

## Brainstem Auditory-Evoked Potential Assessment of Auditory Function and Congenital Deafness in Llamas (*Lama glama*) and Alpacas (*L pacos*)

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Auditory function of llamas and alpacas was assessed objectively by means of brainstem auditory-evoked response audiometry (BAER) to establish the normal hearing range and to test the hypothesis of a correlation between blue eyes, white coat, and deafness. Sixty-three camelids were available for the study. Thirteen animals had blue irides; 1 animal had 1 blue and 1 pigmented iris. Wave latencies, amplitudes, and interpeak latencies were measured under general anesthetic. Click stimuli (dB [HL]) were delivered by an insert earphone. Four to five positive peaks could be detected; waves I, II, and V were reproducible; wave II appeared infrequently; and wave IV generally merged with wave V to form a complex. Peak latencies decreased and peak amplitudes increased as stimulus intensity increased. A hearing threshold level of 10–20 dB (HL) was proposed as the normal range in llamas and alpacas. None of the animals with pigmentation of coat and iris showed any degree of hearing impairment. Seven of the 10 blue-eyed, pure-white animals were bilaterally deaf and one of them was unilaterally deaf. However, 2 blue-eyed, white animals exhibited normal hearing ability. Three blue-eyed animals with pigmented coat did not show any hearing impairment. All white animals with normal iris pigmentation had normal auditory function; so did the 1 animal with 1 normal and 1 blue iris. The high frequency (78%) of bilaterally deaf animals with pure white coat and blue iris pigmentation supports the hypothesis of a correlation between pigmentation anomalies and congenital deafness in llamas and alpacas.

**Key words:** Hearing level; Pigmentation; South American camelids.

Congenital deafness has long been known to occur in cats with white coat color and blue eyes.<sup>1</sup> A blue iris results from an absence of pigment in the iris (heterochromia iridis). Reduction or loss of the tapetal pigment may also be present. Hair coat color associations with deafness have also been reported in several other species, including mice, dogs, mink, and humans.<sup>2–4</sup> Similar associations have been reported in South American camelids,<sup>5</sup> but no objective auditory assessments have been made. The domesticated South American camelid species, the llama (*Lama glama*) and the alpaca (*L pacos*), are increasingly being bred internationally for fiber and meat and as beasts of burden.<sup>6</sup> The identification of congenital defects that may be inherited is essential to maintain a sound genetic base from which to breed future generations.

Peripheral deafness or hearing loss can be characterized by three pairs of descriptors: inherited or acquired, congenital or later onset, and sensorineural or conductive.<sup>7,8</sup> Three classifications are commonly seen in humans and animals: inherited congenital sensorineural, acquired later-onset sensorineural, and acquired later-onset conductive. The deafness observed in white-colored South American camelids with blue irides is probably caused by an inherited congenital sensorineural form.<sup>5</sup>

The histopathologic pattern that occurs in most dog breeds and white cats is known as cochleo-saccular type of end-organ degeneration. The deafness results from initial

degeneration of the stria vascularis, probably caused by the absence of melanocytes in the stria tissue.<sup>9,10</sup> Degeneration of the stria is followed by collapse of Reissner's membrane and the cochlear duct, degeneration of hair cells of the organ of Corti, and collapse of the saccule.<sup>11</sup> Secondary loss of spiral ganglion cells is also seen at later stages.<sup>12</sup> The function of melanocytes in normal stria is not known,<sup>9,13</sup> but appears to tie in with hair pigment associations with deafness in humans,<sup>10</sup> cats,<sup>12</sup> mink, and mice.<sup>14,15</sup>

Objective assessment of auditory function may be measured with the brainstem auditory-evoked response test (BAER), also referred to as the brainstem auditory-evoked potential test (BAEP) or the auditory brainstem response test.<sup>11,16,17</sup> A computer-based system detects electrical activity in the cochlea and auditory pathways in the brain through small subdermal electrodes in these tests.<sup>16</sup> The response waveform consists of a series of peaks identified by Roman numerals: peak I is produced by the cochlea and cochlear nerve and later peaks are produced within the brain.

The aim of the current study was to establish the normal range of hearing levels in llamas and alpacas and to estimate auditory function in relation to coat color and iris pigmentation using the BAEP.

