Electrodiagnostic evaluation of peripheral nerve function in rheas and barred owls

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Objective—To establish reference values for electrodiagnostic evaluation of peripheral nerve function in birds.

Animals—6 rheas and 6 barred owls.

Procedure—Birds were anesthetized with propofol or isoflurane in oxygen. Using a computer-based electromyography system and needle electrodes for stimulation and recording, electromyography (EMG) was performed on the pectoral, biceps brachialis, and gastrocnemius muscles, and evoked EMG was performed on the tibial and ulnar nerves. Motor nerve conduction velocity (MNCV) was calculated. Repetitive stimulation was performed on these 2 nerves. Late F waves were recorded for each nerve, when possible.

Results—Activity was evident during insertion of the electrodes, but muscles tested were electrically quiescent after spontaneous EMG. Motor nerve conduction velocity was faster in the tibial nerve than ulnar nerve but did not differ significantly between species. Mean ± SEM MNCV was 132.3 ± 7.8 m/s for the tibial nerve and 59.7 ± 7.8 m/s for the ulnar nerve. A significant difference was not observed in responses at the fourth or ninth stimulation during repetitive stimulation. Subsequent to the initial stimulation, amplitudes were ± 22.7% of the initial motor potential amplitude. Recorded F waves were inconsistent, which may have been associated with technique.

Conclusions and Clinical Relevance—Reference range (mean ± 2 SEM) for MNCV was 34.1 to 75.3 m/s for the ulnar nerve and 116.7 to 147.9 m/s for the tibial nerve in barred owls and rheas. After repetitive stimulation, motor potential amplitudes may be ± 22.7% of the initial amplitude response.


Electromyography (EMG) is used to study the electrical activity of muscle membranes. Electrical activity elicited when the electrode is inserted (ie, insertion activity) and the duration and amplitude of the potential at the motor unit can be used to evaluate the integrity of that motor unit. Evoked EMG is used to study the ability of a compound muscle associated with peripheral nerve to propagate an action potential in response to electrical stimulation (ie, the M response). The latency of this response, which represents the time required for conduction of the stimulus through the axon, across the neuromuscular junction, and into the muscle, can be used to calculate motor-nerve conduction velocity (MNCV). The latency of the F wave, a smaller action potential that follows the M response, represents the time required for the stimulus to return to the muscle via retrograde conduction through the ventral nerve root and ventral horn cell, and it is used to assess proximal segments of peripheral nerves. Repetitive stimulation of a nerve assesses the ability of the neuromuscular junction to transmit a series of motor-action potentials without substantial alteration in amplitude, as well as the ability of the postsynaptic membrane to respond to transmission. Changes in MNCV, latency of the F wave, and amplitude of the repetitive stimulation may be used, along with spontaneous EMG findings, to localize a disorder within the motor unit.14 However, these tests cannot be used routinely in birds until reference values are established.

Spontaneous EMG was used to determine prognosis and to direct treatment in 7 wild birds with traumatic injuries involving peripheral nerves and musculoskeletal disease. Widespread fibrillation potentials and lack of typical interference patterns suggested denervation in 3 of these 7 birds that had muscle atrophy and wing paralysis or paresis. The remaining 4 birds were rehabilitated after results of spontaneous EMG provided evidence that the motor units were functional, despite clinical signs of muscle weakness and diminished neurologic responses. In another report, results of spontaneous EMG revealed fibrillation potentials and a few bizarre high-frequency discharges in the muscles of the wings of 2 red-tailed hawks with trauma to the brachial plexus. Results of EMG were interpreted as normal in a northern goshawk that had progressive hemiplegia, which was subsequently found to be secondary to local Sarcocystis encephalitis.7

The MNCV of the tibial nerve in 2- to 4-week-old chickens is 32 m/s, whereas that in 1-week-old chickens is 22 m/s and that in 15-week-old chickens is 53 m/s.8 Chickens inoculated intraperitoneally with Marek's disease virus had signs of depression, paresis, and electroencephalographic abnormalities, but there was not any difference in MNCV of the tibial nerve in these chickens, compared with uninfected control chickens.9 In addition, a linear association has been found between MNCV of the tibial nerve and cloacal temperature.9 Motor-nerve conduction velocity decreases with decreasing body temperature in healthy chickens, and there is an age-related effect on tem-
perature-associated variation in MNCV of the tibial nerve.\textsuperscript{11} Results of evoked EMG are linearly affected by tissue temperature in dogs\textsuperscript{12,13} and horses\textsuperscript{14} and by aging in cats,\textsuperscript{15} dogs,\textsuperscript{16-17} and horses.\textsuperscript{18} The objective of the study reported here was to establish reference values for electrodiagnostic evaluation of peripheral nerve function in rhesus and barred owls.

