterozygous animals.

Materials and Methods

Animals—Fourteen back-crossed Abyssinian-crossbred cats of unknown genotype for progressive rod-cone degeneration were included in the study. Cats were 8 to 18 months old. All had a normal-appearing fundus at the start of the study. These cats were obtained through breeding a homozygous-affected Abyssinian cat with an Abyssinian-crossbred cat that had apparently normal vision but was heterozygous for the recessive gene. The latter was obtained through breeding a clinically affected Abyssinian cat and a clinically normal unrelated mixed-breed cat. The back-crossed generation of the cats with the unknown genotype would be 50% homozygous recessive and 50% heterozygous carrier as determined on the basis of Mendelian genetics for an autosomal recessive trait.[18]

Twenty-four clinically normal age-matched mixed-breed control cats were obtained from another study and used in the study reported here. The study also included 6 age-matched Abyssinian-crossbred cats that were heterozygous for the trait and 6 Abyssinian cats that were homozygous for the trait.

Care and use of the cats and the experimental protocol adhered to the Association for Research in Vision and Ophthalmology resolution regarding use of animals in research. Additionally, the study was conducted in accordance with the guidelines of the Animal Care and Use Committee at the University of Missouri, where the research was performed.

Equipment and recording procedures—Routine ophthalmoscopy was performed on the 14 back-crossed Abyssinian-crossbred cats with unknown genotype at 3-month intervals beginning when cats were 12 weeks old. Any retinal appearance consistent with retinal degenerative disease was recorded.

We performed ERG on each cat (2 to 5 times/cat) at intervals of 3 to 6 months. Cats were allowed to adapt to a dark room for 2 hours. Then the pupils of each cat were dilated by topical ophthalmic administration of 1% tropicamide, and anesthesia was induced by IM injections of medetomidine[a] (0.1 mg/kg) and ketamine hydrochloride[b] (5 mg/kg).

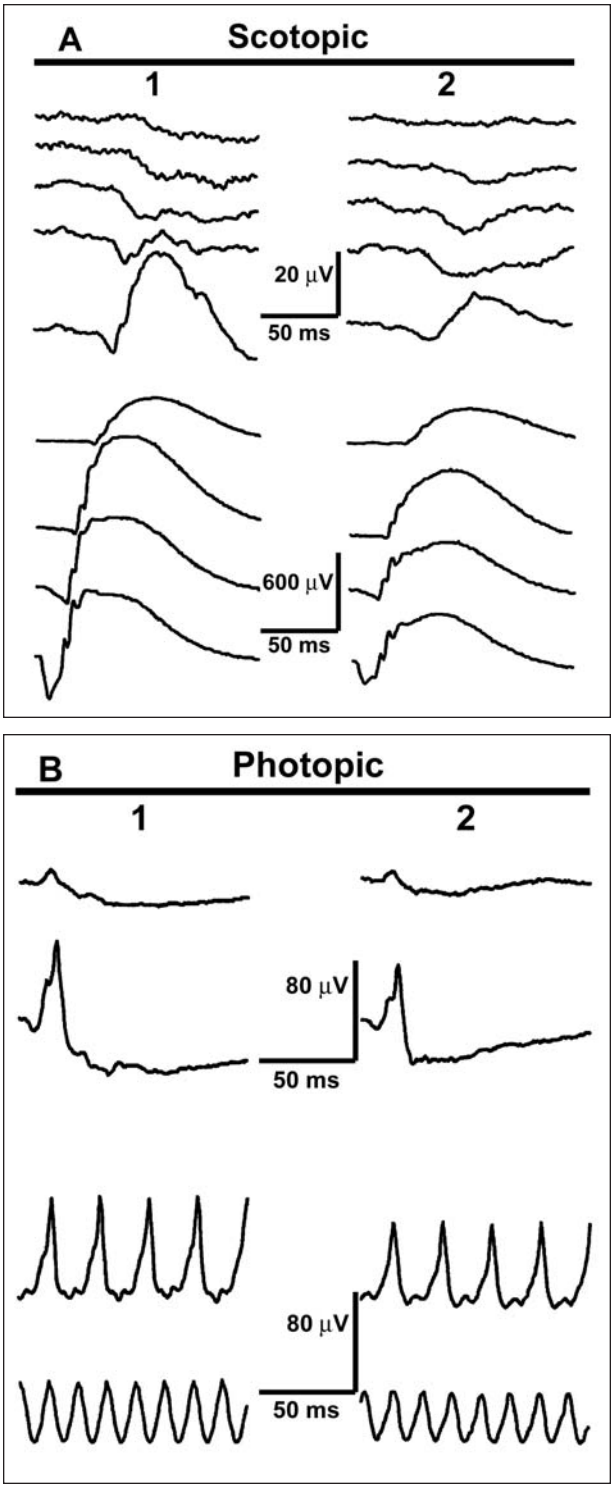

Figure 1—Scotopic (A) and photopic (B) waveforms during electroretinography (ERG) performed on 2 age-matched Abyssinian cats (a heterozygous-carrier cat [1] and a homozygous-affected cat [2]). Brightness of the flash stimulus was increased at intervals of 1.0 from –6.0 to 0.0 (candela × s)/m^2 and intervals of 0.3 log from 0.0 log to 0.6 log (candela × s)/m^2. Notice the difference in amplitude of the a-waves and b-waves, the slight changes in implicit times, and the patterns of the waveforms between the 2 cats. These are seen specifically for the low-amplitude a- and b-waves in the affected cat, which give the ERG recording (especially at maximum stimuli) a more reduced appearance, compared with the ERG of the clinically normal cat at the same light intensity.