### Materials and Methods

#### Animals

Auditory potentials were collected from llamas on a farm in southern Germany and alpacas on a farm in eastern Australia. Thirty-two females (12 llamas, 20 alpacas) and 31 males (13 llamas, 18 alpacas) were tested. Forty-seven animals (23 llamas, 24 alpacas) were aged between 3 and 6 years, 13 (2 llamas, 11 alpacas) between 7 and 12 years, and 3 alpacas were 13 years of age. Animals were kept and raised under similar conditions within each farm. The total number of animals on each farm was 100 llamas and 800 alpacas, respectively. Deafness (behavior) was not known to occur in the selected animals.

Twenty llamas and 16 alpacas had pigmented coats and irides, whereas 5 llamas and 22 alpacas had white coats, of which 1 llama and 13 alpacas also had blue irides. Three of these animals had some

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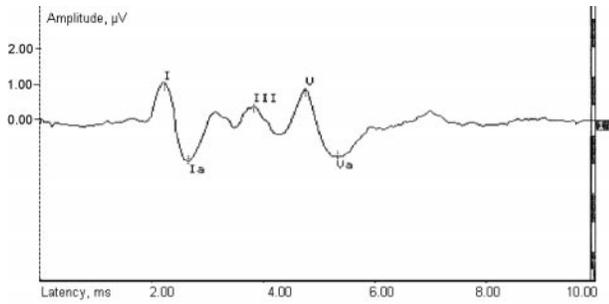
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Submitted June 24, 2004; Revised October 22 and December 17, 2004; Accepted March 30, 2005.

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0891-6640/05/1905-0017/\$3.00/0



**Fig 1.** Typical brainstem auditory-evoked responses of South American camelids (*Lama glama*) at 90 dB hearing level.

dark color spots in their white coats. One alpaca with a white coat had 1 colored and 1 white iris.

The animals were anesthetized before the testing procedure with 0.4 mg/kg xylazine and 4.0 mg/kg ketamine mixed in the same syringe and administered intravenously.<sup>18</sup>

**Brainstem Auditory-Evoked Potential Test Protocol**

Brainstem auditory-evoked potential measurements were performed by means of portable equipment (EPOSYS 4 SEMANTIK GmbH, Germering, Germany). Subdermal recording needle electrodes were placed at the estimated point of the midline of the middle of the cerebral hemispheres and just rostral to the base of each ear, with a ground electrode placed just caudal to the intraorbital line.

Each ear was individually exposed to alternating rarefaction clicks by means of insert earphones at an intensity of 90 dB HL (hearing level). Intensity was decreased in a stepwise manner (90, 60, 40, 30, 20, 10) to a minimum of 10 dB HL. Averages of at least 1,000 responses of 10-milliseconds duration were collected once for each ear. Data were collected with a computer-based electrodiagnostic signal averaging system.

Latencies and amplitudes of waves I, III, and V and interpeak latencies of waves I-III, I-V, and III-V were measured. Latencies include the time of each click-stimulus pulse through the earphone of approximately 1 millisecond.

Hearing level was defined as that intensity (dB) where waves I-V could no longer be generated.<sup>19,20</sup> Animals were considered deaf when BAEP recording produced a flat line or no recognizable waveform at an intensity of 90 dB HL.<sup>4,8,11</sup>

**Statistical Analyses**

The Statistical Package for Social Sciences (SPSS) Version 9.0 and Statistical Analysis System (SAS) GLM procedure were used to analyze the hearing level and associations between hearing level and condition of individual phenotypic markers based on likelihood chi-square statistics.

The following statistical model was used to analyze the latency measurements and hearing level:

$$y_{ijkl} = \mu + sp_i + s_j + a_k + e_{ijkl}$$

where  $y_{ijkl}$  = latency,  $\mu$  = overall mean,  $sp_i$  = fixed effect of species ( $i = 1, 2$ ),  $s_j$  = fixed effect of sex ( $j = 1, 2$ ),  $a_k$  = fixed effect of birth age ( $k = 1-13$ ),  $e_{ijkl}$  = residual error.

**Results**

**Brainstem Auditory-Evoked Potential Characteristics**

Brainstem auditory-evoked potential characteristics were described at an intensity of 90 dB HL. Each camelid showed a highly repeatable series of 4-5 peaks (Fig 1).