Materials and Methods

Experimental Animals—Six adult male rhesus (Rhesus cynomolgus) and 6 adult barred owls (Strix varia) of unknown sex were used in the study. These species were chosen to represent the diversity among birds and to potentially determine differences in primary neuromuscular activity. Rhesus, which are flightless raptors, have highly developed muscles in the pelvic limbs and relatively underdeveloped muscles in the wings, which reflects daily activities of walking or running. Conversely, barred owls, which are ratites, have highly developed muscles in the pelvic limbs, which reflects daily activities of soaring and diving in search of prey.

The protocol used was approved by a university Institutional Animal Care and Use Committee. Birds were acclimatized for 36 hours after a 2-hour-long transport to the study location. Birds were weighed and identified at the beginning of the study. Results of observations and physical examinations did not reveal neurologic abnormalities in any bird.

Experimental design—Anesthesia was induced in rhesus with propofol\textsuperscript{1} (5 mg/kg of body weight, IV) and in barred owls with isoflurane\textsuperscript{2} in oxygen delivered via face mask. Birds were intubated, and anesthesia was maintained with isoflurane in oxygen. A pulse oximeter, esophageal stethoscope, and cloacal temperature probe were used to monitor birds, and a recirculating warm water pad and heat lamp were used to maintain core body temperature throughout anesthesia. Intermittent positive-pressure ventilation was used as needed. Blood was obtained for measurements of serum activities of creatine kinase as part of another project. Ambient temperature and cloacal temperature were maintained in constant ranges.

The tibial and ulnar nerves were evaluated, using spontaneous and evoked EMG. A computer-based electromyographic (EMG) system\textsuperscript{3} interfaced with differential amplifiers, filters, a stimulator, and a signal-averaging system was used for stimulation and recording during electrophysiologic evaluation. Responses were monitored visually, using a computer monitor, and audibly, using an amplifier and loudspeaker. Concentric platinum-core needle electrodes\textsuperscript{4} (0.46 diameter, 0.07 mm\textsuperscript{2} area, 37 mm long) were used to register electrical activity.

Spontaneous EMG was performed on the lateral head of the gastrocnemius, superficial pectoral, and biceps brachii muscles on one side of each bird. Insertional and resting activities were observed and recorded. Evoked EMG was performed on the tibial and ulnar nerves by use of an established protocol\textsuperscript{5-9,11} that was modified when used on the ulnar nerve.

Each stimulating electrode had a pair of concentric bipolar needle conductors (anode and cathode). The proximal stimulating electrode for the tibial nerve (TS\textsubscript{2}) was placed percutaneously, caudal to the greater trochanter of the femur near the ischiatic nerve, whereas the distal stimulating electrode for the tibial nerve (TS\textsubscript{9}) was placed percutaneously, caudal to the proximal portion of the middle of the tibiotarsus close to the tibial nerve over the gastrocnemius muscle. The monopolar recording needle for the tibial nerve (TR) was placed distal to the tarsometatarsal joint in the abductor muscle of the fourth digit. A grounding electrode (TG) was placed subcutaneously over the lateral aspect of the hock between TS\textsubscript{2} and TR.

The proximal stimulating electrode for the ulnar nerve (US\textsubscript{2}) was placed percutaneously, caudal to the humerus near the median portion of the ulnar nerve, and the distal stimulating electrode for the ulnar nerve (US\textsubscript{9}) was placed percutaneous, ventrocaudal to the middle portion of the ulna near the ulnar nerve. The monopolar recording needle for the ulnar nerve (UR) was placed in the ventral interosseous muscle within the intermetacarpal space. A grounding electrode for the ulnar nerve (U9) was placed subcutaneously over the lateral aspect of the carpus between US\textsubscript{2} and UR.

