

Morphometric Parameters of Peripheral Nerves in Calves Correlated with Conduction Velocity

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Background: Peripheral nerve injuries are the most frequent neurologic disorder in cattle. So far, no physiologic values have been established for the motor nerve conduction velocity (mNCV) in this precocial species.

Objectives: The electrophysiologic and morphometric reference values of peripheral nerves in calves were determined. It was hypothesized that these parameters would correlate to the high degree of maturity in the first days of life in this species compared to other species.

Animals: Twenty-six healthy calves were used in this study.

Methods: The mNCV of the radial and the sciatic/common peroneal nerve was measured in all 26 calves. Nerve biopsies from a group of 6 calves were taken to correlate the obtained electrophysiologic data with morphological parameters.

Results: The mean mNCV of the radial nerve was 48.3 ± 10.6 m/s, whereas the mean mNCV of the sciatic/peroneal nerve was with 83.8 ± 5.9 m/s significantly faster ($P < .0001$). The average fiber diameter was 8.40 ± 2.80 μm (range, 1.98–17.90 μm) and the average g-ratio was 0.61 ± 0.04 SD.

Conclusion and Clinical Importance: The established reference values for mNCV in calves correlate well with the evaluated morphometric parameters. Attributable to their comparably fast mNCV and high fiber diameters, juvenile calves appear to be much more mature individuals than other mammals. Electrophysiologic characterization of peripheral nerve injury now is feasible in this species.

Key words: g-ratio; Myelination; Precocial species.

Evaluations of morphometric and neurophysiologic parameters of peripheral nerves have been performed in various species to elucidate the relationship between structure and function in the peripheral nervous system. Axon and fiber diameter, myelination, and the internodal distance have been shown to influence conduction velocity in peripheral nerves. Large, heavily myelinated fibers with a longer internodal distance (saltatory conduction) tend to have faster conduction velocity.^{1–4} In addition to these morphological parameters, nerve conduction velocity is influenced by more subtle factors such as density of ion channels at the node of Ranvier.^{5,6} The external diameters (including the myelin sheath) of the largest peripheral myelinated fibers in mammals evaluated so far, range from approximately 20 μm in horses to 1 μm in dogs and cats.^{7–9} In cross-section, the parameters axon diameter, fiber diameter (including the myelin sheath), myelin

Abbreviations:

bw	body weight
CMAP	compound muscle action potential
MNCV	motor nerve conduction velocity
SD	standard deviation

thickness, total fiber density, frequencies of axon and fiber diameters, frequencies of myelin thickness, and the g-ratio (axon diameter divided by fiber diameter) can be evaluated morphometrically. These parameters were determined for humans,^{10,11} dogs,^{4,8,12} cats,^{2,3,13} horses,^{9,14,15} and sheep^{16,17} and were correlated with the motor nerve conduction velocity (mNCV). It was concluded that during maturation axon and fiber diameter, myelination, and internodal distance increase, leading to an increase in mNCV.^{4,8,12,18}

In the first part of this study, reference values for mNCV in calves in our laboratory were established. In addition to these neurophysiologic examinations, a biopsy technique for collection of peroneal nerve samples from calves was used to study the morphometric parameters of this nerve and to correlate these parameters with mNCV in calves.

Previously, electrophysiologic and morphometric reference values in cattle have not been evaluated, despite the fact that peripheral nerve injuries are one of the most frequent neurologic disorders in cattle.¹⁹

Materials and Methods

Animals

For evaluating reference values for mNCV of calves in a clinical setting, a group of 20 healthy calves (group 1) was used. The animals were of different breeds (12 Holstein Friesian, 2 red

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Holstein Friesian, 6 half-breeds from Holstein and Limousine) and their age ranged from 16 to 85 days.

In an additional group of 6 healthy calves (group 2), the thickness of the myelin sheath, the axonal area/diameter, and the fiber area/diameter were evaluated after measurement of the mNCV to compare these morphometric findings with the mNCV results. All 6 calves were Holstein Friesian, with age ranging from 14 to 30 days. All the animals in this study were treated in accordance with the German Animal Welfare Law (animal experiment number AZ 04/755).

Anesthesia

The animals were fasted 12 hours before anesthesia. Anesthesia was induced with xylazine (Xylazin 2%^a) at a dosage of 0.1 mg/kg body weight (bw) and ketamine (Ketamin 10^b) at a dosage of 4 mg/kg bw IV. After successful intubation, anesthesia was maintained using isoflurane (Isofluran CP^a) at 1.5–3% and oxygen via a Draeger^c respirator. To obtain nerve biopsies, the 6 calves in group 2 also received caudal epidural anesthesia with procaine hydrochloride (Procaïn 2%^b) at a dosage of 0.3 mL/kg bw. Duration of anesthesia was 30 minutes to measure mNCV in all 4 limbs and an additional 45 minutes to obtain the nerve biopsies.

