Quantitative analysis of motor unit action potentials in the subclavian muscle of healthy horses

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Objective—To evaluate the application of analysis of motor unit action potentials (MUAP) in horses and to obtain values of MUAP for the subclavian muscle of horses.

Animals—10 healthy adult Dutch Warmblood horses.

Procedure—Electromyographic examination of the subclavian muscle in conscious nonsedated horses was performed to evaluate insertional activity, spontaneous activity, MUAP variables, and recruitment patterns. Muscle and body temperatures were measured at the beginning and end of the procedure. Amplitude, duration, number of phases, and number of changes in direction (ie, turns) for all representative MUAP were analyzed to determine values for this muscle in this group of horses.

Results—Mean ± SD duration of insertional activity was 471.7 ± 33.45 milliseconds. Mean MUAP amplitude in the examined horses was 379 μV (95% confidence interval [CI], 349 to 410 μV). Mean MUAP duration of the subclavian muscle was 7.27 milliseconds (95% CI, 6.84 to 7.71 milliseconds). Mean number of phases was 2.9, and mean number of turns was 3.0. Prevalence of polyphasic MUAP, defined as MUAP with > 4 phases, was 7.7%. Number of MUAP that had > 5 turns was 2.4%. Satellite potentials were found in 1.0% of the MUAP.

Conclusions and Clinical Relevance—This study revealed that electromyography including MUAP analysis can be performed in horses, and values for the subclavian muscle in healthy adult horses can be obtained. Analysis of MUAP could be a valuable diagnostic tool for use in discriminating between myogenic and neurogenic problems in horses. (*Am J Vet Res* 2002;63:198–203)

In horses, it can be difficult to discriminate between myogenic and neurogenic muscle atrophy. It is not always easy to conduct an examination of the nervous system in horses, and interpretation of results can be difficult or subjective.[1] **Electromyography (EMG)** is routinely used for diagnosis of neuromuscular disorders in humans. Quantitative analysis of **motor unit action potentials (MUAP)** was first used in the 1950s.[2] Since then, the technique has been developed to retrieve the maximum amount of detail about the disease processes being investigated.[3-11] If the technique can be applied to horses, it would provide a more objective examination method for discriminating between neurogenic and myogenic problems.

In EMG, electrical activity generated by muscle fibers is recorded, analyzed, and interpreted. Variables of interest include insertional activity, spontaneous activity, shape of action potentials for a single motor unit, and recruitment patterns. Insertional activity is spontaneous activity generated by mechanical effects on muscle fibers attributable to insertion of the EMG needle and can be inapparent or prolonged depending on the underlying abnormality.[12-15] After cessation of activity attributable to insertion of the needle, resting muscle is electrically silent. Spontaneous activity in normal resting muscle is evident in the end-plate region and is caused by spontaneous release of acetylcholine in this region (end-plate noise) or by discharges of muscle fibers secondary to mechanical activation of the nerve terminal (end-plate spikes).[12-15] Pathologic spontaneous activity can result from membrane instability as a result of myogenic or neurogenic activity. Some types of spontaneous activity are considered neurogenic, such as neuromyotonic discharges and doublets; however, spontaneous activity such as fibrillation potentials and positive sharp waves can be detected in both types of disorders (ie, neurogenic and myogenic).[4,9,10,13-17]

Shape of the MUAP combined with information about spontaneous activity and recruitment patterns enables investigators to discriminate between neurogenic and myogenic disorders.[4,9,10,13-17] When EMG is performed in horses,[18,19] the main emphasis has been on detection of pathologic spontaneous activity of muscle fibers, and systematic MUAP analysis has not been described. Therefore, it appears that the full potential of EMG has not yet been explored in horses. The study reported here was conducted to determine whether MUAP analysis can be applied to horses and to acquire values for the normal subclavian muscles of healthy horses.

Materials and Methods

Horses—Ten healthy adult Dutch Warmblood horses (4 geldings and 6 mares) were used in the study. Horses were 4 to 10 years old (mean ± SD, 7.0 ± 1.73 years old) and weighed between 540 and 703 kg (mean, 608 ± 43.7

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kg). Height of horses, as measured to the top of the shoulder, ranged from 1.62 to 1.71 m (mean, 1.67 ± 0.03 m). Horses were owned by the Faculty of Veterinary Medicine and used for educational and recreational purposes. They did not have a history of health problems, and none had received medication.