Interelectrode distance from proximal to distal (ie, S\textsubscript{1} to S\textsubscript{9}) was measured in millimeters, using a flexible string. The stimulator delivered 100-μs pulses at 2 pulses/s (pps). The intensity of the stimulus was increased until a muscle-action potential of maximal amplitude was induced. The intensity was then increased 20 to 25% to ensure stimulation of all axons. Recordings were made at this supramaximal threshold potential. Stimulus intensity, action-potential amplitude, latency, interelectrode distance, and conduction velocity were recorded for each evoked EMG. Motor nerve conduction velocity was calculated as the interelectrode distance divided by the difference between the latency measurements at S\textsubscript{1} and S\textsubscript{9}.

Each nerve was repetitively stimulated at S\textsubscript{9}. The change in amplitude response as a percentage of initial amplitude response (A0) was determined for the fourth (A4) and ninth (A9) stimulation. Supramaximal stimulation was delivered at 5 to 10 pps. The late-wave response (ie, F wave) was recorded by using the same stimulation variables but at 1 pps. These procedures were repeated 2 to 6 times in each bird.

Biopsy of the gastrocnemius and biceps brachialis muscles was performed for histologic and morphometric study immediately after the electrophysiologic study as part of another project. Nerves were not biopsied. At the end of the study, birds were returned to the lending institutions.

Statistical analyses—The mean MNCV for the tibial and ulnar nerves were calculated for each bird. A split-plot analysis\textsuperscript{12} was used to evaluate the effects of species and nerve tested. Mean percentage of initial amplitude response after repetitive stimulations was calculated for each nerve of each bird. A paired Student t-test was used to evaluate the difference between motor potential amplitudes at the A0, A4, and A9 stimulations.

Results

After the initial insertional activity, we did not detect spontaneous electromyographic activity, such as fibrillation potentials, positive sharp waves, complex repetitive discharges, or myotonic potentials.

Nerve-conduction velocities on evoked EMG recordings were repeatable in each species (Table 1). Overall MNCV (mean ± SEM) for the tibial nerve was 132.3 ± 7.8 m/s. There was not a significant difference between MNCV for the tibial nerve in barred owls (mean, 134.2 m/s; median, 113.8 m/s; range, 101.8 to 222.7 m/s) and rhesus (mean, 129.8 m/s; median, 126.2 m/s; range, 108.2 to 176.0 m/s). Overall MNCV for the ulnar nerve was 59.7 ± 7.8 m/s. There was not a significant difference between MNCV for the ulnar nerve in barred owls (mean, 56.6 m/s; median, 54 m/s; range, 39.7 to 77.3 m/s) and rhesus (mean, 62.9 m/s; median, 61.1 m/s; range, 50.9 to 83.9 m/s). The effects of species and nerves tested were not significant (P = 0.652 and 0.911, respectively). A correlation was not observed between results of evoked EMG and body weight (barred owls 0.7 to 1.4 Kg; rhesus, 27.3 to 35.5 Kg) or cloacal temperature (barred owls, 38.9 to 40.1 C; rhesus, 36.7 to 40.1 C). Motor-nerve conduction velocity was significantly (P = 0.001) faster in the tibial nerve than in the ulnar nerve.

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Motor-action potentials were consistent on repetitive stimulation. Amplitude at A4 ranged from –12.6 to +17.6% of that at A0, whereas that at A9 ranged from –22.7 to +21.6% of that at A0. The differences between amplitudes at A4 and A0 (P = 0.710), A9 and A0 (P = 0.638), and A9 and A4 (P = 0.624) did not differ significantly.

The F waves were inconsistently recorded and could not be analyzed statistically. The H-reflex response was not observed in the course of this study. Histologic evaluation of effector muscles associated with the nerves tested confirmed that angular atrophy of nerve fibers were not evident; thus, we did not detect evidence indicative of abnormal nerve function or denervation. The H-reflex was blocked by the intensity of supramaximal stimulation.