Measuring mNCV

Measurement of mNCV was performed in lateral recumbency in the clinics for ruminants during late spring and summer at room temperature between 18°C and 24°C. All stimulations of peripheral nerves and recording of compound muscle action potentials (CMAP) in the particular muscles were performed using a Vicking Quest electrodiagnostic device (Nicolet Viking Quest IV^d). The nerves of each side and limb were stimulated supramaximally with a rectangular stimulation impulse. The strength of the supramaximal stimulation ranged mainly between 18.75 and 25 mA with a frequency of 1.0 Hz and duration of 0.1 ms. The common supramaximal stimulus strength in our laboratory ranges between 15 and 40 mA. Stimulation electrodes had a diameter of 0.5 mm, a length of 7.5 cm and were coated with Teflon (Part No. 019-411500^d). For recording CMAP in the extensor carpi radialis (thoracic limb) or the fibularis tertius (pelvic limb), a bipolar concentric needle electrode (Part No. 019-721700^d) measuring 0.6 mm in diameter and having a length of 60 cm was used. The CMAPs were displayed and stored before calculating mNCV on the electrodiagnostic device. Calculation of mNCV was performed automatically by the electrodiagnostic device using the following formula and the rectally measured body temperature as a correction factor:

$$\text{mNCV (m/s)} = \frac{\text{distance between proximal and distal stimulation point (m)}}{(\text{latency of proximal stimulus} - \text{latency of distal stimulus}) (\text{s})}$$

The radial nerve was stimulated at the distal third of the humerus between the brachial muscle and the lateral head of the brachial triceps muscle and proximally in the angle between the head of the humerus and the ventral part of the scapula. The sciatic/peroneal nerve was stimulated distally in the popliteal fossa and proximally in the trochanteric fossa between the trochanter major and the tuber ischiadicum. The ground electrode was placed between the distal stimulation point and the recording electrode. All results are expressed as mean \pm SD and median.

Repeated Measurements

To evaluate the reliability of the measurement procedure itself and the influences of interindividual (stimulated nerve, side of stimulated nerve) and environmental effects, repeated measurements were performed. Six randomly chosen calves in group 1 were measured 5 times on different days (every other day) according to the aforementioned measurement procedure.

Morphometric Evaluation

Calves in group 2 were used to evaluate the correlation between the thickness of the myelin sheath and the axonal diameter and the mNCV. After measuring mNCV of the sciatic/peroneal nerve, a biopsy of the peroneal nerve was taken. The area of skin incision (located 5 cm proximal to the distal stimulation point of the peroneal nerve in the popliteal fossa) was prepared using conventional surgical disinfection methods. After palpating the nerve in the lateral aspect of the distal femur an 8 cm long incision was made in the fascia of the biceps femoris muscle. To prevent inadvertent damage to the nerve, the fascia was elevated with rat-toothed forceps before incision. To visualize the nerve as completely as possible, surrounding fat and fascia were dissected. Thus, damage to the nerve itself was avoided. A 5-0 USP silk suture was inserted through the caudal one-third to one-half of the nerve at the proximal and the distal end of the biopsy site allowing minimal gentle traction to excise a 2- to 3-cm fascicular biopsy using a no.#11 scalpel blade. Two-thirds of the nerve diameter remained in the animal. The fascial layer was closed with absorbable suture material (3-0 USP polydioxanone) and the skin was apposed with a 1-0 USP monofilament nylon fiber. After recovery from anesthesia, calves showed no motor impairment.

Tissue Preparation and Morphometric Evaluation

After surgery, biopsies of the peroneal nerve were processed for morphometric evaluation. Methods for processing biopsies have been described in detail elsewhere.^{20–22} Briefly, biopsies initially were fixed in Karnovsky's fixative for 24 hours. Post fixation in 1% osmium oxide was carried out for 1.5 hours, and after several dehydration steps the biopsies were embedded in Epon blocks. Semi-thin transverse sections of 1 μ m were cut and mounted onto uncoated glass slides and stained with 1% paraphenylenediamine solution for 5 minutes to stain myelin. These slices were assayed using light microscopy^e at 400 \times magnification and digitized for further analyses.^f

With the exception of 1 calf (C), 2 slices showing different fascicles were taken from each calf for analysis (only 1 slice was available for evaluation in calf C). To evaluate an entire fascicle, the images displaying just a part of the nerve fascicle were merged to form 1 image using a multiple imaging alignment procedure. The area of all axons and the diameter of all myelin sheaths were measured on 4 slices from 4 different calves. On the remaining slices, samples of 100 randomly chosen axons from each calf were analyzed using the Analy-SIS ProR Software.^f Axon diameter and, fiber area/diameter were calculated from the measurements assuming that the nerve fiber and the axon were circular. The generated data were used to calculate the g-ratio (sum of axon diameter divided by sum of fiber diameter) of measured fascicles in all 11 slices. Furthermore, the areas of these fascicles and the number of all nerve fibers were examined to calculate fiber density. All results are expressed as mean \pm SD, minimum and maximum. The data were pooled for all calves.

Statistical Analysis

The aim of statistical evaluation was to identify differences in mNCV between the radial and sciatic/peroneal nerves, the influence of left or right side as well as variation in repeated measurements of the same calf on several days, and within days (day-to-day variability). The resulting linear 4-way mixed model comprises nerves and sides as fixed effects and contains day and 2 measuring repetitions consecutively as random effects. Furthermore, correlation between the thickness of the myelin sheath, axonal diameter, and the mNCV was examined by calculating the correlation coefficient (r^2). Normal distribution of the data was confirmed by visual assessment of normal probability plots of the model residuals. In addition, descriptive statistics with several diagrams (boxplots, histograms) were performed. Analyses were carried out with the statistical software SAS, version 9.1.[§] For analyzing the linear mixed model, the “mixed” procedure was used.

