Electrophysiological studies in American Quarter horses with neuroaxonal dystrophy

Carrie J. Finno,* Monica Aleman,† Ron Ofri,‡ Steven R. Hollingsworth,§ John E. Madigan,** Laramie Winfield,† Danika L. Bannasch,*

*From Population Health and Reproduction, University of California, Davis, CA 95616, USA; †William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, CA 95616, USA; ‡The Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot 76100, Israel; §Department of Surgical and Radiological Sciences, University of California, Davis, CA 95616, USA; and **Medicine and Epidemiology, University of California, Davis, CA 95616, USA

Abstract

Objective: Neuroaxonal dystrophy (NAD) is a disease characterized by the sudden onset of neurologic signs in horses ranging from 4 to 36 months of age. Equine degenerative myeloneuropathy (EDM), a disease that has been associated with low vitamin E concentrations, is considered a more advanced form of NAD. The objective of this report is to describe the electrophysiological features of NAD/EDM in American Quarter horses (QHs).

Horses: Six NAD/EDM-affected QHs and six unaffected QHs were evaluated by ophthalmic examination and electroretinography. Five of the NAD/EDM-affected QH and five unaffected QHs were also evaluated by electroencephalography (EEG).

Results: Ophthalmic examination, ERGs, and EEGs were unremarkable in NAD/EDM cases.

Conclusions: Neuroaxonal dystrophy/EDM does not appear to cause clinical signs of ocular disease or functional ERG/EEG deficits in QHs.

Key Words: electroencephalogram, electroretinogram, equine degenerative myeloneuropathy, lipofuscin, neuroaxonal dystrophy, vitamin E

Across species, neuroaxonal dystrophy (NAD) is a pathological diagnosis of bilateral symmetrical neuroaxonal degeneration of anatomically localized nuclei and axonal processes in the central nervous system. Equine degenerative myeloneuropathy (EDM) is a neurologic disease associated with low vitamin E concentrations and a genetic predisposition in various breeds of horses, which is considered a pathologically more advanced form of equine NAD. Clinically, NAD and EDM are indistinguishable. Neurologic abnormalities with NAD and EDM consist of symmetric ataxia, dysmetria, and proprioceptive deficits of all limbs that typically develop during the first year of life.

In humans, mice, monkeys, and dogs with dietary vitamin E deficiencies, retinal degeneration is often observed. In horses with equine motor neuron disease (EMND), a distinct neurodegenerative condition associated with vitamin E deficiency, lipofuscin deposits are often observed on examination of the retina. Flash electroretinogram (ERG) of EMND-affected horses has demonstrated decreased B-wave amplitudes. Although NAD/EDM has been associated with vitamin E deficiency, complete ophthalmic examinations, including ERGs, have not been previously reported in horses affected with NAD/EDM. Additionally, a unique feature of NAD/EDM in the American Quarter horse (QH) is an inconsistent menace response and abnormal mentation, ranging from quiet to obtunded. In our previous work, we describe the electroencephalogram (EEG) findings in only one NAD/EDM-affected QH. The purpose of this report is to describe the ophthalmic examinations and ERG and EEG findings of NAD/EDM-affected QHs as compared to unaffected QH controls.

MATERIALS AND METHODS

Horses

Six NAD/EDM-affected Quarter horse mares weighing between 390 and 600 kg (mean ± SD, 456 ± 90 kg) and eight unaffected QHs (seven mares, one gelding) weighing between 385 and 546 kg (461 ± 60 kg) were used in this
All six NAD/EDM QHs (aged 2 \( n = 2 \), 5 \( n = 2 \) and 9 \( n = 2 \) years) were available for ophthalmic examination and ERG, and 6/8 unaffected QHs (aged 2, 5, 10, 11, 26, and 28 years) were used as control cases. Five of the six NAD/EDM-affected QHs (aged 2, 5 \( n = 2 \) and 9 \( n = 2 \) years) were available for electroencephalography (EEG), and five unaffected control QHs (aged 2, 5, 7, 10, and 11 years) served as controls. None of the horses were administered vitamin E supplementation before or during the study.