Discussion

Electrophysiologic studies were conducted on the ulnar and tibial motor nerves of neurologically normal barred owls and rheas to define reference values prior to use of these techniques in clinical situations on birds with peripheral neuropathy. To our knowledge, repetitive stimulation and F-wave evaluation have not been performed on birds. Repetitive stimulation techniques were easily applied; however, F-wave techniques were technically more difficult to record.

For determination of MNCV, the suggested distance between S1 and S2 is 10 cm, whereas that between each cathode and anode is 1 to 3 cm. However, the size of the barred owls used in the study reported here precluded use of these distances in this species. Despite differences in the interelectrode distances used in the 2 species evaluated in this study, differences in MNCV of the ulnar or tibial nerve were not significantly different between barred owls and rheas. Placement of electrodes on the basis of an interelectrode distance that was determined by measuring the distance along the skin has inherent errors because of variations attributable to nerve anatomy and location of nerves in relation to joints in each bird. Necropsy evaluation of other adult barred owls and rheas had been performed to determine the location and course of the ulnar and tibial nerves to aid in the placement of the measuring tape used during this study.

A greater stimulus intensity was required to evoke an action potential in rheas, compared with barred owls. This difference may have been associated with an increased amount of soft tissue between the needle electrode and tested nerves in rheas, which are larger birds, compared with barred owls. One rhea and 1 barred owl had MNCV values for the tibial nerve that were faster than in the other birds of that species, but these faster velocities were not correlated with physiologic variables or MNCV values for the ulnar nerve in these 2 birds. These differences may have been the result of technical error. Use of a greater number of birds would have minimized any effect of outlying values on establishment of a reference range. A split-plot analysis was used for statistical analysis rather than an ANOVA, because ANOVA assumes independence among variables tested. Two measurements (ie, 1 each of the ulnar and tibial nerves) were made on each bird, and an association was expected between these 2 measurements as a result of the length of the limb and each bird’s body weight and phylogenetic order.

Mean nerve-conduction velocity was significantly faster in the tibial nerve than in the ulnar nerve of birds studied, which is consistent with the direct association between nerve fiber diameter and MNCV. On the basis of results of necropsy examinations, the diameter of the tibial nerve is greater than that of the ulnar nerve at the sites we chose for stimulation. Gross nerve size could be related to axon size, axon number, or amount of connective tissue but was not defined because histologic examination of nerves was not included in the investigation.

The difference between tibial MNCV in chickens, compared with that of rheas and barred owls, is unclear but may be a combination of previously mentioned factors in association with immaturity of the chickens in other studies, which consisted of birds 4 months old. Motor-conduction velocity in the tibial nerve of barred owls and rheas was faster than that of chickens, cats, dogs, pigs, sheep, monkeys, and humans. Motor-conduction velocity in the ulnar nerve of barred owls and rheas appears to be similar to that of dogs and humans. The effects of age and ambient temperature on MNCV were minimized in the study reported here by use of birds that were adults, by maintaining ambient temperature and cloacal temperature of the birds as constant as possible throughout testing, and by testing birds immediately after anesthetic induction to avoid excessive cooling of the limb. A correlation was not found when cloacal temperatures were plotted against

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conduction velocity. Cloacal temperature and room temperature were more variable in rheas than in barred owls, because the study environment was not climate controlled for the rheas. However, these variations appeared to be independent of one another. A more accurate assessment of the effect of temperature may have been accomplished by subcutaneous monitoring of perineural tissue temperature, rather than cloacal temperature. Birds were handled, anesthetized, and maintained in a manner similar to that in a clinical setting to maximize information gained and its usefulness in clinical evaluations.

Mean ± 2 SEM provided a reference range for MNCV of the ulnar (34.1 to 75.3 m/s) and tibial (116.7 to 147.9 m/s) nerves for owls and rheas. After repetitive stimulation, amplitudes of the initial amplitude response. Further investigation of F-wave latency is necessary to determine its value in birds. Because of the great diversity among avian species, additional research must be conducted to determine whether these reference values may be applied to other species of birds and whether changes in electrodiagnostic variables in diseased birds follow a course analogous to that in mammals.

References