

Establishment of a Method to Measure Length of the Ulnar Nerve and Standardize F-wave Values in Clinically Normal Beagles

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ABSTRACT. We designed a new method of measuring the length of the ulnar nerve and determining standard values for F-wave parameters of the ulnar nerve in clinically normal beagles. Nerve length must be precisely measured to determine F-wave latency and conduction velocity. The length of the forelimb has served as the length of the ulnar nerve for F-wave assessments, but report indicates that F-wave latency is proportional to the length of the pathway traveled by nerve impulses. Therefore, we measured the surface distance from a stimulus point to the spinous process of the first thoracic vertebra (nerve length 1) and the anterior horn of the scapula (nerve length 2) as landmarks through the olecranon and the shoulder blade acromion. The correlation coefficients between the shortest F-wave latency and the length of nerves 1, 2 or the forelimb were 0.61, 0.7 and 0.58. Nerve length 2 generated the highest value. Furthermore, the anterior horn of the scapula was easily palpated in any dog regardless of well-fed body. We concluded that nerve length 2 was optimal for measuring the length of the ulnar nerve.

KEY WORDS: canine, central nerve, forelimb, latency, motor nerve

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F-wave examination is one method of measuring the performance of peripheral nerves, such as motor and sensory nerves. F-waves are usually clinically applied as a diagnostic test for peripheral nerve disease. Electrical stimulation is required to elicit F-waves. Stimulating a peripheral nerve causes antidromic conduction in motor nerves as far as the alpha motor nerve of the ventral horn of the spinal cord. Thereafter, motor nerves peripherally conduct impulses in the antegrade direction. F-waves comprise the myoelectric potential induced from a muscle through the myoneural junction [6, 9, 10, 16]. Abnormalities of nerves in a proximal portion of a stimulus site are undetectable in motor nerve conduction studies. In contrast, F-waves can reflect motor nerve conduction along the entire length of a motor axon as well as the function of the cell body of alpha motor nerves. Therefore, values for F-waves have contributed to assessments of neuropathies caused by disorders of the spinal cord or motor nerves [1, 4, 7, 8, 17, 19, 20].

F-waves have been measured, and their applicability has been evaluated in dogs. Knecht *et al.* established a stimulus with which to produce F-waves of the ulnar and tibial nerves of dogs [11]. Machida *et al.* described the applicability of

F-wave analysis to evaluations of the severity of experimentally induced myelopathy at the proximal portion of the spinal cord in dogs [12]. Moreover, F-wave evaluation can help to diagnose polyneuropathy [14] and polyradiculoneuropathy [3] in dogs as well as in humans.

Nerve conduction can be evaluated based on F-wave latency, which is the elapsed time between a stimulus artifact and the first F-wave, and it closely correlated with the length of the alpha motor nerve. Therefore, the length of the nerve must be precisely measured for accurate F-wave evaluation. Steiss *et al.* has identified a close correlation between F-wave latency and the length of the forelimb or hindlimbs [18]. Okuno *et al.* reported that F-wave latency correlates with the length of the tibial nerve measured along the pathway [15].

The ulnar nerve is generally used for assessing F-waves in the forelimbs of dogs. We measured the length of the ulnar nerve using two landmarks. We also measured the length of forelimb as described by Steiss [18]. We then compared correlation coefficients between the shortest F-wave latency and the length of each nerve or forelimb length to determine the optimal way to measure the length of the ulnar nerve for F-waves in dogs. We also determined normative values for F-wave parameters in the ulnar nerve of beagles.

MATERIALS AND METHODS

Animals: The right forelimbs of 21 clinically normal beagles (age, 1–7 years; weight, 7.3–15.2 kg; male, n=10, of which four were neutered; female, n=11, of which eight were spayed) that were free of neurological impairment or hematological abnormalities were assessed in strict accor-

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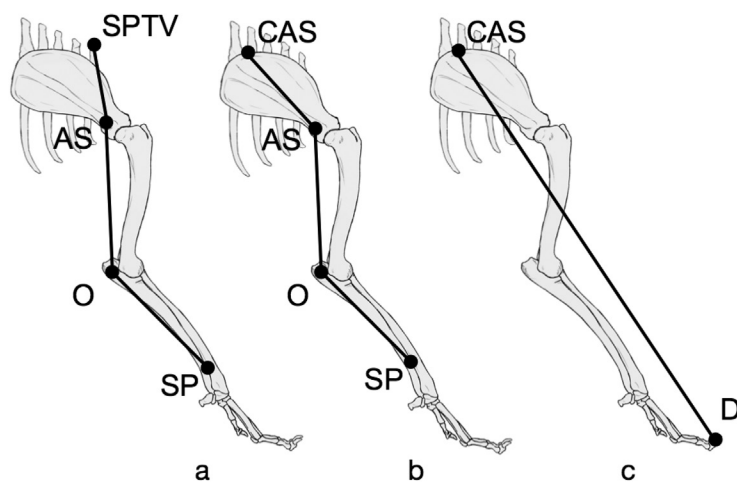