Results

Reference Values of mNVC in Calves

The radial nerve and the sciatic/peroneal nerve were measured on the left and on right side of each calf in group 1. In 2 single stimulations, the supra-maximal stimulus strength was 62 and 80 mA. These recordings with abnormal high stimulation strength were excluded from further analysis. The results of these measurements are shown in Figure 1. The calculated mean mNCV of the radial nerve was 48.3 ± 10.6 m/s (median, 46 m/s) whereas the mean mNCV of the sciatic/peroneal nerve was 83.8 ± 5.9 m/s (median, 84 m/s). The calculated SD for the sciatic/peroneal nerve was lower than the SD of the radial nerve (Fig 1).

An analysis of variance was performed on the collected data from 71 measurements of the radial nerve and 78 measurements of the sciatic/peroneal nerve. The time or the day of the measurement ($P = .2519$) and the side (left/right) of the measured nerve ($P = .859$) had no significant influence on mNCV. The mNCV of the sciatic/peroneal nerve differed significantly ($P < .0001$) from the mNCV of the radial nerve. The age of the examined animals from group 1 had no significant effect on the mNCV ($P_{[\text{radial nerve}]} = .732$, $P_{[\text{sciatic/peroneal nerve}]} = .199$). The correlation between age and mNCV is shown in Figure 3A.

Repeated Measurements of mNCV

Interindividual differences and environmental effects were evaluated by taking repeated measurements from 6 calves in group 1. The stimulated nerve and the side of stimulated nerve were considered as interindividual effects. The values of mNCV of the peroneal nerve relating to repeated measurement at different time points on consecutive days are demonstrated in Figure 3B.

The data of these 6 calves were used for analyzing variance components (vc). Higher values for the vc imply less reliability of the measurements and a greater range of obtained values in repeated measurements.

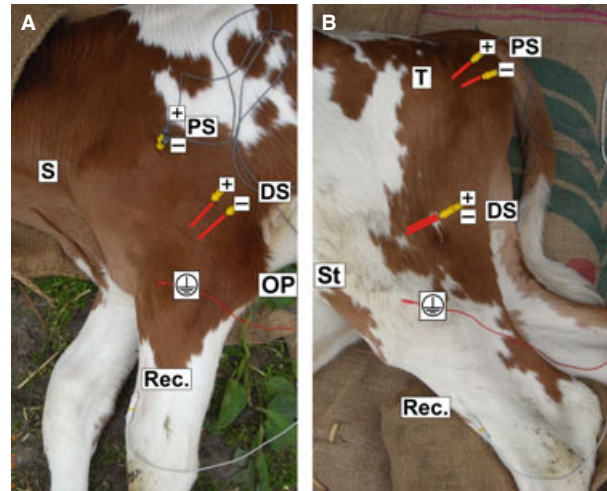


Fig 1. (A) Photograph demonstrating position of electrodes during motor nerve conduction testing of the radial nerve showing proximal stimulation electrodes (=PS, angle between the head of the humerus and the ventral part of the scapula), distal stimulation electrodes (=DS, distal third of the humerus between the brachial muscle and the lateral head of the brachial triceps muscle), ground electrode (=), and bipolar concentric needle electrode (=Rec.) for recording the compound muscle action potential in the extensor carpi radialis muscle. Anatomic landmarks are S, shoulder joint; OP, olecranon process. (B) Photograph demonstrating position of electrodes during motor nerve conduction testing of the sciatic/peroneal nerve showing proximal stimulation electrodes (=PS, trochanteric fossa between the trochanter major and the tuber ischiadicum), distal stimulation electrodes (=DS, in the popliteal fossa), ground electrode (=), and bipolar concentric needle electrode (=Rec.) for recording the compound muscle action potential in the fibularis tertius muscle. Anatomic landmarks are T, major trochanter; St, stifle.

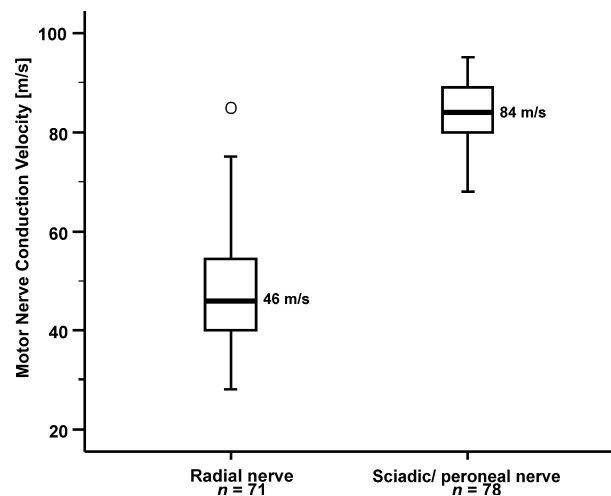


Fig 2. Box plotting of the motor nerve conduction velocity in 20 calves. mNCV, motor nerve conduction velocity; \top , maximum; \perp , minimum; —, median; O, outlier; n, number of measurements per nerve. The box includes 50% of the values.

The analysis identified greater influences of the individual calf and the time of measurement on different days for the radial nerve (vc for calf, 20.1; vc for time,

7.5) rather than for the sciatic/common peroneal nerve (vc for calf, 3.6; vc for time, 0.3). Therefore, we concluded that mNCV measurements of the sciatic/peroneal nerve are much more reliable than those of the radial nerve.

Morphometric Evaluations of Nerve Biopsies

To correlate the electrophysiologic data with morphological parameters, nerve biopsies of 6 calves (group 2) were taken. The samples were labeled “calf A–E” (abbreviated CA, CB, etc.) and 2 slices per calf, each containing 1 fascicle, were evaluated, apart from calf C, where only 1 slice was available for morphometric analysis. Figure 3 shows the typical appearance of one fascicle used for measuring the fascicle area, the

total fiber count, axon area, and thickness of the myelin sheath.