Cats were positioned in sternal recumbency throughout the procedures and maintained in a temperature-controlled environment.

Both eyes of each cat were tested concurrently. Jet contact lenses[c] were placed on the corneas by use of a cushion of 2% hydroxypropyl-methylcellulose.[d] Platinum subdermal

Table 1—Median and 95th and 5th percentiles of the confidence limit (CL) for the amplitude of the a- and b-waves recorded during electroretinography (ERG) conducted on both eyes of 6 heterozygous-carrier and 8 homozygous-affected Abyssinian-crossbred cats (2 to 5 ERG sessions/cat at intervals of 3 to 6 months during a 15-month period) by use of various light intensities.*

		a-wave amplitude (μV)				b-wave amplitude (μV)													
Cats		S 1.0	S 2.0	S 4.0	P 1.0	S –6.0	S –5.5	S –5.0	S –4.5	S –4.0	S –3.0	S –2.0	S –1.0	S 1.0	S 2.0	S 4.0	P –1.0	P 1.0	F 30 Hz	F 50 Hz
Heterozygous																				
1	Median	305.60	357.35	371.25	10.35	NR	NR	NR	26.90	93.10	372.40	627.30	613.40	787.05	837.65	858.50	15.90	81.65	80.55	58.95
	95% CL	351.90	411.18	424.08	14.05	NR	NR	NR	32.55	113.20	446.00	692.70	687.50	877.33	937.35	937.35	20.68	86.83	85.93	66.88
	5% CL	298.52	342.60	314.83	9.20	NR	NR	NR	21.58	61.80	340.20	593.73	566.00	729.20	775.45	706.05	11.98	76.15	72.80	53.40
2	Median	322.55	366.70	386.35	13.00	NR	NR	NR	NR	65.70	406.05	708.30	643.50	858.50	876.40	900.30	14.50	92.60	86.65	59.20
	95% CL	348.80	390.80	412.08	14.53	NR	NR	NR	NR	93.09	440.25	748.61	671.73	913.37	948.40	950.01	16.39	97.28	92.93	61.24
	5% CL	280.58	318.97	338.87	11.39	NR	NR	NR	NR	35.34	352.39	652.27	632.95	801.85	801.85	838.87	12.61	87.84	83.18	55.55
3	Median	293.60	342.60	366.60	13.90	NR	NR	NR	12.30	41.00	329.60	673.65	638.85	824.05	861.15	895.55	16.30	90.95	88.95	55.40
	95% CL	312.03	350.51	381.96	13.90	NR	NR	NR	13.92	46.97	349.11	684.51	651.75	843.67	901.85	926.78	44.83	94.40	95.33	60.26
	5% CL	275.43	315.83	329.65	12.71	NR	NR	NR	10.68	39.20	218.89	619.44	584.73	763.55	820.37	817.32	13.11	83.93	79.35	46.81
4	Median	280.10	328.70	324.05	14.55	NR	NR	NR	NR	59.05	314.55	555.55	541.70	703.70	777.80	763.90	26.45	83.80	104.30	79.25
	95% CL	304.21	332.84	364.84	17.04	NR	NR	NR	NR	82.48	359.75	619.80	573.28	764.33	794.45	813.42	37.78	96.30	112.60	96.78
	5% CL	246.22	324.56	299.08	13.00	NR	NR	NR	NR	34.35	263.74	497.26	492.36	631.26	761.15	675.03	15.89	69.35	100.00	60.87
5	Median	305.55	341.05	362.00	15.50	NR	NR	NR	37.95	99.45	399.20	666.70	634.25	819.45	834.20	856.30	17.70	81.90	80.25	55.55
	95% CL	330.53	368.61	391.82	16.61	NR	NR	NR	57.38	117.53	454.15	682.43	665.31	867.63	901.77	916.86	21.76	90.44	83.65	57.76
	5% CL	296.30	333.30	336.09	14.05	NR	NR	NR	28.47	88.09	367.80	650.89	599.29	763.46	780.58	789.79	14.50	74.64	71.84	52.92
6	Median	314.80	360.45	358.45	12.50	NR	NR	NR	17.30	76.45	411.50	685.15	689.80	847.20	918.60	913.25	16.75	81.25	80.60	56.10
	95% CL	330.53	372.10	391.82	14.88	NR	NR	NR	22.29	88.49	413.80	714.63	718.03	884.73	940.15	948.40	23.00	91.80	87.60	57.04