Fig. 1. Landmarks for measuring nerve lengths 1, 2 and forelimb length. Solid circles, landmarks. Solid line measured from body surface using a soft and flexible measuring tape. Nerve length was measured in neutral position (a, b). Forelimb length was measured at extended position (c). a, Nerve length 1; b, nerve length 2; c, forelimb length. AS, acromion of scapula; CAS, cranial angle of scapula; D, tip of third digit; O, olecranon; SP, stimulus point; SPTV, spinous process of first thoracic vertebra.

dance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Institute of Experimental Animal Sciences of Tokyo University of Agriculture and Technology approved the protocol (Permit Number: 24–87).

Procedures: All dogs were subcutaneously injected with 0.04 mg/kg of atropine sulfate (Atropine Sulfate Injection 0.5 mg; Mitsubishi Tanabe Pharma Corp., Osaka, Japan), then anesthesia was induced by the intravenous administration of 6 mg/kg propofol (Rapinivet; Intervet K.K., Tokyo, Japan), and a tracheal tube was inserted. Anesthesia was maintained throughout the experimental period by isoflurane inhalation (Isoflurane for Animal; Intervet K.K.). Rectal temperatures were monitored, and the dogs were maintained at temperatures between $\geq 37.0^{\circ}\text{C}$ and $< 38.8^{\circ}\text{C}$ using heated pads. All measurements were obtained within 15 min of anesthesia induction.

Measurement method: We used a Neuropack MEB-9102 polygraph system (Nihon Kohden, Tokyo, Japan) for electrical stimulation and a disk-electrode to measure the compound muscle action potential of a wide area. Percutaneously inserted needles for electrical stimulation were placed on the ulnar nerve immediately proximal to the carpal joint of the right forelimbs of dogs placed in the left lateral recumbent position. The cathode of the stimulating electrodes was located proximally and approximately 1 cm from the anode. Electric stimulation was applied as a rectangular wave under the following conditions: duration, 0.2 msec; frequency, 1 Hz; intensity, approximately 20% above the supramaximal stimulus for the M response; number of recorded responses, $n=32$. A surface disk-electrode was placed one-third distal over the interosseous muscle of the fifth digit, and action potentials of the muscles were measured and recorded. A reference disk-electrode was located on the dorsum of the

fifth digit, and evoked F-waves were stored on a polygraph.

The length (mm) of the nerve was measured at the body surface using a soft, flexible measuring tape. Anesthetized dogs were placed in the left lateral recumbent position in a state of muscular relaxation. The F-waves of the arms in humans are measured between a stimulating point and the spinous process of the seventh cervical vertebra. The ulnar nerve in dogs originates mainly from the eighth cervical and first thoracic nerves, and runs to the carpal region [2]. Therefore, we selected the cranial border of the spinous process of the first thoracic vertebra as a landmark. We measured the surface distance from the stimulus point to the cranial border of the spinous process of the first thoracic vertebra through the olecranon and the acromion of the scapula (nerve length 1; Fig. 1a). However, length measured from the body surface might exceed the length of the actual nerve. Moreover, the spinous process of the first thoracic vertebra might be difficult to palpate in an overweight dog. We thus selected the anterior horn of scapula as a second landmark, because it is easily palpated in any dog and it approximately corresponds to the intervertebral foramen between the seventh cervical vertebra and first thoracic vertebra at the origin of the eighth cervical nerve. We measured the surface distance from the stimulus point to the anterior horn of the scapula through the olecranon and the acromion of the scapula (nerve length 2; Fig. 1b). The length of each nerve was measured with the joint of the forelimb placed in a neutral position. The length (mm) of the forelimb was measured from the tip of the third digit to the anterior horn of the scapula in the extended position as described by Steiss [18] (forelimb length; Fig. 1c).