The fascicle area of the 11 evaluated fascicles ranged from 25,066 to 119,591 μm^2 (median, 39,363 μm^2) and the range for the total fiber number per fascicle from 117 to 1,247 (median, 248) fibers. On the basis of these parameters, the fiber density of each fascicle was calculated (total fiber count divided by fascicle area). The fiber density ranged from 2,545 to 11,940 fibers/ mm^2 .

Data obtained from the measurements (thickness of the myelin sheath, axonal area/diameter, fiber area/diameter, g-ratio) of each calf are summarized in Table 1. Axon diameter, fiber area, and fiber diameter were calculated from the measured parameters assuming that axon, fiber, and myelin sheath are circular in shape. As a parameter for the myelination of axons,

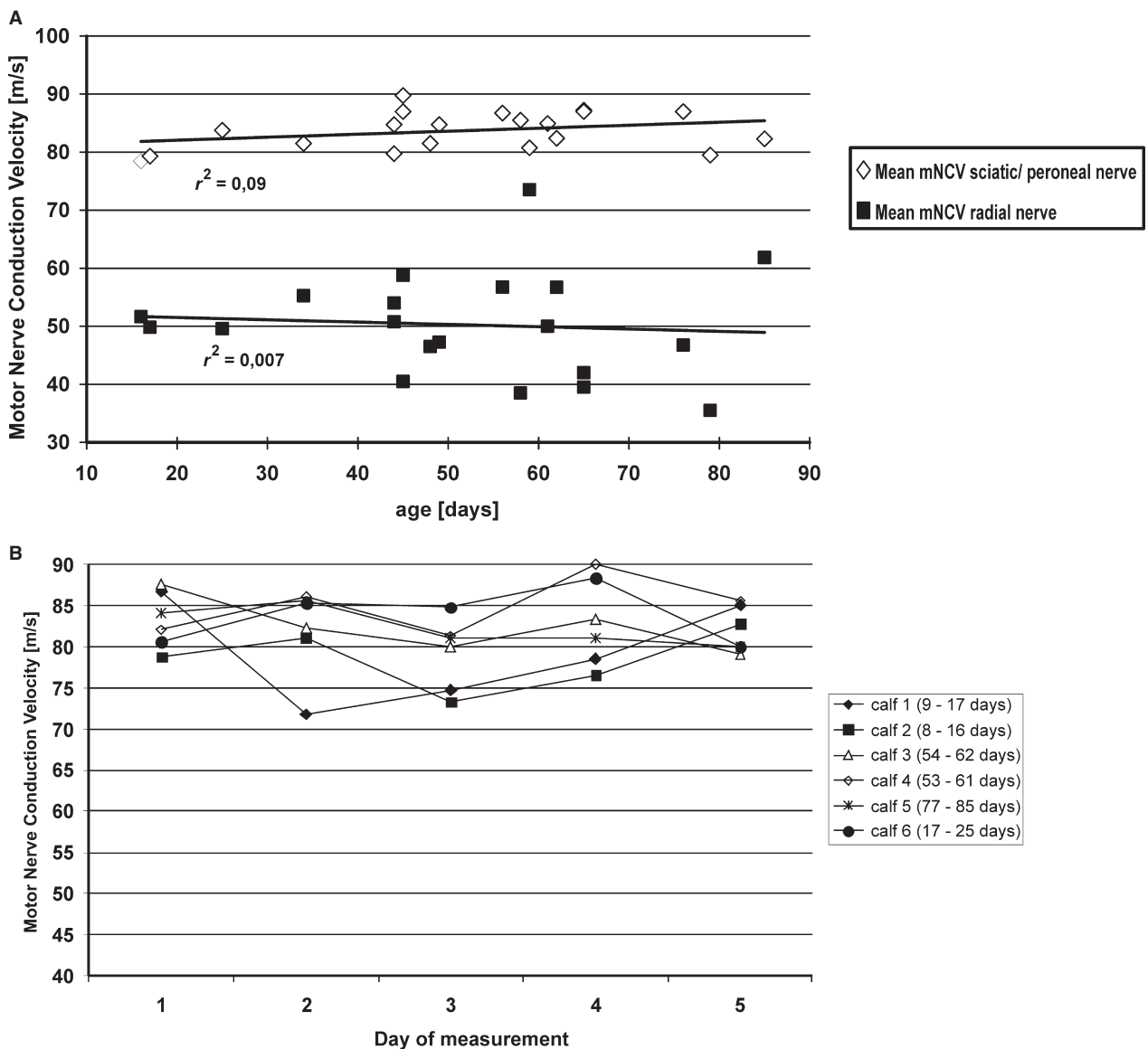


Fig 3. (A) Correlation between age and motor nerve conduction velocity. mNCV, motor nerve conduction velocity; r^2 , correlation coefficient. (B) Repeated measurements of the motor nerve conduction velocity of sciatic/peroneal nerve of 6 calves on 5 consecutive days. mNCV, motor nerve conduction velocity, the age of the calves is shown in brackets; d, day.

Table 1. Obtained centralized data of 11 (n = 11) slices from 6 calves (group 2).