	5% CL	299.08	348.80	344.80	11.31	NR	NR	NR	12.40	65.44	405.29	573.65	669.48	833.30	887.20	878.61	11.18	72.83	78.70	54.57
Homozygous																				
1	Median	185.20	195.60	204.85	10.95	NR	NR	NR	NR	51.75	312.95	593.75	559.05	662.05	690.95	694.45	19.65	73.15	85.10	60.30
	95% CL	203.70	235.15	222.20	13.62	NR	NR	NR	NR	65.21	346.89	699.67	586.13	700.93	761.56	753.74	39.16	75.90	97.36	63.00
	5% CL	154.89	163.87	169.82	9.30	NR	NR	NR	NR	48.92	297.97	523.28	514.21	579.83	588.90	595.81	14.76	60.97	70.89	51.48
2	Median	110.40	111.10	131.25	5.50	NR	NR	NR	14.85	49.30	268.05	465.00	447.40	505.00	535.00	545.50	12.50	46.75	56.15	44.10
	95% CL	115.45	129.54	146.59	8.72	NR	NR	NR	28.57	72.24	346.45	514.67	493.75	573.25	583.30	618.75	15.90	54.93	63.14	56.23
	5% CL	89.47	101.33	105.00	4.68	NR	NR	NR	11.33	43.24	209.43	387.50	363.95	451.32	435.44	453.12	7.14	39.25	46.29	39.45
3	Median	159.75	173.65	183.50	10.05	NR	NR	NR	30.70	84.25	354.00	569.40	545.15	638.90	649.30	678.95	14.55	73.60	89.70	57.90
	95% CL	166.70	186.47	185.20	13.62	NR	NR	NR	33.04	88.62	383.44	610.80	579.15	676.52	679.55	697.78	17.64	75.67	94.89	63.65
	5% CL	152.80	166.70	174.07	7.25	NR	NR	NR	28.36	79.89	332.38	545.86	528.84	619.14	625.00	651.79	13.25	71.53	80.18	54.28
4	Median	142.25	160.30	166.85	7.40	NR	NR	NR	28.65	91.35	359.00	642.35	631.95	720.15	759.00	752.25	18.20	73.85	69.00	38.35
	95% CL	157.00	182.57	187.24	11.06	NR	NR	NR	35.80	104.26	403.43	682.05	667.00	784.45	791.52	798.35	35.10	78.02	74.60	44.95
	5% CL	117.58	137.88	137.88	3.72	NR	NR	NR	14.40	46.07	242.92	562.73	579.29	661.02	692.48	693.42	11.96	48.15	46.70	25.35
5	Median	189.80	185.20	203.70	9.20	NR	NR	NR	30.55	94.25	397.70	676.65	625.00	763.90	787.05	777.80	29.10	77.05	82.70	54.55
	95% CL	194.40	210.68	210.68	12.50	NR	NR	NR	39.58	117.68	439.10	768.53	719.90	796.30	863.45	821.78	38.10	92.05	90.23	56.43
	5% CL	169.00	185.20	178.23	7.48	NR	NR	NR	23.98	19.55	335.18	642.80	576.35	726.83	740.70	708.33	20.11	75.90	72.80	49.78
6	Median	114.60	138.90	142.35	8.35	NR	NR	NR	14.65	41.55	236.10	486.10	452.20	517.35	559.05	569.45	10.65	58.80	74.95	50.20
	95% CL	123.97	138.90	151.75	10.83	NR	NR	NR	18.86	54.34	243.58	517.68	478.15	539.62	568.37	583.30	12.38	60.97	82.73	55.94
	5% CL	111.10	132.95	138.90	7.40	NR	NR	NR	12.74	27.40	214.43	442.71	412.65	477.41	520.16	531.97	10.20	54.26	65.73	43.45
7	Median	145.75	152.70	159.70	9.35	NR	NR	9.50	24.15	74.00	306.30	478.45	480.55	588.80	614.55	586.75	19.65	64.80	73.85	48.60
	95% CL	166.03	180.56	198.25	12.59	NR	NR	10.31	34.03	86.56	352.91	584.74	552.14	656.55	660.40	671.84	26.17	75.77	92.92	55.80
	5% CL	118.05	128.11	131.21	7.40	NR	NR	8.69	15.65	51.50	192.19	360.19	351.20	417.35	438.84	428.79	8.77	36.90	50.51	35.06
8	Median	166.70	185.20	190.90	9.60	NR	NR	NR	38.15	103.50	379.50	576.15	569.65	640.00	659.85	692.50	21.65	60.45	74.35	41.15
	95% CL	166.70	189.33	203.25	15.78	NR	NR	NR	57.25	218.73	558.75	635.35	618.00	708.40	698.43	738.28	26.60	66.33	78.70	47.38
	5% CL	135.50	156.50	155.00	5.50	NR	NR	NR	23.70	66.65	309.25	550.65	536.45	619.83	617.33	664.35	10.70	57.85	68.13	38.65