**Table 1.** Means ( $\pm$ SD) of latencies (msec) of waves I, III and V and amplitudes ( $\mu$ V) of waves I and V at different intensities (dB hearing level [HL]) in llamas and alpacas with pigmented coats and irides.

Wave	dB (HL)	Species	Latency (msec)	Amplitude ( $\mu$ V)
			Mean $\pm$ SD	Mean $\pm$ SD
I	90	Llama	2.13 $\pm$ 0.07	1.09 $\pm$ 0.49
		Alpaca	2.06 $\pm$ 0.06	1.14 $\pm$ 0.58
III	90	Llama	3.89 $\pm$ 0.12	—
		Alpaca	3.76 $\pm$ 0.23	—
V	90	Llama	4.80 $\pm$ 0.16	0.83 $\pm$ 0.36
		Alpaca	4.62 $\pm$ 0.15	1.02 $\pm$ 0.64
I	60	Llama	2.36 $\pm$ 0.09	0.23 $\pm$ 0.20
		Alpaca	2.34 $\pm$ 0.10	0.36 $\pm$ 0.16
III	60	Llama	4.21 $\pm$ 0.33	—
		Alpaca	4.10 $\pm$ 0.21	—
V	60	Llama	5.25 $\pm$ 0.33	0.42 $\pm$ 0.18
		Alpaca	4.96 $\pm$ 0.24	0.63 $\pm$ 0.35
I	40	Llama	2.98 $\pm$ 0.42	0.08 $\pm$ 0.03
		Alpaca	3.25 $\pm$ 0.23	0.30 $\pm$ 0.01
III	40	Llama	4.28 $\pm$ 0.31	—
		Alpaca	4.49 $\pm$ 0.49	—
V	40	Llama	5.64 $\pm$ 0.23	0.45 $\pm$ 0.07
		Alpaca	5.47 $\pm$ 0.18	0.33 $\pm$ 0.07
I	30	Llama	2.99 $\pm$ 0.56	0.10 $\pm$ 0.10
		Alpaca	2.85 $\pm$ 0.08	0.03 $\pm$ 0.05
III	30	Llama	4.34 $\pm$ 0.45	—
		Alpaca	4.38 $\pm$ 0.41	—
V	30	Llama	5.77 $\pm$ 0.37	0.33 $\pm$ 0.08
		Alpaca	5.64 $\pm$ 0.30	0.32 $\pm$ 0.17
I	20	Llama	2.16 $\pm$ 0.18	—
		Alpaca	2.15 $\pm$ 0.20	0.10 $\pm$ 0.01
III	20	Llama	4.50 $\pm$ 0.22	—
		Alpaca	4.45 $\pm$ 0.21	—
V	20	Llama	6.07 $\pm$ 0.12	0.18 $\pm$ 0.05
		Alpaca	5.88 $\pm$ 0.09	0.25 $\pm$ 0.13

The distances between the peaks were about 1 millisecond. Peaks I, III, and V were repeatable. Peak II was not always present and was therefore not included in the statistical analyses of latencies and amplitudes. Peak IV was present infrequently. Most of the time, peak IV merged into wave V to build I complex.

Peak latencies of the potential components reflected the time from application of the auditory click stimulus until it was transformed into a measurable action potential. A reduction of the intensity led to increased latencies and declining amplitudes until they were completely flat at low intensities (Table 1). At 10 dB (HL), no more potential could be generated from any of the animals.

Amplitude responses of different potential components varied extremely among animals. They are of limited importance for clinical diagnosis.<sup>17</sup> Amplitudes of waves I and V were constant over all intensities and were included in the analyses. Wave V was of principal importance for the definition of a hearing level (Table 1).

There were differences between species in latencies at different auditory stimulus intensities (Table 2). Coat pigmentation and sex had no significant effects ( $P > .05$ ) on latencies of waves I, III, and V. However, latencies at the following auditory stimulus intensities were significantly longer in older animals ( $>6$  years; average 10.9 years)

**Table 2.** Effect of species on latencies of waves I, III, and V in llamas and alpacas with pigmented coats and irides.

Wave	dB (Hearing Level)			
	30	40	60	90
I	$P < .05^a$	ns	ns <sup>b</sup>	$P < .001^a$
III	ns	$P < .05^c$	ns	$P < .05^a$
V	ns	$P < .05^a$	$P < .01^a$	$P < .05^a$

<sup>a</sup> Llama > alpaca.