Collection of data—Each horse was weighed, and its height was measured. Then, each horse was restrained in stocks. Rectal and intramuscular temperatures were recorded. Rectal temperature was recorded by use of an electronic thermometer.[a] Intramuscular temperature was measured in the subclavian muscles. A small bleb of lidocaine hydrochloride was administered in the subcutaneous tissues over the right subclavian muscle, and a probe[b] was inserted through a 16-gauge 60-mm needle into the muscle. The probe was connected to a thermometer,[c] and intramuscular temperature was recorded. The MUAP then were recorded in the left subclavian muscle, which was followed by injection of lidocaine, insertion of the probe, and measurement of the intramuscular temperature in the left subclavian muscle. This method was chosen to prevent local anesthetic or damage of muscle fibers as a result of insertion of the temperature probe from interfering with recording of MUAP.

Electromyographic examination of the left subclavian muscle was conducted in all horses. A portable EMG apparatus[d] was connected to a portable computer[e] to record and store signals. A portable recorder[f] was connected to the console to enable recording and storage of the complete EMG signal. Activity in resting muscle was recorded with an amplifier gain of 50 µV/division and a sweep speed of 20 ms/division. Filter settings were 5 Hz and 10 kHz. For recording of MUAP, amplifier gain was 100 to 500 µV/division, depending on the size of obtained MUAP, and sweep speed was set at 20 ms/division. Recordings were made, using a disposable concentric 26-gauge EMG needle[g] (length, 50 mm; diameter, 0.45 mm; sampling area, 0.068 mm^2). A surgical pad was attached to the horse, using a girdle, and connected to the preamplifier to serve as the ground electrode.

Insertional activity was determined at least 3 times. Spontaneous activity was measured after insertion of the EMG needle. The MUAP were generated by voluntary contraction of the subclavian muscle; contractions occurred spontaneously or were induced by movements of the horse (eg, bearing weight on the left forelimb). The EMG needle was inserted in at least 6 locations in the left subclavian muscle, and recordings were obtained for detection of sharp-sounding MUAP while the needle was being withdrawn in 3-mm increments. This technique was used to ensure that recordings were obtained throughout the entire muscle.

Recordings were partially semiautomatic, because the program recognized MUAP of the same configuration, using a trigger line that identified identical MUAP that were greater than a selected amplitude. Because the program apparently had difficulty in identifying approximately half of the MUAP, these MUAP were measured from a stored epoch. The MUAP that were identified by the machine had to be verified, and measurements were corrected as necessary.

Definition of variables—Duration of insertional activity was measured semiquantitatively from the initial insertion of the needle to the end of the activity generated by intramuscular movement of the needle plus insertional activity. End-plate noise was defined as an irregular baseline caused by monophasic small negative waves; end-plate spikes were defined as rapid irregular discharges in the same region. Amplitude of each superimposed MUAP was measured from maximum negative value (in relation to baseline value) to maximum positive peak value (peak-to-peak value). Duration was measured from the initial deflection from baseline to final return to baseline. This point of final return was selected on the basis of identical shape of the waveform of the superimposed MUAP. A phase was defined as the part of the signal between each crossing of the baseline and counted as the number of baseline crossings plus 1. A change in direction (ie, turn) was considered to have occurred independent of crossing the baseline.[10] The time for increase of MUAP (ie, risetime), was the duration of the fastest positive (or negative) deflection and was measured automatically.[10,15] Polyphasic MUAP were defined as MUAP with > 4 phases, whereas complex MUAP were

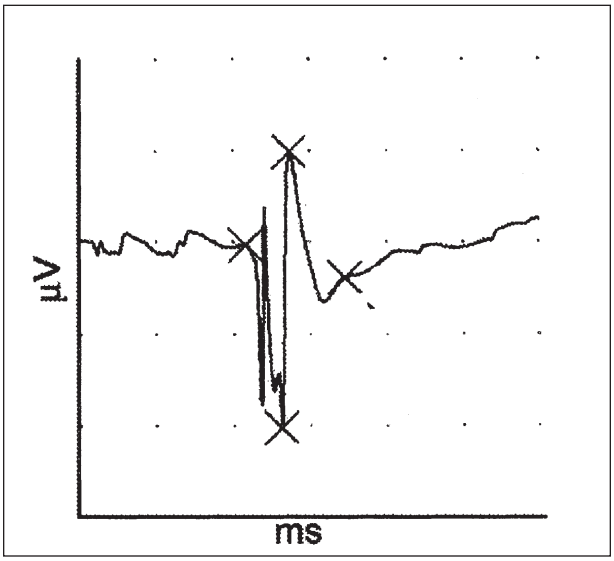

Figure 1—Electromyographic recording of the left subclavian muscle in a healthy adult horse revealing polyphasic complex motor unit action potentials (MUAP). Notice the 5 phases and 7 turns. Electromyography was conducted, using 200 µV/division and 5 ms/division. Important events in the MUAP are indicated (X).