*Each ERG was recorded from –6.0 log (candelas × s)/m^2 to 0.6 log (candelas × s)/m^2. For each scotopic recording (S), brightness was increased in intervals of 0.5 log from –6.0 log (candelas × s)/m^2 to –4.0 log (candelas × s)/m^2, intervals of 1 log from –4.0 log (candelas × s)/m^2 to 0.0 log (candelas × s)/m^2, and intervals of 0.3 log from 0.0 log (candelas × s)/m^2 to 0.6 log (candelas × s)/m^2. Four or more flashes were provided at a mean frequency of 0.1 Hz, and the interval between each recording was 1 minute. Cats were then allowed to adapt to light (to 30 candelas/m^2) for 10 minutes. For each photopic recording (P), ERG responses were elicited by use of –1.0 log (candelas × s)/m^2 and 0.0 log (candelas × s)/m^2, with the latter performed at 2.1, 30.1, and 50.1 Hz (the latter 2 were flicker recordings [F]). Light intensities were controlled by placing neutral-density gelatin filters over a xenon strobe; this resulted in a decrease in intensity of the strobe flash that had 3 computer-specific intensities (0.0 [candelas × s]/m^2, 0.3 [candelas × s]/m^2, and 0.6 log [candelas × s]/m^2).

NR = Nonrecordable.

needle electrodes[e] were used; a reference electrode was placed at the base of each ear, and 1 ground electrode was placed at the occiput.

A computerized ERG stimulating-and-recording system[f] with bilateral full-field light stimulation was used. Light stimulation was generated by a xenon strobe flash, and light was spread by a diffuser located on the inner surface of a Ganzfeld dome. Neutral-density gelatin filters[g] were placed over the strobe flash to decrease intensity of the stimulating light, which had 3 computer-selectable intensities (0.0 log [candelas × s]/m^2, 0.3 log [candelas × s]/m^2, and 0.6 log [candelas × s]/m^2). Light intensity was measured by a light meter[h] positioned at the same height as the tested eyes in the Ganzfeld dome. Electrodes were connected to a preamplifier, and signals were processed through a bandpass filter (0.1 to 500 Hz) before being recorded by use of a specially designated computer.

Each ERG session was recorded from –6.0 log (candelas × s)/m^2 to 0.6 log (candelas × s)/m^2 by use of a selected sequence. Brightness was increased in intervals of 0.5 log

Table 2—Median and 95th and 5th percentiles of the CL for implicit time of the a- and b-waves recorded during ERG conducted on both eyes of 6 heterozygous-carrier and 8 homozygous-affected Abyssinian-crossbred cats (2 to 5 ERG sessions/cat at intervals of 3 to 6 months during a 15-month period) by use of various light intensities.*