F-wave parameter analysis: We calculated the shortest F-wave latency. We considered the first deflection (positive or negative) as the F-wave. The shortest F-wave latency was the minimal amount of time among F-wave latencies ac-

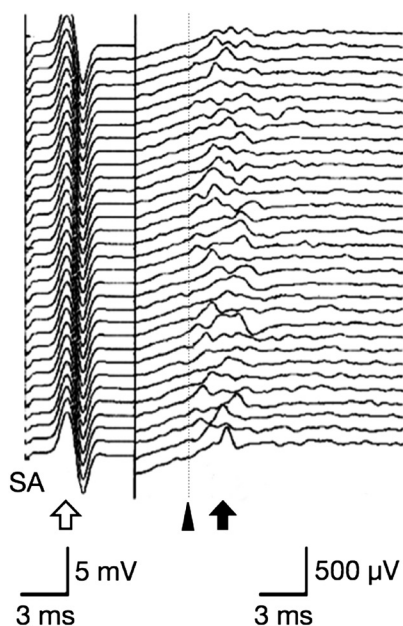


Fig. 2. M responses (outlined arrow) and the F-waves (solid arrow) recorded from ulnar nerve of clinically normal beagle. Recording sensitivity differs between M response and F-wave. F-wave form and latency varied. Shortest F-wave latency (black triangle) is from stimulus artifact (SA) to first deflection of F-wave among 32 stimuli.

quired by 32 stimuli. The shortest F-wave latency was plotted against nerve length 1, 2 or forelimb lengths. Correlation coefficients were determined using linear regression analysis with a least-squares method [16]. We evaluated difference among three values measured using the two landmarks and forelimb length. We postulated that the length of the ulnar nerve would be overestimated using nerve length 1 and forelimb length compared with nerve length 2. We compared the possibility of overestimation using Bland-Altman analysis. Data were statistically analyzed using GraphPad Prism version 5.0 (GraphPad Software Inc., La Jolla, CA, U.S.A.), and significance was set at $P < 0.05$.

We also determined normative values of other F-wave parameters comprising F-wave conduction velocity, F-wave persistence, F-wave peak-to-peak amplitude and the ratio of F/M amplitude in the ulnar nerve of beagles. F-wave conduction velocity based on nerve length (FWCV nerve) was calculated using the equation [15]:

$$\text{FWCV nerve 1 (or 2)} = \frac{\text{nerve length 1 (or 2) (mm)} \times 2}{(\text{shortest F-wave latency (msec)} - \text{M response latency (msec)} - 1)}$$

M response latency is the amount of time required to generate compound muscle action potentials.

F-wave conduction velocity based on forelimb length (FWCV limb) was calculated using equation [16]:

$$\text{FWCV limb (m/s)} = \frac{\text{forelimb length (mm)} \times 2}{(\text{shortest F-wave latency (msec)} - \text{M response latency (msec)} - 1)}$$

F-wave persistence was calculated by dividing the number of recorded F-waves by 32 (stimuli). F-wave peak-to-peak

amplitude was measured, and the ratio of F/M amplitude (%) was considered as the ratio of the average of the maximal F-wave and M-wave peak-to-peak amplitudes. All values are expressed as means \pm SD.

RESULTS

The anterior horn of the scapula was easily palpated as the landmark from the body surface in all beagles, whereas the spinous process of the first thoracic vertebra in overweight dogs was not.

Supramaximal electrical stimuli applied to the ulnar nerve elicited detectable F-waves in all dogs. F-waves were polymorphic, and the amplitude was far smaller than that of M-waves. The latency of F-waves varied, and the shortest latency was used to calculate F-wave conduction velocity (Fig. 2).

Figures 3–5 show linear regression analysis of the shortest F-wave latency and nerve lengths 1 and 2 or forelimb length, and the correlation coefficients were 0.61, 0.70 and 0.58, respectively ($P < 0.01$). The value generated using nerve length 2 was the highest. Each linear regression analysis yielded the following regression equations:

$$\text{Shortest F-wave latency (msec)} = (0.023 \times \text{nerve length 1}) + 3.26.$$

$$\text{Shortest F-wave latency (msec)} = (0.027 \times \text{nerve length 2}) + 2.68.$$

$$\text{Shortest F-wave latency (msec)} = (0.021 \times \text{forelimb length}) + 2.45.$$

The results of the Bland-Altman comparison indicated that nerve length 1 and forelimb length overestimated conduction distance compared with nerve length 2 (Fig. 6).

The normative value of shortest F-wave latency was 11.4 ± 1.0 msec, FWCV nerves 1, 2 and limb were 84.8 ± 6.9 , 79.4 ± 5.7 and 103.0 ± 9.3 m/s, respectively, F-wave persistence was $92.5 \pm 9.8\%$, F-wave peak-to-peak amplitude was $160 \pm 89 \mu\text{V}$, and the F/M amplitude ratio (%) was 1.3 ± 0.7 in the ulnar nerve of the beagles.