Parameter	Pooled Data of 6 Calves
Axon diameter (μm)	
Mean	5.17
SD	2.35
Min.	0.95
Max.	14.91
Fiber diameter (μm)	
Mean	8.40
SD	2.80
Min.	1.98
Max.	17.90
Axon area (μm^2)	
Mean	25.29
SD	22.62
Min.	0.71
Max.	174.46
Fiber area (μm^2)	
Mean	61.57
SD	39.62
Min.	3.09
Max.	251.44
Myelin thickness (μm)	
Mean	1.62
SD	0.64
Min.	0.38
Max.	3.20
g-ratio	
Mean	0.61
SD	0.04

SD, standard deviation; min., minimum; max., maximum.

the g-ratio also was calculated from the 11 samples (Table 1). The g-ratio ranged from 0.55 to 0.68, with a mean of 0.61 ± 0.04 SD. No differentiation between smaller ($<4 \mu\text{m}$) and larger fibers was made for the calculations in this study. The average g-ratio of each calf was correlated with the corresponding mNCV measured in the segment the biopsy was taken from and had a 40.44% correlation (Fig 4).

To visualize the distribution and frequency of the evaluated morphometric parameters, bar histograms were plotted for axon and fiber diameter, axon and fiber area and myelin sheath thickness (Fig 5A–E). These frequency histograms represent the pooled data of the 6 calves.

Discussion

To evaluate the relationship between structure and function of the peripheral nervous system in cattle, morphometric and neurophysiologic parameters of peripheral nerves were investigated. In the first part of the study, the function of the peripheral nerves in cattle was studied by measuring mNCV of the radial and the sciatic/peroneal nerve in 20 healthy calves. Because of technical reasons, we were not able to measure the limb temperature. As an alternative, we used rectal temperature to account for temperature decrease under anesthesia over time. Because anesthesia never took

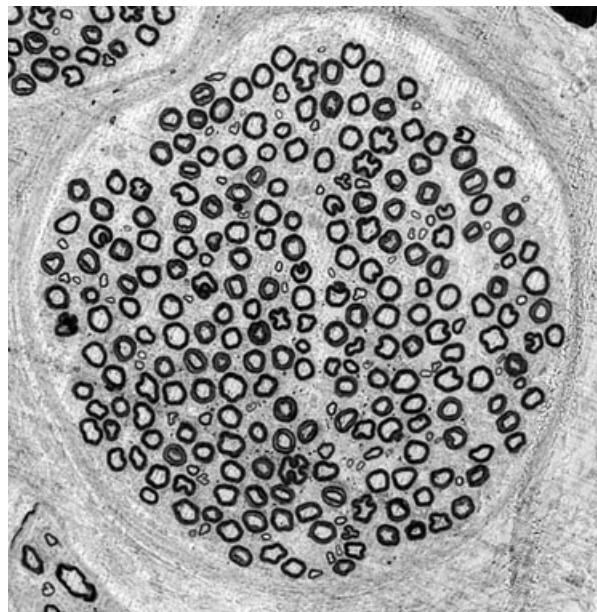


Fig 4. Transverse section of a typical fascicle from peroneal nerve, paraphenyldiamine staining of a biopsy specimen of peroneal nerve (calf E), 400 \times magnification.

>30 minutes, the influence of the mild temperature decrease on mNCV was minimal. The stimulus intensity in 2 single stimulations was relatively high compared to the common supramaximal stimulus intensity (15–40 mA) in our laboratory. The explanation may be improper positioning of the stimulation electrodes or other technical difficulties. These 2 stimulations were excluded from further analysis. The mean mNCV of the radial nerve was 48.3 ± 10.6 m/s and that of the sciatic/peroneal nerve 83.8 ± 5.9 m/s. An analysis of variance identified a highly significant difference in mNCV between the 2 evaluated nerves, indicating that the sciatic/peroneal nerve has a faster mNCV than the radial nerve. This finding is not unique to cattle and also has been described in cats, dogs, and sheep.^{16,23–25} Especially in sheep, comparable large discrepancies between mNCV of radial and sciatic nerve have been described.¹⁶ Age and side of stimulation had no influence on measured mNCV in the examined group of calves. Repeated measurements were performed to identify interindividual and environmental effects, such as stable temperature or artifacts caused by atmospheric electromagnetic noise, and to evaluate the reliability of the method used. Data of the repeated measurement were investigated by analysis of variance. This statistical model demonstrated that the individual calf and environmental effects of the measurement day had greater impact on the acquisition of the mNCV of the radial nerve than on the acquisition of the mNCV of the sciatic/peroneal nerve. It can be concluded that the measurements of the sciatic/peroneal nerve result in more reliable values for mNCV than measurements of the radial nerve. A possible explanation for this observation could be the short distance between the proximal and distal stimulation sites for the radial