The six NAD/EDM-affected QHs originated from a farm with a high incidence of NAD/EDM and were all classified as affected with a mean ataxia score \( \geq 2 \) as previously described (scores 2, 2.17, 2.17, 2.67, 2.75, 3). A horse with a mean score of \( \geq 2 \) was classified as affected. This cut-off was used based upon our previous findings in QH with NAD/EDM, where a horse with a mean score of 2 was confirmed to have NAD/EDM on postmortem examination.

Horses with a score >0 but <2 have neurologic gait abnormalities; however, we have not had the opportunity to perform a complete histologic assessment and verify NAD/EDM on a case graded within this range. The six affected horses were related within three generations and had been previously demonstrated to have low (<1.5 ppm) serum vitamin E concentrations. The eight unaffected horses were from a university research herd and determined to be neurologically normal after a complete neurologic evaluation by a board-certified equine internist (CF). All protocols were approved by the University of California, Davis Institutional Animal Care and Use Committee (Protocol # 16272).

**Serum Vitamin E concentrations**

Measurement of serum vitamin E was repeated by high-performance liquid chromatography with fluorescence detection, as previously described, in two NAD/EDM horses (aged 9 years) at the time of ERG recording.

---

**Figure 1.** Rod responses of a normal (a) and an affected (b) Quarter horse. Responses to dim flashes of light were recorded every 4 min over 20 min. There was a progressive increase in responsiveness (from black to red to green to pink to yellow) as the animals spent more time in the dark, but no obvious difference between the two horses. Similarly, there were no obvious differences between the two horses in their cone responses, recorded following light adaptation (C-normal, D-affected), nor were there any significant differences in any of the parameters analyzed. (E) Electroencephalogram in a horse with neuroaxonal dystrophy (NAD). Note sleep spindles (*), vertex waves (oval), and K-complex (square) consistent with no rapid eye movement (REM) sleep. Also note second-degree atrioventricular block (arrow) on the electrocardiogram. Other channels were removed for this figure. Calibration bar is at the bottom right (25 \( \mu \text{V}, 1 \text{s} \)).
**Ophthalmic examination**

1% tropicamide (Tropicacyl®; Cardinal Health, Dublin, OH, USA) was applied to each eye 15 min prior to examination. Horses were then sedated with a combination of detomidine hydrochloride (Dormosedan®; Pfizer Animal Health, New York, NY, USA) (0.01 mg/kg) and xylazine (Rompun®; Mobay Corporation, Animal Health Division, Shawnee, KS) (0.2 mg/kg), intravenously. A complete ophthalmic examination, including visualization of the retina, was performed by a board-certified ophthalmologist (SH).

**Electroretinogram (ERG)**

Electroretinograms were performed by an experienced board-certified ophthalmologist (RO) using an ERG unit with a handheld mini Ganzfeld stimulator (HMsERG; RetVetCorp, Columbia, MO, USA). Randomly selected unilateral recordings (ocula dextra [OD], four affected, four controls; ocular sinistra [OS], two affected, two controls) were conducted in standing stocks. ERGs were performed one or more hours after the ophthalmic examination in all horses. Horses were re-sedated with combination of detomidine hydrochloride (Dormosedan®) (0.01 mg/kg) and xylazine (Rompun®; Mobay Corporation) (0.2 mg/kg) intravenously, and topical 0.5% proparacaine (Proparacaine Hydrochloride Ophthalmic Solution, Cardinal Health, Dublin, OH, USA) was applied to both eyes. A Jet contact lens electrode (ERG-Jet disposable contact lens electrode, Fabrinsal, SA, Switzerland) was placed on the cornea using 0.5% proparacaine (Proparacaine Hydrochloride Ophthalmic Solution, Cardinal Health, Dublin, OH, USA). Subcutaneous needles placed at the lateral canthus and midline at the level of the nostrils served as reference and ground electrodes, respectively. Electrode impedance was checked and maintained at <5 kΩ. All preparations were conducted in ambient lighting. A comprehensive ERG recording, aimed at assessing rod and cone function, was conducted based on a published protocol. This included a 20-min dark adaptation period, during which rod function was tested every 4 min using a dim stimulus (average of 10 flashes, 0.5 Hz, 10 μcd/m² per s). Subsequently, the mixed rod-cone response to a standard (average of 4 flashes, 0.1 Hz, 3 cd/m² per s) and high intensity (average of four flashes, 0.05 Hz, 10 cd/m² per s) stimulus was assessed. Cone function was assessed following 10 min of light adaptation (30 cd/m²) using a high intensity flash (average of 32 flashes, 2 Hz, 3 cd/m² per s) and the cone flicker test (128 flashes, 31 Hz, 3 cd/m² per s).