DISCUSSION

Among the shortest F-wave latencies (ms) and forelimb lengths (mm), the regression coefficient (0.021) in the present study was similar to that (0.022) reported by Steiss [18]. It was indicated that the present results agreed with their findings. We confirmed the accuracy of the present F-wave findings. The correlation coefficients between the shortest F-wave latency and the nerve lengths 1 and 2 or forelimb length were similar, but higher for nerve length than forelimb length. These findings were identical to those of the tibial nerve in dogs [15]. Furthermore, the correlation coefficient was higher with nerve length 2 than nerve length 1. The anterior horn of the scapula was easier to palpate than the spinous process of the first thoracic vertebra in overweight dogs. Therefore, the length of the ulnar nerve measured from the stimulus point to the anterior horn of scapula through the olecranon and the shoulder blade acromion (nerve length 2) was more accurate than that determined using the other two

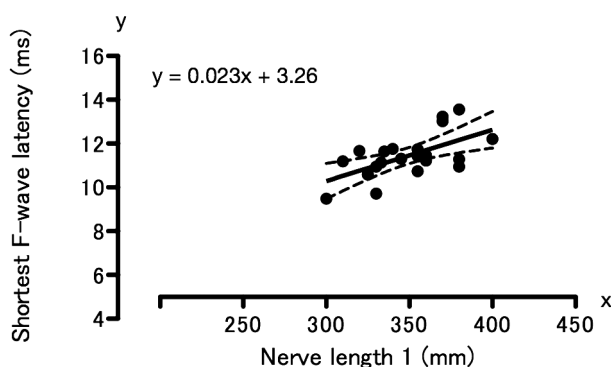


Fig. 3. Linear regression analysis of shortest F-wave latency and ulnar nerve length 1 in 21 clinically normal beagles. Correlation coefficient, 0.61; $P < 0.01$ (analyzed by linear regression using least-squares method). Solid line, regression line; dotted lines, 95% of confidence intervals; solid circles, values from one dog.

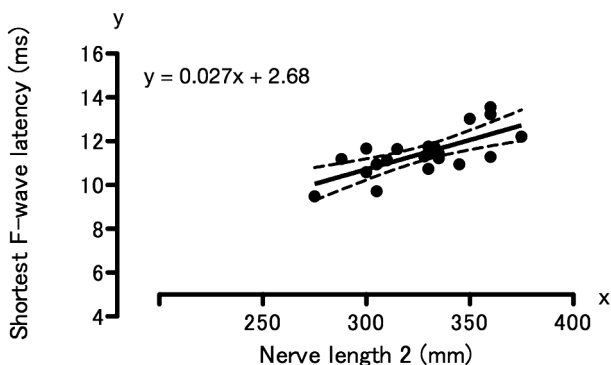


Fig. 4. Linear regression analysis of shortest F-wave latency and ulnar nerve length 2 in 21 clinically normal beagles. Correlation coefficient, 0.70; $P < 0.01$ (analyzed by linear regression using least-squares method). Solid line, regression line; dotted lines, 95% of confidence intervals; solid circles, values from one dog.

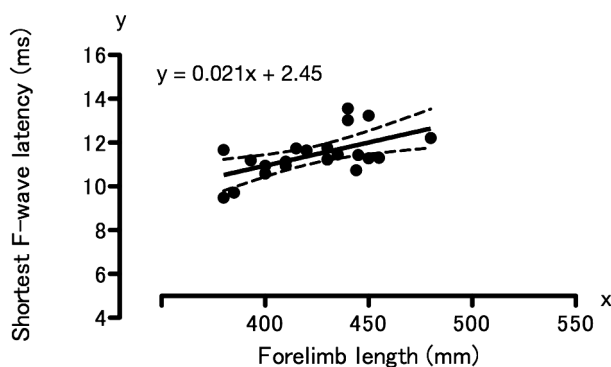


Fig. 5. Linear regression analysis of shortest F-wave latency and forelimb length in 21 clinically normal beagles. Correlation coefficient, 0.58; $P < 0.01$ (analyzed by linear regression using least-squares method). Solid line, regression line; dotted lines, 95% of confidence intervals; solid circles, values from one dog.