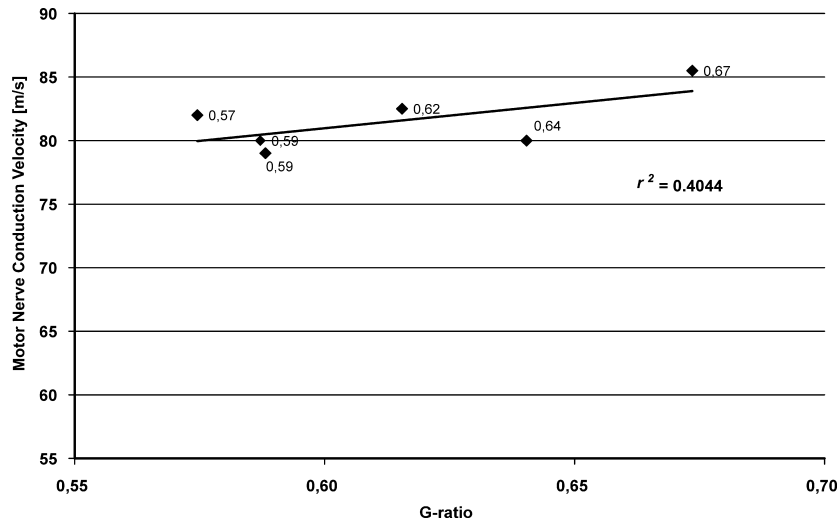


Fig 5. Correlation of g-ratio with the motor nerve conduction velocity in 6 calves. r^2 , correlation coefficient. A correlation of 40.44% of the g-ratio to the motor nerve conduction velocity is demonstrated by the correlation line, indicating that a good myelination of the peripheral nerve (low g-ratio) correlates positively with an increasing conduction velocity. mNCV, motor nerve conduction velocity.

nerve (4–7 cm). An ideal distance between the 2 stimulation points is at least 10 cm to decrease possible distance measurement failures.²⁶ Distances >10 cm improve the reliability of mNCV evaluations.^{27,28} However, because of the anatomic conditions in calves, it was not possible to increase the distance between the 2 stimulation sites of the radial nerve. Another possible explanation would be an underestimation of the actual length of the measured radial nerve segment, which would lead to a falsely lower mNCV. Dissections of the nerve would have been necessary to assess this possibility. Additional nerve biopsies of the dissected nerve would have allowed a morphological (eg, fiber diameter, myelination, g-ratio) comparison between radial and sciatic/peroneal nerve. These examinations (dissection and biopsy) should be part of future research to clarify the reason for the mNCV discrepancy between radial and sciatic/peroneal nerves. These minor limitations and the broader reference

range for the radial nerve mNCV must be considered in the diagnostic use of the obtained reference values. The reliability analysis of our results performed by repeated measurements and the statistical model of variance component analysis supported the accuracy of the obtained reference values for mNCV in calves. Because measured mNCV of the animals in group 1 were relatively high, the measurements should be further validated using a second morphology-based approach. The age of the calves in group 2 was chosen to provide a relatively homogeneous population. Examining calves in this age group should prove our hypothesis, namely that cattle display early maturation of locomotion considering morphology and electrophysiology.

Comparing the collected mNCV of calves with reference ranges of mNCV of other species such as dogs, cats, and humans of similar age, the results in cattle appear relatively high^{12,25,29} (Table 2). Moreover, the

Table 2. Morphometric parameter and nerve conduction velocities of different species compared with calves.

Species, Nerve, and Age	Fiber Diameter (μm)					g-Ratio		NCV (m/s)		
	Mean	SD	Median	Min.	Max.	Mean	SD	Mean	SD	Author
Calves, N. fibularis, 0.06 years (Current study)	8.4	2.8	8.24	2	17.9	0.61	0.04	83.8	5.9	Current study
Horse, N. palmaris lateralis, 0.5 years ¹⁵	8.12	6.68	7.71	2	14	0.65	0.0067	48		Wheeler ¹⁴
Dog, N. fibularis, 0.25 years ³⁷	3.82	0.239		1	12			50.07	1.31	Sims et al ¹²
Sheep, N. fibularis, 6 days before birth ¹⁷	5.62	0.16		2.8	12			50.3	1.8	Same as left
Human, N. suralis, 2.0 years ¹⁰				2	12	0.65–0.75 ^a		38		Hopf et al ^b
Cat, N. tibialis, adult ⁷				1	18			95.4	10.8	Pillai et al ²⁵
Sheep, N. fibularis, adult ¹⁶				1	18			103.9	12.7	Same as left

NCV, nerve conduction velocity, depending on the nerve if motor or sensory; N., nervus; min., minimum; max., maximum; SD, standard deviation.

^aRange instead of mean and standard deviation.

^bHopf HC, Dengler R, Röder R, Vogt T. *Elektromyographie-Atlas*. Stuttgart, New York: Georg Thieme; 1996

obtained mNCV of calves are higher than the mNCV ranges of adult dogs and humans.^{23,29} This phenomenon can be explained by the influence of 2 crucial factors.

The first influencing factor is the measurement method, especially positioning of the electrodes for recording the CMAP in the muscle and the type of electrodes used for recording as described by Steiss and Argue in 1987. In that study the mNCV (measured in radial, tibial, and peroneal nerves) of adult dogs and sheep are compared to each other.¹⁶ Sheep had a mean mNCV in the peroneal nerve of 103.9 ± 12.7 m/s, which was on average 35 m/s faster compared with the mNCV seen in the peroneal nerve of dogs. The difference in mNCV between these 2 species may be based upon anatomic variation caused by digitigrade (dogs), versus unguligrade locomotion (ruminants). This interspecies difference has the consequence that no distal limb equivalents to the interosseous muscle in dogs or the extensor digitorum brevis muscle in humans are available to record CMAPs in ruminants. Therefore, it is necessary in these animals to record CMAPs from more proximal muscles such as the tibialis cranialis or the fibularis tertius.^{16,30} However, the more proximal positioning of the recording electrode has an influence on the measured mNCV. The conduction is faster in proximal segments of nerves.^{31,32} Recordings in dogs with a more proximally positioned recording electrode resulted in a faster proximal mNCV of 88.1 ± 8.3 m/s.¹⁶ Because of anatomical peculiarities caused by unguligrade locomotion in calves, sheep, and goats, it is not possible to record CMAPs from muscles distal to the ankle. Only values originating from species with the same type of locomotion, same stimulation, and same recording sites are directly comparable. These anatomic variations make it essential to establish reference values for each species and measurement method.