		a-wave implicit time (ms)				b-wave implicit time (ms)													
Cats		S 1.0	S 2.0	S 4.0	P 1.0	S –6.0	S –5.5	S –5.0	S –4.5	S –4.0	S –3.0	S –2.0	S –1.0	S 1.0	S 2.0	S 4.0	P –1.0	P 1.0	F 30 Hz	F 50 Hz
Heterozygous																			
1	Median	12.00	10.00	9.40	10.00	NR	NR	NR	76.50	78.50	73.50	65.00	62.25	61.00	61.90	62.50	21.65	25.00	32.10	19.00
	95% CL	12.30	10.50	9.40	10.75	NR	NR	NR	86.63	82.88	79.73	68.53	65.20	65.23	62.83	64.45	22.35	25.45	32.30	19.35
	5% CL	11.50	9.63	9.08	9.55	NR	NR	NR	75.00	74.00	70.50	61.48	59.45	58.50	58.25	56.50	21.00	24.63	32.00	18.93
2	Median	11.40	10.10	9.50	10.50	NR	NR	NR	NR	76.90	74.25	63.00	58.75	60.15	55.80	57.50	20.75	24.75	32.25	19.00
	95% CL	11.93	10.46	9.93	10.93	NR	NR	NR	NR	79.88	75.85	63.93	59.93	62.47	57.18	58.85	20.98	25.43	32.50	19.26
	5% CL	10.88	9.49	9.42	10.50	NR	NR	NR	NR	73.07	72.23	60.38	56.73	57.58	54.17	55.73	20.53	24.50	32.00	18.83
3	Median	11.00	9.50	9.25	9.75	NR	NR	NR	78	69.25	68.90	56.75	50.10	51.50	53.50	52.75	20.50	24.50	32.25	19.00
	95% CL	11.00	9.93	9.39	10.43	NR	NR	NR	78	71.20	71.63	59.03	58.46	53.00	56.20	53.26	20.93	24.93	32.50	19.00
	5% CL	10.58	9.50	9.03	9.42	NR	NR	NR	78	66.45	66.35	54.30	41.15	47.03	50.38	49.53	20.50	24.08	32.00	19.00
4	Median	11.00	10.00	9.40	9.35	NR	NR	NR	NR	77.00	73.50	68.00	49.25	59.65	63.75	57.35	21.50	26.50	28.75	21.75
	95% CL	11.00	10.45	11.63	9.91	NR	NR	NR	NR	83.83	89.48	75.93	62.43	64.70	64.88	67.53	21.84	28.00	32.50	24.50
	5% CL	11.00	9.55	9.05	9.26	NR	NR	NR	NR	72.73	66.88	58.38	36.08	57.58	62.63	54.23	20.91	24.58	24.58	19.00
5	Median	11.50	9.50	9.15	10.50	NR	NR	NR	85.25	80.75	77.25	60.25	52.25	57.75	56.50	54.50	21.75	25.50	32.25	19.00
	95% CL	12.00	9.50	9.39	11.18	NR	NR	NR	87.20	82.00	78.50	65.40	66.78	63.85	63.05	61.83	22.00	26.43	32.50	19.00
	5% CL	11.00	9.33	8.83	10.08	NR	NR	NR	80.75	76.53	75.58	57.65	39.00	50.38	45.11	50.15	20.65	25.00	32.00	19.00
6	Median	11.00	9.75	9.50	9.75	NR	NR	NR	72.50	73.75	68.25	57.75	45.75	49.00	47.00	45.50	21.65	25.75	32.40	19.00
	95% CL	12.11	10.43	9.93	10.00	NR	NR	NR	73.78	75.78	70.93	59.28	59.40	54.44	52.48	52.90	21.97	26.43	32.93	19.00
	5% CL	11.00	9.50	9.08	9.50	NR	NR	NR	70.38	70.03	63.45	56.23	35.08	44.50	43.91	38.53	21.08	25.50	32.05	19.00
Homozygous																				
1	Median	11.15	10.25	9.38	10.65	NR	NR	NR	NR	81.75	76.25	67.00	62.25	59.50	63.00	59.25	22.25	27.25	32.25	19.00
	95% CL	12.15	10.93	9.50	11.00	NR	NR	NR	NR	87.18	80.20	69.43	65.30	62.13	68.31	60.43	22.93	28.26	32.50	19.00
	5% CL	10.15	10.00	8.61	10.30	NR	NR	NR	NR	79.73	70.60	62.45	61.08	56.45	59.23	57.65	21.15	26.50	32.00	19.00
2	Median	11.75	9.45	9.00	10.40	NR	NR	NR	84.75	87.50	75.50	65.50	61.70	56.65	56.15	56.75	22.20	27.00	31.85	18.80
	95% CL	12.00	10.55	9.46	11.76	NR	NR	NR	87.28	112.40	80.65	70.75	67.10	61.28	58.42	59.82	23.83	28.20	32.33	19.06
	5% CL	10.39	8.70	8.59	9.03	NR	NR	NR	80.53	81.72	71.75	59.18	53.48	52.28	50.50	53.45	20.90	24.90	31.70	18.70
3	Median	12.45	11.20	9.50	12.00	NR	NR	NR	99.65	93.00	83.35	73.00	64.25	65.50	62.00	65.40	24.00	26.25	32.35	19.00
	95% CL	14.88	12.85	11.63	13.70	NR	NR	NR	102.04	758.18	90.86	78.25	67.80	69.13	65.21	69.80	24.43	26.93	32.50	19.00
	5% CL	12.06	9.89	9.16	11.15	NR	NR	NR	97.27	86.15	78.65	72.00	26.88	59.75	60.58	64.58	22.47	25.58	32.03	18.92
4	Median	12.00	10.00	9.35	10.55	NR	NR	NR	93.05	85.25	82.00	78.00	72.00	67.50	66.75	65.00	23.75	28.60	32.00	19.00
	95% CL	12.83	11.50	10.55	12.50	NR	NR	NR	99.38	88.83	84.78	79.44	73.55	70.33	68.13	69.15	26.55	38.25	32.41	19.11
	5% CL	11.00	9.40	9.00	9.53	NR	NR	NR	84.64	83.60	76.63	73.45	69.42	66.18	65.89	61.95	22.00	27.50	32.00	18.90
5	Median	11.00	10.00	9.28	10.65	NR	NR	NR	88.00	88.25	78.00	68.75	64.75	61.25	61.90	62.50	22.25	26.25	32.50	19.05
	95% CL	11.73	10.38	9.50	11.44	NR	NR	NR	93.45	98.13	83.05	73.13	70.75	69.03	63.70	65.00	22.93	27.00	32.50	19.28
	5% CL	10.63	9.50	9.00	10.00	NR	NR	NR	84.13	83.13	72.00	67.25	62.63	57.50	58.25	61.00	22.00	26.00	32.13	19.00
6	Median	11.25	9.70	9.20	10.00	NR	NR	NR	96.00	86.25	77.50	68.35	64.25	58.75	60.00	60.75	16.25	26.75	32.15	19.00
	95% CL	11.93	10.00	9.40	11.28	NR	NR	NR	102.75	87.38	77.95	73.17	65.38	60.33	62.25	65.03	21.43	27.43	32.29	19.00
	5% CL	10.58	9.40	9.00	9.49	NR	NR	NR	89.25	85.13	77.05	63.54	63.13	57.18	57.75	56.48	11.08	26.08	32.02	19.00
7	Median	11.95	10.60	9.45	10.40	NR	NR	99.70	91.15	85.40	83.70	71.00	66.75	65.65	64.05	62.80	23.20	26.00	31.85	19.00
	95% CL	12.50	11.32	9.87	11.00	NR	NR	101.77	94.10	96.12	89.23	75.12	72.58	70.86	65.90	72.29	23.96	28.65	34.81	19.20
	5% CL	10.60	9.23	8.73	8.86	NR	NR	97.63	87.73	82.06	78.99	68.58	63.64	57.47	56.98	57.42	21.35	22.12	31.70	18.70
8	Median	11.00	9.65	9.00	10.50	NR	NR	NR	100.70	79.25	76.50	69.00	69.10	70.05	65.00	65.00	23.05	27.00	31.70	19.00
	95% CL	11.38	10.00	11.50	10.95	NR	NR	NR	105.75	102.00	83.33	85.83	77.50	73.30	68.48	67.23	23.85	28.10	32.00	19.20
	5% CL	10.63	9.50	8.50	10.33	NR	NR	NR	90.23	44.60	74.00	67.43	63.25	58.50	59.50	57.50	22.60	17.70	31.33	18.70

*See Table 1 for key.