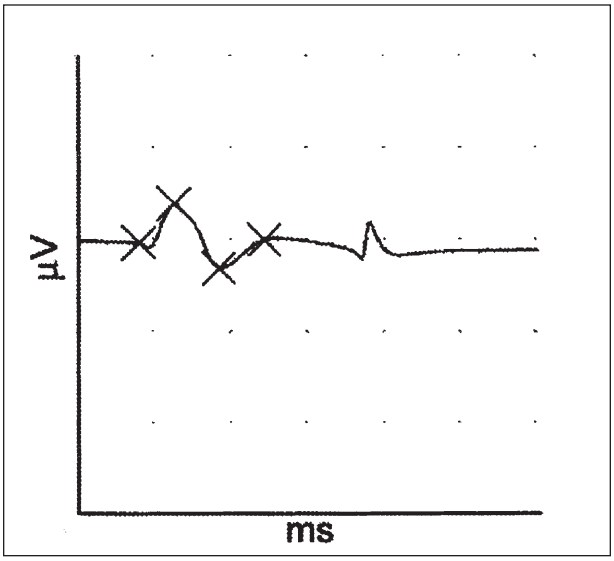

Figure 2—Electromyographic recording of the left subclavian muscle in a healthy adult horse, revealing a MUAP followed by a satellite potential. Electromyography was conducted, using 200 µV/division and 5 ms/division. *See* Figure 1 for key.

defined as MUAP with > 5 turns (**Fig 1**). A satellite potential was a late component in close relationship to the main potential (**Fig 2**). Resulting interference patterns were categorized as a single pattern, poorly mixed pattern, well-mixed pattern, or interference pattern (**Fig 3**).

Statistical analysis—Only representative MUAP with a maximal risetime of 0.80 milliseconds were used for statistical analysis. A representative MUAP was defined as MUAP that could be recorded with an identical superimposed shape at least 4 times. At least 20 representative MUAP were analyzed. Amplitude, duration, number of phases, number of turns, and risetime were determined. Finally, recruitment of additional motor units during activation of the muscle with increasing strength was evaluated.

Descriptive statistics were used to characterize measured variables. Correlations between variables were assessed by use of Pearson correlation coefficients. A Student 2-tailed *t*-test was used to compare temperature measurements. Normality of the data was analyzed, using the Kolmogorov-Smirnov test. Significance was set at $P < 0.05$.

Results

Mean ± SD intramuscular temperature of the subclavian muscles did not differ significantly between the beginning (37.2 ± 0.11 C) and end (37.3 ± 0.27 C) of the recording period, but mean rectal temperature was higher, but not significantly so, at the end of the recording period (38.3 ± 0.23 C), compared with rectal temperature at the beginning of the recording period (37.9 ± 0.29 C). Intramuscular temperature at the beginning of the recording period was positively correlated ($r = 0.84$; $P = 0.002$) with rectal temperature at the beginning of the recording period. We did not detect a significant correlation between rectal or intramuscular temperatures and MUAP amplitude, MUAP duration, number of phases, and number of turns.

Data for insertional activity and all MUAP variables were normally distributed ($P < 0.001$). Mean ± SD dura-

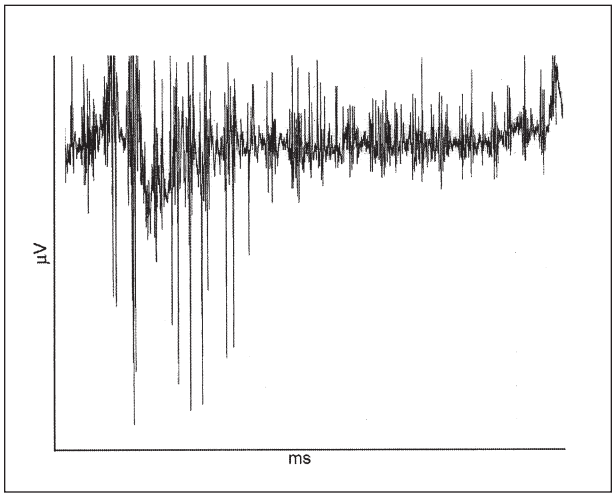

Figure 3—Electromyographic recording of the left subclavian muscle in a healthy adult horse revealing an interference pattern induced by strong muscle contraction. Electromyography was conducted, using 200 μV/division and 200 ms/division.