**Electroencephalogram (EEG)**

On a separate day, horses were sedated with a combination of 0.01 mg/kg detomidine hydrochloride (Dormosedan®) and 0.02 mg/kg xylazine (Rompun®, Mobay Corporation) intravenously prior to the placing of EEG electrodes. All EEGs were recorded on a digital telemetry electroencephalographical system (EEG 9000-Neurofax 9100; Nihon Kohden America Inc, Foothill Ranch, CA, USA). Electrode nomenclature and placement was based on a modified human 10–20 system, as described in horses previously. In brief, subcutaneous needle electrodes (Grass S48; Grass Technologies, World Headquarters, Astro-Med Industrial Park, West Warwick, RI, USA) were placed in the prefrontal (two electrodes), frontal (three), central (three), parietal (three), and occipital (two) regions. Additional electrodes included one that served as a ground (between the two prefrontal electrodes, one in the intercanthus region and one at the base of each ear to evaluate for ear movement artifacts on the EEG. Concurrently, an electrooculogram (two subcutaneous electrodes per eye, one each in the upper and lower eyelids), electromyogram (two subcutaneous electrodes located in the splenius muscle), and electrocardiogram (one subcutaneous electrode in the region of the left heart base and one at the left heart apex) were also performed. A bipolar montage (rostral to caudal and transverse) was used with sensitivities set for recording as described.

Thirty to forty minutes of EEG recordings were obtained. The data obtained were thoroughly examined from EEG tracings and simultaneous video of the horse under study.

**RESULTS**

**Serum Vitamin E concentrations**

Serum Vitamin E concentrations were below the reference range in the 2 NAD/EDM-affected horses (0.96 and 1.3 ppm; reference range >1.5 ppm).

**Ophthalmic examination**

An inconsistent menace response was present in 5/6 NAD/EDM-affected cases and 0/6 control horses. Mild focal areas of depigmentation localized ventral and/or medial to the optic disk were noted in all six NAD/EDM-affected cases (OU [n = 4], OS [n = 2]) and 5/6 unaffected horses (OU [n = 4]; OS [n = 1]).

**Electroretinograms**

Diagnostic ERG recordings were available in all twelve cases. When compared with the QH control values and with published baseline values, there were no abnormalities noted in the ERG recordings of the affected horses (Fig. 1).

**Electroencephalography**

Diagnostic EEG recordings were available in all ten cases. There were no notable abnormalities on the baseline EEG recordings. EEG recordings consisted of alternating periods of drowsiness and slow-wave sleep, because of sedation, and periods of wakefulness. Sleep spindles were intermittently observed 2 min following sedation (Fig. 1). No rapid eye movement (REM) was observed.

**DISCUSSION**

Although neuroaxonal dystrophies and diseases characterized by vitamin E deficiencies in other species have been described in horses, often result in retinal degeneration, we were unable to document...
any clinically significant ophthalmic abnormalities in these QHs affected with NAD/EDM other than an abnormal menace in 5/6 NAD/EDM-affected cases, a finding that has been reported previously in QHs with NAD/EDM. To date, pathological lesions to account for this abnormal menace response in QH with NAD/EDM have not been found. Mild focal areas of depigmentation are often found in horses with no history or signs of ocular disease. These are considered a variation of normal and in this study were found with approximately the same frequency in the NAD/EDM-affected and unaffected horses. Additionally, in this study, EEG and ERG findings were unremarkable in NAD/EDM-affected horses, and horses remained responsive to stimuli throughout the recording period.