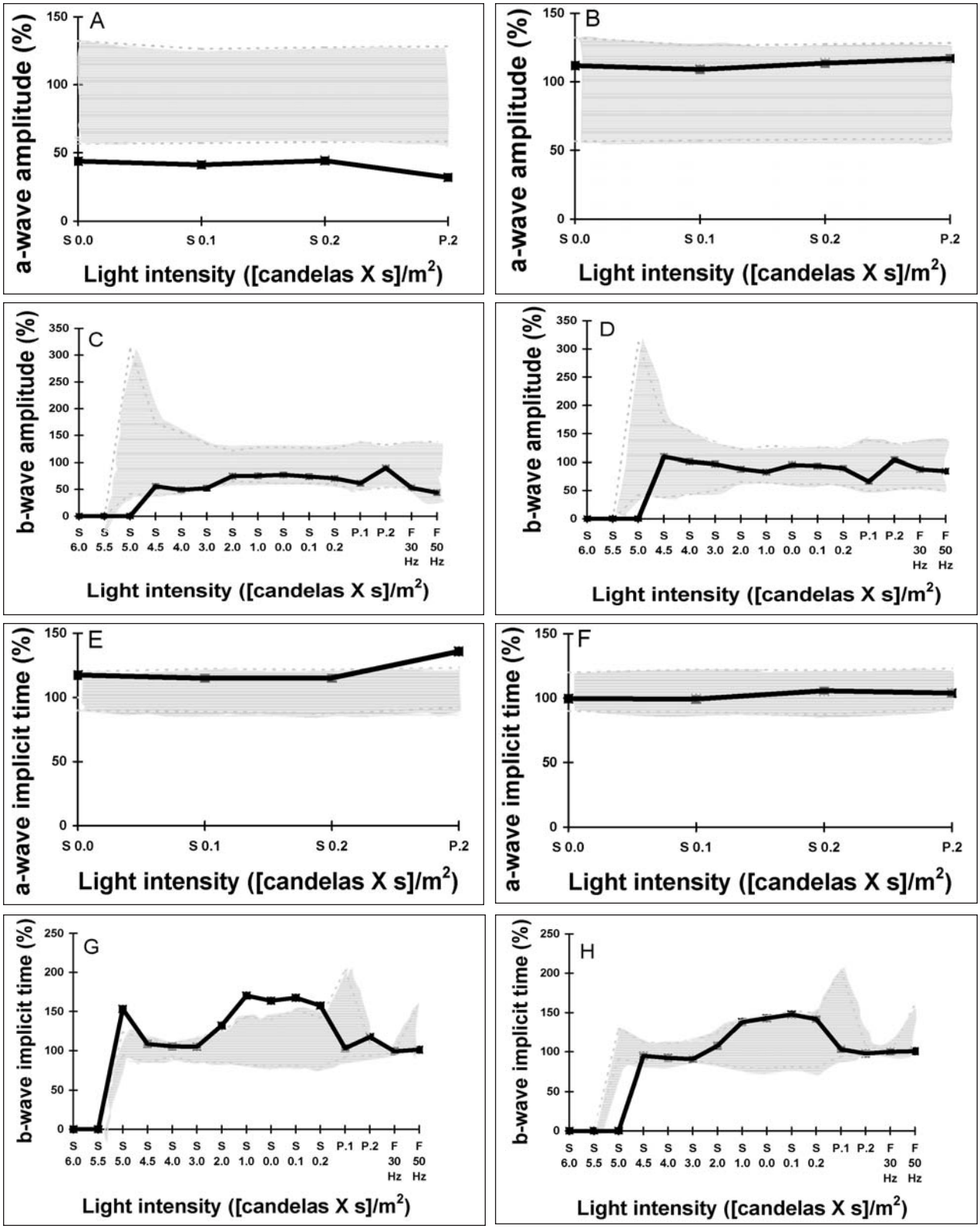

Figure 2—A series of graphs depicting ERG differences between a representative homozygous-affected cat (A, C, E, and G) and a representative clinically normal cat heterozygous for hereditary rod-cone degeneration (B, D, F, and H) for a-wave amplitude (A and B), b-wave amplitude (C and D), a-wave implicit times (E and F), and b-wave implicit times (G and H). Results represent multiple values of a single ERG session for each cat. Values on the y-axis are the percentage of the values obtained for the normal cats. The median for the heterozygous cats was designated as 100% in each graph, and the area between the 5th and 95th percentiles is indicated (gray shading). Notice that scales for the y-axis differ among panels. Values on the x-axis represent scotopic (S), photopic (P), and flicker (F) light intensities. Notice the marked decrease in a-wave amplitude (panel A) and the prolonged b-wave implicit time (panel G) in the homozygous-affected cat, compared with values for the heterozygous cat (panels B and H, respectively).