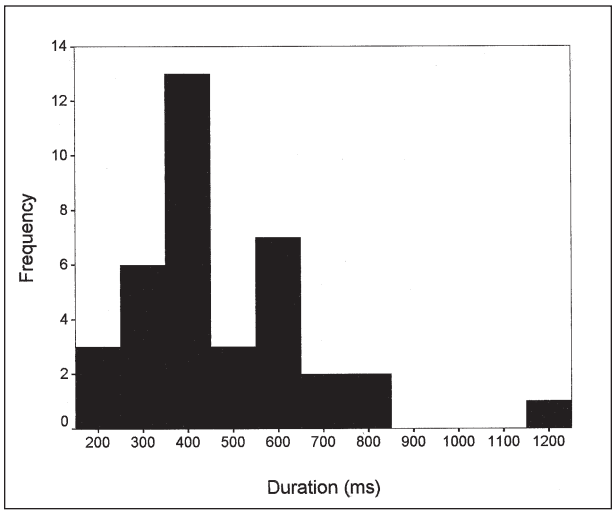

Figure 4—Histogram for duration of insertional activity obtained during electromyography of the left subclavian muscle in 10 healthy adult horses.

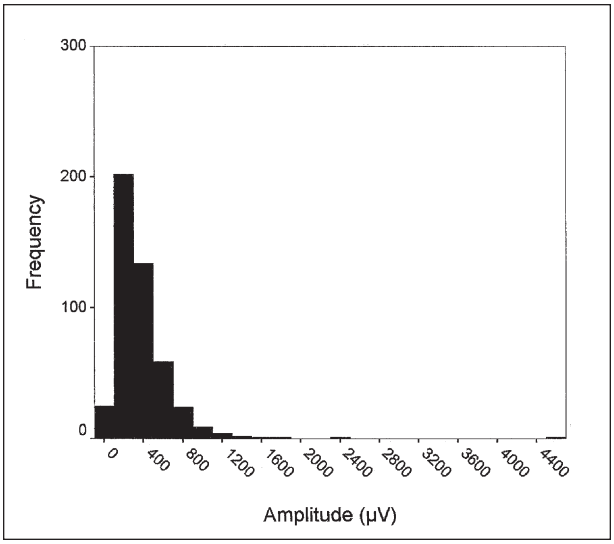

Figure 5—Histogram for MUAP amplitude obtained during electromyography of the left subclavian muscle in 10 healthy adult horses.

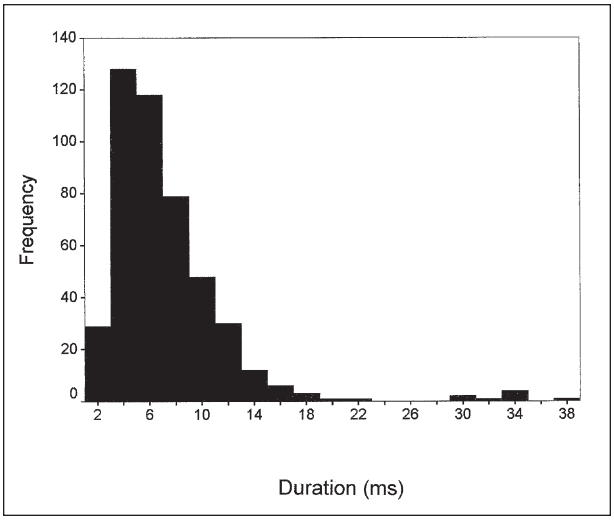

Figure 6—Histogram for MUAP duration obtained during electromyography of the left subclavian muscle in 10 healthy adult horses.

tion of insertional activity was 471.7 ± 33.45 milliseconds (95% **confidence interval [CI]**, 403.9 to 539.6 milliseconds; **Fig 4**). End-plate activity was encountered locally. Pathologic spontaneous activity was not observed in the 10 horses.