<sup>b</sup> Not significant:  $P > .05$ .

<sup>c</sup> Alpaca > llama (see Table 1).

when compared with younger ( $\leq 6$  years; average 4.4 years): at 90 ( $P < .01$ ), 60, and 30 dB (HL) of wave I ( $P < .05$ ) and at 40 dB(HL) of wave V.

Interpeak latencies of waves I–III became shorter with decreasing intensities. The differences at 40 and 30 dB (HL) were probably due to low numbers tested. Interpeak latencies of waves I–V were relatively constant. Interpeak latencies of wave II–V increased with increasing stimuli intensities and vice versa (Table 3).

### Hearing Level

Hearing level was reached in 65% (32/49) of the animals at 10 dB (HL) and in 35% (17/49) at 20 dB (HL). No differences ( $P > .05$ ) were found for species, sex, or age. Figure 2 shows representative brainstem auditory-evoked potentials recorded from a llama with normal auditory function at 90, 60, 40, 30, and 20 dB (HL).

### Brainstem Auditory-Evoked Potentials and Pigmentation

All animals ( $n = 49$ ) with colored irides had hearing levels between 10 and 20 dB (HL). Seven out of 9 alpacas with pure-white coat color and blue irides were bilaterally deaf. The other pure-white-coated animals ( $n = 2$ ) with blue irides and the other mainly white animals with some dark color spots in their coats with blue irides ( $n = 3$ ) exhibited hearing levels in the range of camelids with pigmented irides, as did 1 animal with a white coat and 1 blue and 1 pigmented iris. One llama with a pure-white coat and blue irides was unilaterally deaf, the other ear showing normal hearing.

### Discussion

This article describes typical brainstem auditory-evoked potentials that occur in llamas and alpacas over the range of 10–90 dB (HL) and has defined the range of normal hearing levels in these species to be between 10–20 dB (HL). There was a correlation between having a white coat and blue eyes and deafness, but it was not possible to establish whether the condition is heritable.

Brainstem auditory-evoked potentials of different mammals are similar in configuration as a result of their similar anatomical structure.<sup>16,17,21,22</sup> The 4–5 waves that were found in the current study in llamas and alpacas were similar to those found in humans,<sup>16,17</sup> dogs,<sup>20,23–25</sup> cats,<sup>21,26</sup> cat-

**Table 3.** Means ( $\pm$ SD) of interpeak latencies of waves I–III, I–V, and III–V (in msec) at different intensities (dB hearing level [HL]) in llamas and alpacas with pigmented coats and irides.

Wave	dB (HL)	Llama Mean $\pm$ SD	Alpaca Mean $\pm$ SD
I–III	90	1.75 $\pm$ 0.12	1.69 $\pm$ 0.14
I–V	90	2.65 $\pm$ 0.17	2.52 $\pm$ 0.16
III–V	90	0.92 $\pm$ 0.23	0.82 $\pm$ 0.16
I–III	60	1.84 $\pm$ 0.34	1.73 $\pm$ 0.15
I–V	60	2.89 $\pm$ 0.32	2.52 $\pm$ 0.19
III–V	60	1.03 $\pm$ 0.24	0.83 $\pm$ 0.20
I–III	40	0.95 $\pm$ 0.05	1.96 $\pm$ 0.30
I–V	40	2.54 $\pm$ 0.38	2.97 $\pm$ 0.85
III–V	40	1.30 $\pm$ 0.28	1.08 $\pm$ 0.37
I–III	30	1.11 $\pm$ 0.38	1.99 $\pm$ 0.27
I–V	30	2.68 $\pm$ 0.08	2.96 $\pm$ 0.27
III–V	30	1.44 $\pm$ 0.38	1.22 $\pm$ 0.24