The second crucial aspect for explanation of the relatively high mNCV reference values in calves is the morphological composition of the evaluated peripheral nerves. Large fibers are able to discharge at a faster rate than small ones.¹ Therefore, they are directly responsible for the first recorded signals of the CMAPs, and determine the calculated velocity.^{2,28,33} The myelination of nerve fibers by Schwann cells is responsible for the saltatory conduction of action potentials along the nerve,³⁴ and to a certain degree the extent of myelination directly affects mNCV.³⁴⁻³⁶ The internodal length increases with age during maturation of the individual,⁸ enhancing mNCV during maturation.^{4,25} This influence of internodal length is accompanied by an increase in the growing fiber diameter and myelin sheath thickness during maturation.⁸ Therefore, in the second part of this study, the structural components of the peripheral nerves influencing mNCV in calves were investigated. We focused on "fiber diameter" and "myelination of fibers" as the main influencing factors for mNCV. These morphological parameters were evaluated in cross-sections of peroneal nerve biopsy samples collected from 6 healthy

calves. In addition, fiber and axon area, axon diameter, and g-ratio (degree of myelination) were determined. The average fiber diameter of all calves ranged from 1.98 to 17.90 μm . The mean fiber diameter was 8.40 ± 2.80 μm and histograms indicated a slight bimodal distribution with a lower peak at 7.0 μm and an upper peak at 10.0 μm (Fig 6). In no other species were such high values found in immature individuals (Table 2). Morphometric studies in dogs (age, 0.25 years) or sheep (age, 6 days before birth) demonstrated maximal fiber diameters of 12 μm with corresponding mNCV of 50.07 m/s in dogs and 50.3 m/s in sheep.^{12,17,37} The fiber diameter of the palmaris lateralis nerve in 6-month-old horses reached maximum values of 14 μm with a corresponding sensory NCV of 48 m/s.^{14,15} The frequency histograms of the fiber diameter in calves demonstrated that 9.85% of the fibers had a diameter >12 μm and 2.55% of the fibers had a diameter >14 μm . Only in morphological studies in adult cats and sheep were fiber diameters of the same dimension as in this study obtained.^{16,38} The reference values of mNCV in the peroneal nerve established by electrophysiologic studies in adult cats (95.4 ± 10.8 m/s) and sheep (103.9 ± 12.7 m/s) were higher than those in calves.^{16,24} The frequency histograms in these 2 species demonstrate higher frequencies of fiber diameters thicker than 12 μm (sheep, in approximately 17% of all fibers are thicker than 12 μm ; cats, in approximately 23% of all fibers are thicker than 12 μm) explaining the higher mNCV. In summary, the collected data on fiber diameters offer an explanation for the mNCV in calves which is faster than that observed in young dogs, horses, and humans (Table 2). The thickness of the myelin sheath in calves ranged from 0.38 to 3.20 μm . The g-ratio is a suitable parameter for comparing myelin thickness of calves with data from literature. The reference range of the g-ratio varies between 0.8 and 0.33 for the suralis nerve of humans.^{10,39} Juvenile humans who are age-matched to the calves examined in this study demonstrated g-ratios between 0.65 and 0.75, whereas age-matched horses demonstrated a g-ratio of 0.65 ± 0.0067 .^{10,15} These 2 studies evaluated sensory nerves morphologically, which might limit comparability with this study. However, Lindemuth et al showed in humans that the examined morphometric parameters of myelinated fibers of sensory nerves were comparable with the respective parameters of mixed (motor and sensory) nerves.⁴⁰ The calves evaluated in this study, although juvenile, showed a higher degree of myelination compared with juvenile humans and horses.

Conclusions

In conclusion, we suggest that calves are much more mature after birth (eg, precocial) with respect to locomotion than similarly aged cats, dogs, and humans. This may relate to the differences among species in mother-offspring behavior and different biologic affiliation to precocial ungulate species (eg, horses, sheep,