We obtained 463 MUAP for all 10 horses. For each horse, number of MUAP measured ranged from 25 to 55 representative MUAP (mean, 46 MUAP). Mean amplitude of MUAP was 379 μV (95% CI, 349 to 410 μV; **Fig 5**). Mean duration of MUAP was 7.27 milliseconds (95% CI, 6.84 to 7.71 milliseconds; **Fig 6**). Only 5 of 463 (1.0%) MUAP were accompanied by satellite potentials. Prevalence of polyphasic MUAP was 7.7% (36/463), and prevalence of complex MUAP was 2.6% (12/463; **Fig 7**). Mean number of phases was 2.9 (95% CI, 2.82 to 2.99), and mean number of turns was 3.0 (95% CI, 2.93 to 3.16). Twenty-three of 463 (5%) of the MUAP had > 5 phases or > 5 turns (**Fig 8**). Recruitment of additional motor units at moderate muscle force yielded a well-mixed pattern or interference pattern in all horses.

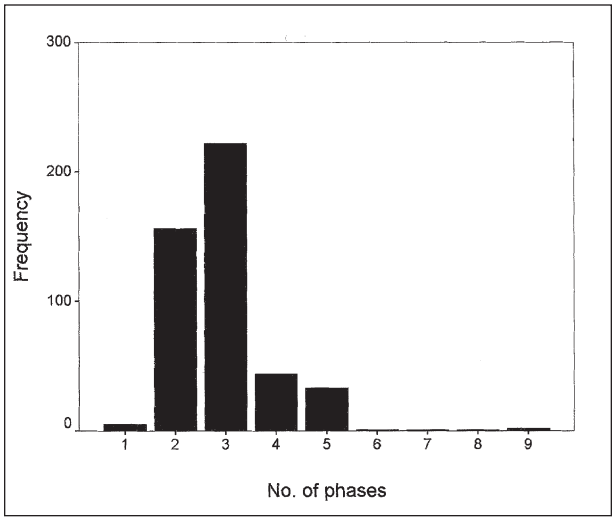

Figure 7—Histogram for No. of phases obtained during electromyography of the left subclavian muscle in 10 healthy adult horses.

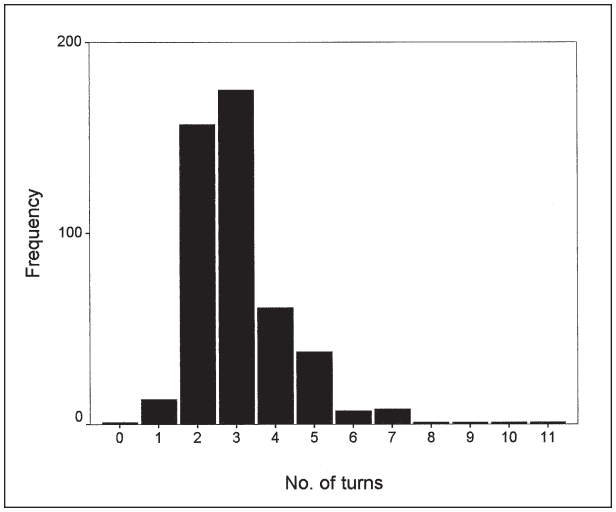

Figure 8—Histogram for No. of changes in direction (turns) obtained during electromyography of the left subclavian muscle in 10 healthy adult horses.

Discussion

In the study reported here, the subclavian muscle was chosen, because we wanted to investigate whether it was possible to perform quantitative analysis of MUAP in horses. For this objective, it seemed logical to start with a muscle in which MUAP activity would be easy to evoke and control. In humans, weight, height, and sex have little, if any, correlation with MUAP variables,[2,3,10,17] and our group of young adult horses of the same breed was relatively uniform in regard to body weight and height. In humans who are between 15 and 55 years old, age does not influence MUAP variables[3,10,12,14,20]; thus, by selecting horses between 4 and 11 years old, we did not expect to detect potential influences attributable to age. Mean values for MUAP variables do not differ significantly between left and right muscles in humans.[3] Therefore, a study to examine muscles on only 1 side of the body seemed justified.