from –6.0 log (candelas × s)/m^2 to –4.0 log (candelas × s)/m^2, intervals of 1 log from –4.0 log (candelas × s)/m^2 to 0.0 log (candelas × s)/m^2, and intervals of 0.3 log from 0.0 log (candelas × s)/m^2 to 0.6 log (candelas × s)/m^2. Four or more flashes were provided at a mean frequency of 0.1 Hz, and the interval between each recording was 1 minute. Cats were then allowed to adapt to light (30 candelas/m^2) for 10 minutes; photopic ERG was then elicited by use of –1.0 log (candelas × s)/m^2 and 0.0 log (candelas × s)/m^2, with the latter intensity performed at frequencies of 2.1, 30.1, and 50.1 Hz. The full-field ERG procedure used in the study induced several responses at scotopic conditions, including rod responses by use of low-light stimuli and combined rod-and-cone responses by use of high-light stimuli. At photopic conditions, single flash responses from cones and flicker responses at 2 high frequencies were recorded.[17]

Evaluation of ERG data—A graphic representation of results was used, similar to methods described for ERG evaluation in dogs.[17] Amplitudes and implicit times were determined for each stimulation intensity.[16,17] Amplitude of the a-wave was measured from baseline to the peak of the first negative deflection, whereas amplitude of the b-wave was measured from peak of the a-wave to the first positive peak of the ERG. Implicit times of the a- and b-waves were measured from the onset of light stimulus to the peak of the a- and b-waves, respectively. Amplitudes and implicit times for the b- and a-waves (16 response curves/eye) were calculated and fitted into graphs that used the median of the results from the clinically normal control cats and the 5th and 95th percentiles of the control cats as limits of normalcy.[16] In addition, values for the **ratio of the amplitude of the maximum scotopic b-wave to the amplitude of the a-wave (b-wave:a-wave)** were also calculated and compared with those of control cats.

Statistical analysis—The Student *t* test was performed to assess differences between b-wave:a-wave of back-crossed cats considered heterozygous-carrier cats and those considered homozygous-affected cats. Differences were considered significant at values of $P < 0.05$.

Results

Analysis of results of preliminary ERG in homozygous-affected Abyssinians and heterozygous-carrier Abyssinian-crossbred cats revealed noticeable differences in ERG waveforms. There also appeared to be differences in amplitudes and implicit times for a- and b-waves. The differences between recordings for heterozygous Abyssinian-crossbred and homozygous Abyssinian cats were more prominent in rod-dominated ERG recordings **(Figure 1)**. Median and 5th and 95th percentiles of amplitude and implicit times of all back-crossed Abyssinian-crossbred cats included in the study were determined **(Tables 1 and 2)**. These results were plotted into graphs for each tested cat **(Figure 2)**.

Eight of 14 back-crossed Abyssinian-crossbred cats had low amplitudes for the a-wave at each ERG session, which is close to or less than the lower limits of the reference range for the control cats in the same age group. The remaining back-crossed cats had a-wave amplitudes that were near the median and within the limits of the reference range.

All 14 back-crossed Abyssinian-crossbred cats had b-wave amplitudes that were within limits of the reference range. A few outlying values were identified, especially at extremely low light intensities (Table 2). However, these were not considered valid data points because of the background noise at that specific recording.

It was also found that the same 8 back-crossed Abyssinian-crossbred cats with decreased a-wave amplitudes had increased implicit times for the b-wave; these values were greater than the upper limits of the reference range for the control cats in the same age group. The remaining back-crossed cats had implicit times for b-waves that were near the median and within the limits of the reference range. All implicit times for a-waves were within the limits of the reference range for the entire group of back-crossed Abyssinian-crossbred cats.

Values for the b-wave:a-wave were calculated for the highest scotopic light intensity used. Mean values for multiple ERG recordings on the same cat were calculated. Analysis of the b-wave:a-wave revealed values that were clearly in 2 groups (higher b-wave:a-wave values for 8 back-crossed Abyssinian-crossbred cats, which had other abnormal results as well, were in 1 group, and the b-wave:a-wave values for the remaining 6 back-crossed cats were in the other group). Use of the Student *t* test revealed a significant ($P = 0.01$) difference between the mean and median values of the 2 groups. Ratios for the 6 heterozygous cats (mean of both eyes) ranged from 2.22 to 2.53 (mean ± SD: 2.35 ± 0.12; median, 2.32), whereas the ratios for the 8 homozygous cats ranged from 3.07 to 3.58 (mean ± SD: 3.37 ± 0.22; median, 3.44).

Discussion

A graphic representation of results by use of median values and limits of reference ranges of age-matched mixed-breed control cats facilitated the clinical interpretation of ERG results. This graphic representation was used to differentiate between heterozygous-carrier and homozygous-affected Abyssinian-crossbred cats before the onset of fundic signs of progressive rod-cone degeneration.