The photoreceptor outer segments of the retina are particularly vulnerable to a deficiency of vitamin E because of the plentiful supply of oxygen, abundant mitochondria, and an unusually high rate of oxidative metabolism, which results in increased free radical species and lipid peroxidation. In addition, more than 65% of the membrane fatty acids within the rod outer segments are polyunsaturated, leaving this tissue particularly vulnerable to peroxidation. In dogs with vitamin E deficiencies and horses with EMND, oxidative damage to the photoreceptors has been documented; however, these animals are typically older (dogs over 4 years of age and horses at peak risk for EMND at 16 years of age) than most NAD/EDM cases (most often diagnosed from 4 months to 3 years of age). Although 4/6 affected QHs were ≤5 years of age, two were 9 years old, confirmed to be vitamin E deficient, and probably deficient in vitamin E throughout their entire lifetime. Therefore, although the argument can be made that chronic long-term vitamin E deficiency (i.e. >9 years) is necessary to produce retinal lipofuscin deposits and ERG changes, this does not appear to occur in horses with NAD/EDM.

The method of EEG analysis in this particular study was by thorough and repeated observations of the video and electrical brain activity recorded simultaneously. Although studies of EEG under anesthesia analyze the data using fast Fourier transforms (FFT), EEG is a mixture of various frequency activities, and the predominant frequency activity observed is what defines the state of vigilance in individuals. Even under ideal circumstances, artifacts can alter EEG signal, and thus, quantitative EEG results should be interpreted with caution as numbers given by FFT may not always correlate with the actual event observed in the patient plus artifacts may be overlooked. The current position taken by the American Academy of Neurology and the American Clinical Neurophysiology Society regarding the use of quantitative EEG in the clinical setting states that advanced quantitative EEG techniques should only be used by physicians highly skilled in clinical EEG and only as an adjunct and in conjunction with traditional EEG interpretation.

In the NAD/EDM-affected horses, vitamin E concentrations were only repeated in 2/6 cases at the time of ERG examination. All six horses had been found to be deficient 6 months before the onset of this study and were not maintained on any vitamin E supplementation nor did they experience any change in feed during that time. A persistent vitamin E deficiency was documented in only 2/6 cases; however, it is likely that the other four cases remained deficient.

Limitations of this study include the absence of vitamin E measurements in the control cases. These horses were maintained on an alfalfa hay diet with grain supplementation as part of a research herd and are unlikely to be vitamin E deficient; however, the possibility that an underlying deficiency existed in control horses cannot be excluded. These horses, however, did not display any neurologic abnormalities. An additional limitation was the characterization of affected NAD/EDM cases based on clinical examination and farm history alone. At this time, a definitive diagnosis of NAD/EDM can only be achieved by postmortem examination with careful histologic evaluation of the brainstem and spinal cord. We have previously reported the diagnostic findings, including complete postmortem examinations on five index cases from the farm where the affected NAD/EDM horses originated. Two of the affected NAD/EDM cases in this study were dams of two of the postmortem-confirmed NAD/EDM index cases, while the other four affected NAD/EDM cases in this study were related to these two dams within three generations. In addition, all horses on this particular farm were vitamin E deficient at the time of diagnosis, which, in conjunction with the degree of relatedness between the six horses, supports a likely clinical diagnosis of NAD/EDM as a cause of their ataxia.

ACKNOWLEDGEMENTS
This project was supported, in part, by the Center for Equine Health with funds provided by the State of California pari-mutuel fund and contributions by private donors. Additional funding was provided by private donations to support equine neurologic research.

REFERENCES