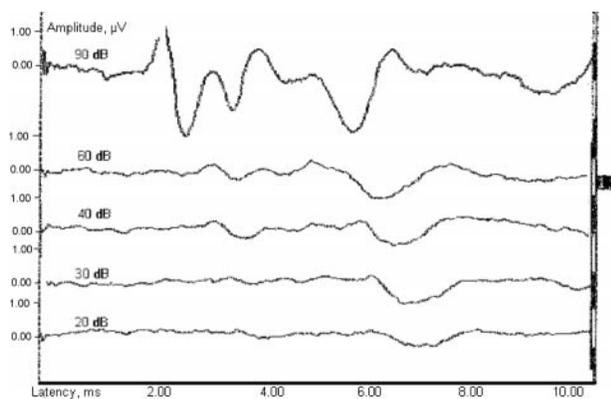
tle,<sup>19</sup> horses,<sup>27</sup> rabbits, and other species.<sup>28</sup> Waves IV and V were not clearly divided in any of the animals in the current study. The merging of waves IV and V was described as normal in humans<sup>17</sup> and cattle.<sup>19</sup> This observation is explained by similar stimulus conduction times of the lemniscus lateralis and colliculus caudalis, the structures that produce waves IV and V, respectively.<sup>19</sup>

The measured latencies and amplitudes behaved as expected.<sup>31</sup> Latencies increased and amplitudes became smaller with decreasing intensity of stimulus.

Hearing level was not significantly different between males and females, which is in agreement with findings in other mammals.<sup>16,29,30</sup>

Hearing loss and increasing latencies as an effect of age has been described in humans.<sup>17</sup> This partial or total loss of auditory function is mainly an effect of continuous and/or repeated exposure to noise and is unlikely to occur in camelids.

Alpacas had a tendency for hearing ability at lower dB levels in comparison with llamas based on the latencies of peaks. Llamas are mainly used in South America as pack animals and meat producers and have been selected for docility and growth. Traditionally, they have been housed and farmed closer to humans. Alpacas, on the other hand, have



**Fig 2.** Representative brainstem auditory-evoked potentials (BAEP) recorded from a llama (*L. glama*) with normal auditory function at 90, 60, 40, 30 and 20 dB hearing level.

been raised for wool production and tend to be farmed extensively,<sup>31</sup> wherein better hearing may confer more rapid escape from approaching predators.<sup>32</sup>

Interpeak latencies (IPL) I–III and I–V showed the same characteristics as described in other animals.<sup>16,17</sup> IPL III–V was physiologically longer in camelids with normal hearing levels.

The development of amplitude of wave V was used to define the normal range of hearing in camelids. In the present study, this range was 10–20 dB (HL). Alpacas and llamas exhibit hearing levels similar to dogs<sup>20,23</sup> and have better hearing ability than many other mammals.<sup>24,27</sup> It is possible that hearing plays a more important role for intraspecies communication in domesticated camelids.

All animals with pigmented coats and irides had normal auditory function. Seven of 9 (78%) alpacas with pure-white coats and blue irides (bilateral) assessed by BAEP were deaf. This is similar to findings in white cats with blue irides.<sup>33</sup> The results support the hypothesis that lack of pigmentation is correlated with deafness in alpacas and llamas. The degree of pigmentation seems to be important because all animals with a white coat and colored irides showed normal hearing levels, as did the animals with colored spots in their white coats.

The cause of deafness in the camelids with nonpigmented irides is probably similar to the mechanism described in other species. During fetal development, most of the pigment-producing cells, or melanocytes, originate in the neural crest. An absence of melanocytes could reflect either a failure of migration from the neural crest or a failure of differentiation after arrival at the inner ear/eye/skin, which is likely to be caused by one or several genes.<sup>34</sup> A lack of melanocytes, and hence pigment, in the ear leads to incomplete development and function of the inner ear.

An animal with nil or reduced auditory function is at an evolutionary disadvantage to animals with normal hearing ability. An animal with unilateral deafness experiences difficulty localizing the source of sound but quickly learns to compensate. On the other hand, a bilaterally deaf animal is unable to anticipate dangers such as predators and may, as a result, fall victim to injury or death. These animals are also limited in intraspecies communication.

The causes of hearing loss and deafness are varied, and implications for management and future breeding vary accordingly. Brainstem auditory-evoked potential recording may be used as a tool to establish hearing function of white-coated animals with blue irides because their association with deafness appears high. The results of hearing tests should be considered when making breeding decisions with blue-eyed, white-coated animals. It is possible that the deafness associated with lack of iris pigmentation in llamas and alpacas is a heritable condition, as occurs in other species such as the cat; however, further testing to establish heritability is required.

### Acknowledgments

This work was financially supported by the German Research Foundation (DFG-Ga 606/4-1). The authors thank Coolaroo Alpaca Stud, Australia, and Pichincha Llamas,

Germany, for their generous contributions of alpacas and llamas to the study.

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