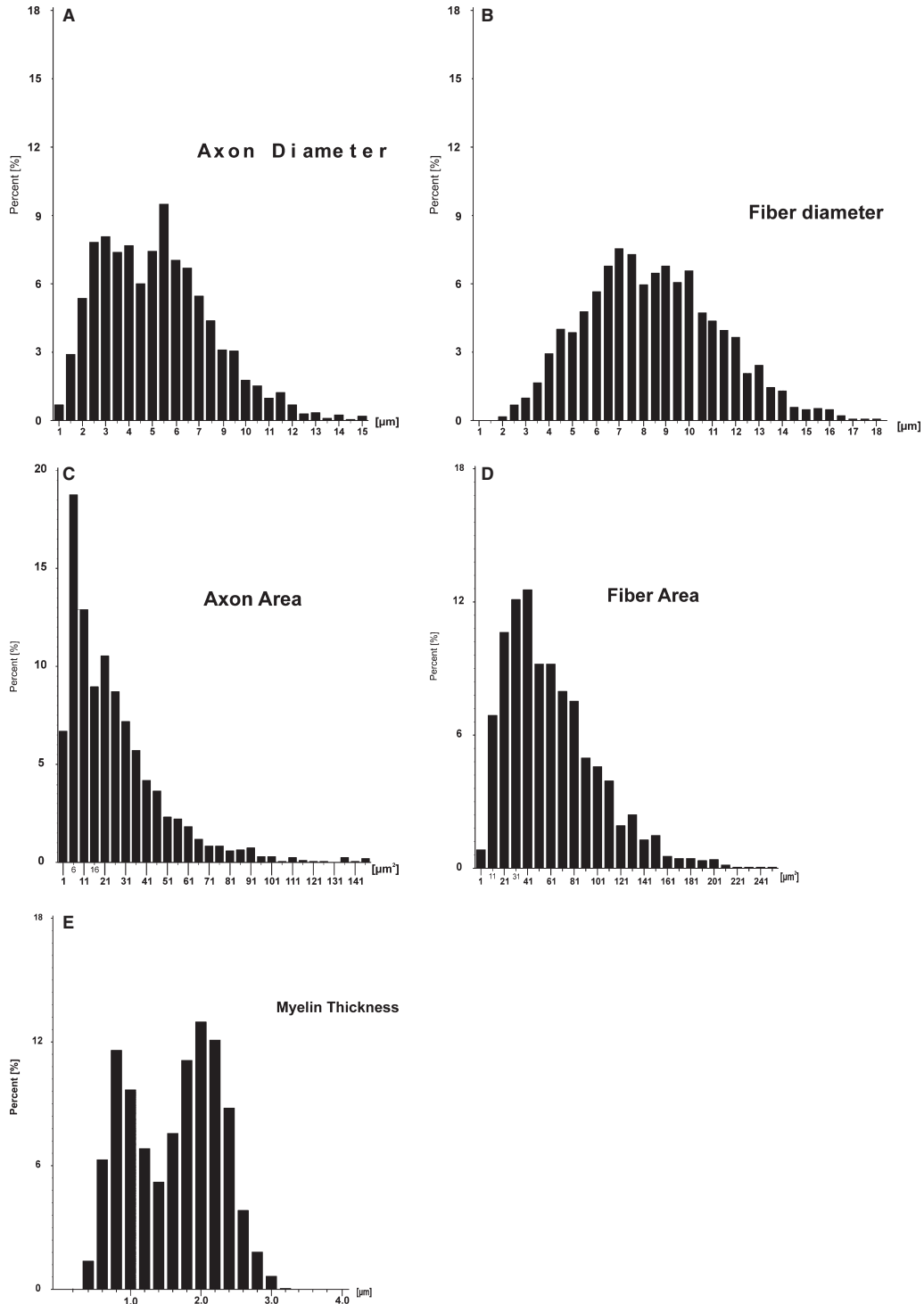


Fig 6. (A) Frequency distribution of the axon diameter (n = 6 calves), the axon diameters range from 0.95 to 14.91 μm; the mean value is 5.17 ± 2.35 μm. Histogram indicating a bimodal distribution with a lower peak at 3.0 μm and an upper peak at 5.5 μm. (B) Frequency distribution of the fiber diameter (n = 6 calves), the fiber diameters ranging from 1.98 to 17.90 μm; the mean value is 8.40 ± 2.80 μm. Histogram indicating a slight bimodal distribution with a lower peak at 7.0 μm and an upper peak at 10.0 μm. The included bars indicate the maximum fiber diameter recognized in the mentioned species. (C) Frequency distribution of the axon area (n = 6 calves), the axon areas ranging from 0.71 to 174.46 μm² (in 3 observations the axon area was greater than 146 μm², this being represented by the last bar on the right); the mean value is 25.29 ± 22.62 μm². Histogram indicating a monopolar distribution with a peak at 6 μm². (D) Frequency distribution of the fiber area (n = 6 calves) the fiber area ranging from 3.09 to 251.44 μm²; the mean value is 61.57 ± 39.62 μm². Histogram indicating a monopolar distribution with a peak at 41.0 μm². (E) Frequency distribution of the myelin thickness (n = 6 calves), myelin thickness ranging from 0.38 to 3.20 μm; the mean value is 1.62 ± 0.64 μm. Histogram indicating a bipolar distribution with a lower peak at 0.8 μm and an upper peak at 2.0 μm.

goats, cattle) and altricial predatory species (eg, cats, dogs, humans). Precocial species are born with a relatively high bw, good equilibrium, and well-developed locomotory abilities.⁴¹

The degree of maturity with respect to locomotion may be comparable with other precocial animals such as horses and sheep. However, it appears that calves are even more mature than these species because of measured mNCV and peripheral nerve morphometry, which presumably help calves to survive in the wild. The reference values for mNCV in calves established in this study are not solely of interest for evolutionary biology and physiologic research. These reference values of healthy calves also can be used for diagnostic evaluations of peripheral nerve diseases in calves.⁴²⁻⁴⁴ As shown in a previous epidemiologic study on neurologic disorders in ruminants, traumatic peripheral nerve injuries are the most frequently recognized neurologic disease in cattle.¹⁹

Footnotes

- ^a CP Pharma, Burgwedel Germany
^b Selectavet, Weyarn-Holzolling Germany
^c Draeger Titus MRT; Draeger Medical Techniques, Lübeck Germany
^d Nicolet EBE GmbH, Kleinostheim Germany
^e BX60; Leica GmbH, Solms, Germany
^f CCD camera; Olympus Photomicrographics System PM20 and Analy- SIS ProR, Version 3.1, Soft imaging System GmbH, Jena, Germany
^g SAS Institute Inc, Cary, NC
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