Rectal temperature of the horses was measured, because deflection of body temperature can potentially influence MUAP amplitude, MUAP duration, and number of phases of the MUAP. Duration increases by 5 to 10% for each degree Celsius that body temperature decreases,[14,15,21] and amplitude can decrease[2] or increase[21] with changes in body temperature. Conduction velocity of action potentials decreases at low temperatures, which results in dispersion of action potentials and, hence, an increase in polyphasic MUAP.[2,10,14] Furthermore, spontaneous activity such as fibrillations and positive waves is reduced at lower temperatures, whereas myotonic discharges can be increased or decreased.[14]

Rectal and intramuscular temperatures were measured. Measurement of rectal temperature is more practical but does not necessarily reflect the actual temperature at the recording site. In our study, rectal temperature at the start of the recordings correlated with intramuscular temperature measured at the same time. However, intramuscular temperature did not change significantly during the recordings, which was in contrast to rectal temperature. This indicates that measurement of rectal temperature is not useful when recording MUAP. In this study in which we used normothermic horses, a correlation was not found between MUAP variables and rectal or intramuscular temperatures.

Furthermore, correlations were not found between MUAP variables, which corresponds with the concept that MUAP variables are independent of each other.[10] The MUAP variables were selected on the basis of maximum amplitude, sharp sound, and reproducibility. Studies[10,11] in humans indicate that MUAP amplitude depends to a great extent on the fibers closest to the recording tip (within 0.5 mm). Sharp crisp sound, maximal amplitude, and a small value for the risetime indicates the MUAP was recorded from a muscle fiber close to the tip of the recording electrode.[3,10,14]

When the risetime was < 0.80 milliseconds, the MUAP was included in statistical analyses. Selection

on the basis of a value of < 0.50 milliseconds for the time for the risetime, which is commonly used in EMG of humans,[14] resulted in inappropriate exclusion of good-quality MUAP in the study reported here. Automatic selection of the MUAP involves the risk of biased selection, depending on the selection criteria.[3,6,11] We avoided this by selecting MUAP from multiple recordings that were obtained with various settings for amplifier gain (100, 200, and 500 μV/division). However, the process of automatic selection failed frequently, resulting in manual selection of the MUAP. This time-consuming method has the advantage that all the MUAP in the recordings are evaluated.

In humans, automatic analysis defines the number of phases on the basis of deviations in amplitude and duration that are greater than a specified minimal value, thereby excluding values that cross the baseline as a result of noise. A minimal change in amplitude is required to avoid influences of noise when evaluating number of turns.[3] This entails the risk of underestimating the number of phases and number of turns in low-amplitude MUAP such as those observed in humans with early denervation or myogenic problems.[4,14,22] Deviations of the signal because of a noisy baseline were generally not hard to recognize. To avoid biased counting, all number of phases and turns were counted automatically, and automatic counts were manually corrected.

A random sample of the activated MUAP was obtained by recording MUAP at several areas of the muscle. This was necessary to obtain a representative sample of the MUAP in the muscle, because MUAP amplitude and MUAP duration depend on fiber type, diameter, and distribution in the motor unit,[20,23,24] which, in turn, depend on depth in the muscle.[10,23,25]

It was difficult to measure amplitude for insertional activity. Amplitude varied within a needle insertion; therefore, this variable was excluded from the study.

Data for MUAP amplitude, MUAP duration, number of phases, and number of turns of the recorded MUAP were normally distributed ($P < 0.001$). For clinical use with respect to the subclavian muscle, the mean amplitude of the MUAP for horses between 4 and 10 years old will be between 349 and 410 μV. The mean duration of MUAP of the subclavian muscle for horses between 4 and 10 years old will be between 6.8 and 7.7 milliseconds. Mean and median number of phases was the same (ie, 3 for both variables), and it is expected that the number of phases for similar horses will be 3. The same is true for the number of turns. These normative data are in agreement with the findings in muscles of humans, particularly the deltoideus, biceps brachii, and triceps brachii.[3,13-15,26] In humans, MUAP duration of these muscles varies from 4.2 to 18.0 milliseconds, and MUAP amplitude varies from 80 to 1,531 μV. In general, the amplitude varies from a few to several hundred millivolts.[2,3,13] In humans, number of phases is ≤ 4 in normal muscles, and the mean of 3 is also a typical finding.[3,14,15] Prevalence of polyphasic MUAP in healthy men varies from 1 to 15%.[10,11,13,14] The percentage found in the horses of our study (7.7%) is within this range. To our knowledge, data on prevalence of complex MUAP in healthy humans are not available.