Eight of 14 back-crossed Abyssinian-crossbred cats had decreased a-wave amplitudes, increased implicit times for b-waves, abnormal ERG curve forms, and increased b-wave:a-wave values, compared with results for the other 6 back-crossed cats and the age-matched mixed-breed control cats. Of the 14 Abyssinian-crossbred cats, those 8 were classified as homozygous-affected cats. These results matched the expected ratio of 50% carrier:50% affected as a result of the selected matings. Furthermore, the ERG identification of cats believed to be affected was confirmed over time because they developed clinical fundic signs characteristic of progressive rod-cone degeneration. The other 6 back-crossed Abyssinian-crossbred cats remained funduscopically normal.

In a previous study,[8] investigators detected significant differences in b-wave maximum amplitudes between clinically normal, affected, and carrier cats for hereditary retinal degeneration. However, the differences in amplitude were not sufficiently consistent for use as a single criterion for differentiation of homozygous and heterozygous cats. In another study,[19] evaluation of multiple factors, such as variables for a- and b-waves, b-wave:a-wave, age at examination, and

funduscopic findings, resulted in an array of variables that could be used to definitively differentiate affected from carrier cats. In that study, heterozygous-carrier cats were effectively differentiated from homozygous-affected cats before onset of clinical changes by use of a mathematical model (ie, principal component factor analysis).

Analysis of the study reported here revealed that a graphic representation of results for the amplitudes and implicit times of a- and b-waves, which were described for use in dogs with retinal degeneration,[17] was also an effective method for differentiating clinically normal but heterozygous cats from affected-homozygous Abyssinian-crossbred cats. In addition, by use of the b-wave:a-wave, even more confidence in the results was obtained. These methods for ERG evaluation are simple to apply in clinical situations when groups of clinically normal animals of the same age and breed can be compared.

Homozygous-affected back-crossed Abyssinian-crossbred cats consistently had decreased a-wave amplitudes, even as young adults. Because the progressive rod-cone degeneration of Abyssinian cats is an outer retinal disease that affects primarily photoreceptors,[4–7,11,15] this finding was not surprising. Marked changes in a-wave variables have also been observed in this strain of cats during in vivo intraretinal recordings performed by use of bright-flash ERG.[i]

Decreased b-wave amplitudes were not found in the study reported here until later in the disease process, although there were early changes observed in the ERG curve form. Several morphologic events are likely to have been responsible for these late changes in b-wave amplitude. It is only after changes are observed in photoreceptor cells that horizontal and bipolar cells undergo morphologic alterations.[4] Furthermore, the photoreceptor-bipolar cell synapses degenerate as photoreceptor cells die, which results in decreased summation of stimuli for initiation of b-waves.[20] Also, there is substantial remodeling of the inner retina in conjunction with generalized degenerative processes of the photoreceptors.[21] This may alter ERG recordings, especially waveform and timing variables, of young affected animals.

Between 2 and 5 ERG sessions were performed on each of the cats within a period of 6 to 15 months, with minor changes in values for the ERG variables among recording sessions. This documents that the degenerative process is extremely slow, which is consistent with findings in another study,[6] and that the recordings obtained were reproducible for each cat.

In homozygous-affected Abyssinian cats, some rods undergo incomplete maturation whereas others develop normally and then slowly degenerate.[7] Morphologic analysis of heterozygous cats needs to be performed to determine whether there are any morphologic alterations consistent with the reduction of maximum scotopic amplitudes observed at an early stage in carrier cats. Mice heterozygous for the rds mutation have been characterized morphologically as having abnormal development of photoreceptors followed by an extremely slow loss of photoreceptors, similar to the degeneration in homozygotes but on a smaller scale.[22] In Labrador Retrievers with inherited retinal degeneration, reduced sensitivity of the DC-ERG in dogs heterozygous for the defect has been documented.[23] There may be many possible mechanisms for decreased photoreceptor function, such as decreased ability to generate electrochemical changes necessary for production of the a-wave or abnormal inhibition of stimuli.

The study reported here was conducted to find a simple and reliable way to clearly differentiate heterozygous, clinically normal cats from those that were homozygous-affected cats and to find a method that could be used in a clinical setting. We documented that the graphic representation of results in combination with evaluation of the b-wave:a-wave were useful and reliable methods that could be applied to any retinal degeneration characterized electroretinographically in which several ERG variables are analyzed. Additional variables could be included, and any species could be studied. The limits of the reference range for the equipment, species, and group of animals would need to be defined before evaluating suspect animals. Once the variables have been established, both hereditary and nonhereditary types of retinal degeneration could be assessed by use of the graphic representation of results and analysis of values for the b-wave:a-wave ratio.

a. Domitor, Vetpharma, Lund, Sweden.
b. Ketalar, Park-Davies, Morris Plains, NJ.
c. Jet lens, Universo Plastique, Grenchen, Switzerland.
d. Methocel, Novartis, Basel, Switzerland.
e. Platinum subdermal needle electrode, Grass Instrumental Division, Astro-Med Inc, West Warwick, RI.
f. Tor, Global Eye, Reymyre, Sweden.
g. Kodak Wratten Nos. 98 and 96, Eastman Kodak Co, Rochester, NY.
h. IL 1700, International Light, Newburyport, Mass.
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