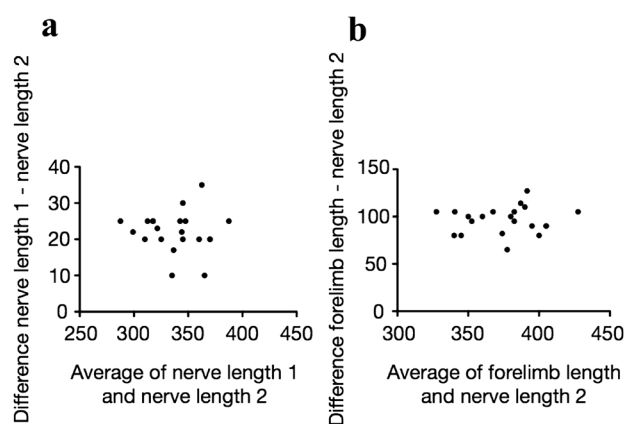


Fig. 6. Bland-Altman comparison. Differences between lengths of (a) nerves 1 and 2 and between lengths of (b) forelimb and nerve 2. Solid circles, values from one dog.

methods. We concluded that the length of the ulnar nerve was determined more accurately using nerve length 2. This is important, because nerve length is a critical factor in assessments of nerve conduction velocity. We also proposed based on the mean \pm 2SD of nerve length 2 that the upper and lower limits of the normal range for F-wave conduction velocity are 68.0 and 90.8 m/s, respectively.

However, the correlation coefficient was 0.7 at the maximum. We did not find any correlations with age, body weight, build, anesthesia and type of landmark used to measure nerve length in the dogs beside 95% confidence intervals. We measured nerve length in only beagles. It might be insufficient as a population and have influenced the correlation coefficient. Nerve length and F-waves should be assessed in small and large dogs. However, all values were within normal ranges.

F-wave data differ between the ulnar and tibial nerves of humans. F-wave conduction velocity is faster (65 vs. 53 m/s), and F-wave persistence is lower (70–100 vs. 100%) in the ulnar, than the tibial nerve [13]. We also found that F-wave conduction velocity in the ulnar nerves using the length of nerves 1 and 2 and forelimb (84.8 ± 6.9 , 79.4 ± 5.7 and 103.0 ± 9.3 m/s, respectively) was faster than that in the tibial nerve reported in a previous study of dogs (77.98 ± 8.62 m/s) [15], and F-wave persistence (92.5 \pm 9.8%) was lower than that in the tibial nerve reported in a previous study of dogs (100%) [15]. Thus, F-waves should be examined in individual target nerves in dogs to accommodate differences in F-wave parameters among nerves.

Human neurological function is determined from F-wave conduction velocity, F-wave persistence, F-wave peak-to-peak amplitude, the F/M amplitude ratio and the F-ratio in addition to F-wave latency [5–7, 13, 21]. These parameters can estimate conduction function in motor nerves, the rate of the motor neuron pool activated by an electrical stimulus and the number of target motor nerve cell bodies [13]. Unlike the shortest F-wave latency, F-wave conduction velocity is fixed regardless of the size of an animal [15]. The amplitude of muscle action potential, such as M-wave amplitude and F-

wave peak-to-peak amplitude, changes with the position of the surface and reference-disk electrode and with the thickness of the tissues between the electrode and muscle, fat and skin. However, these factors have little effect on the F/M amplitude ratio, because the amplitude of the F-wave is comparable with that of the M-wave measured under the same conditions. F-wave parameters in the dog, such as shortest F-wave latency, F-wave conduction velocity, F-wave persistence, F-wave peak-to-peak amplitude and F/M amplitude ratio, have also been useful in assessing spinal cord involvement, polyneuropathy and polyradiculoneuropathy [3, 12, 14]. Therefore, we established F-wave parameters of the ulnar nerve in beagles. Changes in tissue temperature influence F-waves, because they pass through the entire length of the peripheral axons. Although we avoid cooling the forelimb by maintaining rectal temperatures [15, 18] and completing all measurements within 15 min of anesthesia induction, tissue temperature should be monitored. F-ratio is a two-latency ratio that compared proximal and distal nerve conduction. The F-ratio, which is $(F-M-1)/2M$, where $(F-M-1)/2$ represents the conduction time from the stimulus site at the elbow joint to the spinal cord, and M is that taken from the remaining distal nerve segment to the muscle [10]. We plan to establish a standard value for the F-ratio in dogs to collect basic data about F-waves.

We established a new method of measuring the length of the ulnar nerve and determining standard values for F-wave parameters in the ulnar nerve of beagles. These standard values are applicable to F-wave examinations of the ulnar nerve in dogs and should be applicable to the diagnosis and evaluation of strategies to treat diseases of the peripheral nervous system, such as diabetic neuropathy, hypothyroid neuropathy and polyradiculoneuropathy and of the central nervous system, such as cervical intervertebral disk herniation, cervical vertebral instability and degenerative myelopathy. By carrying out clinical application of this method and accumulating clinical data, the usefulness of the electrophysiological study in veterinary medicine will be established.

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