It was difficult to evaluate the recruitment pattern. In the study reported here, recruitment pattern was subjectively evaluated by assessing the visual aspect of the signal when the horse was forced to make a jerky movement with its head. Because this method rules out a reproducible identical measurement for all 10 horses, the value of this observation is uncertain, and interpretation of the results may not be feasible.

In general, it can be concluded that analysis of MUAP in horses can be conducted in accordance with the same principles that are used in humans, although differences and difficulties were observed. Nevertheless, analysis of MUAP in horses can be performed without major problems. Therefore, normative values of additional muscles of horses should be determined. In humans, several advanced computer-aided selection processes or methods for analysis of MUAP or interference patterns are available and have potential for use in future studies.[6-8,10] In addition, many of the modern computer-aided EMG techniques can potentially enhance the possible uses of conventional EMG in horses.

[a]Model 8011 Fritz thermometer, Vetin-Aacofarma, Boxtel, The Netherlands.
[b]Voltcraft 500, thermocouple-K type, Conrad Electronic, Berlin, Germany.
[c]Voltcraft 500, Conrad Electronic, Berlin, Germany.
[d]Nicolet Meridian EMG apparatus, Nicolet Biomedical Inc, Madison, Wis.
[e]Topline 8000,Topline, Hoevelaken, The Netherlands.
[f]Sony Walkman professional WM-D3, Tokyo, Japan.
[g]EMG concentric needle electrode, Nicolet Biomedical Inc, Madison, Wis.

References

1. Knottenbelt DC. Equine neurological disease and dysfunction: a diagnostic challenge for the practitioner. Part 1: objectives and limitations of a neurological examination. *Equine Vet Educ* 1996;8:196–199.
2. Buchthal F, Pinelli P. Action potentials in normal muscle. *Acta Physiol Scand* 1951;25(suppl 89):13–14.
3. Bischoff C, Stålberg E, Falck B, et al. Reference values of motor unit action potentials obtained with multi-MUAP analysis. *Muscle Nerve* 1994;17:842–851.
4. Fuglsang-Frederiksen A, Sheel U, Buchthal F. Diagnostic yield of analysis of the pattern of electrical activity and of individual motor unit potentials in myopathy. *J Neurol Neurosurg Psych* 1976;39:742–750.
5. Kamen G, Caldwell E. Physiology and interpretation of the electromyogram. *J Clin Neurophysiol* 1996;13:366–384.
6. Nirkko AC, Rösler KM, Hess CW. Sensitivity and specificity of needle electromyography: a prospective study comparing automated interference pattern analysis with single motor unit potential analysis. *Electroencephalogr Clin Neurophysiol* 1995;97:1–10.
7. Sanders DB, Stålberg EV, Nandedkar SD. Analysis of the electromyographic interference pattern. *J Clin Neurophysiol* 1996;13: 385–400.
8. Stålberg E, Bischoff C, Falck B. Outliers, a way to detect abnormality in quantitative EMG. *Muscle Nerve* 1994;17:392–399.
9. Stålberg E, Chu J, Nandedkar SD, et al. Automatic analysis of the EMG interference pattern. *Electroencephalogr Clin Neurophysiol* 1983;56:672–681.
10. Stålberg E, Nandedkar SD, Sanders DB, et al. Quantitative motor unit potential analysis. *J Clin Neurophysiol* 1996;13:401–422.
11. Stewart CR, Nandedkar SD, Massey J, et al. Evaluation of an automatic method of measuring features of motor unit action potentials. *Muscle Nerve* 1989;12:141–148.
12. Daube JR. Needle examination in clinical electromyography. *Muscle Nerve* 1991;14:685–700.
13. Kimura J. Techniques and normal findings. In: *Electro-*

diagnosis in diseases of nerve and muscle: principles and practice. 2nd ed. Philadelphia: FA Davis Co, 1989;211–270.

14. Oh SJ. Needle electromyography study. In: *Principles of clinical electromyography case studies*. Baltimore: The Williams & Wilkins Co, 1998;77–119.

15. Sethi RK, Thompson LL. The EMG examination. In: *The electromyographer's handbook*. Boston: Little, Brown & Co, 1989;128–142.

16. Buchthal B, Behse F. Peroneal muscle atrophy (PMA) and related disorders. I. Clinical manifestations as related to biopsy findings, nerve conduction and electromyography. *Brain* 1977;100:41–66.

17. Dorfman LJ, Robinson LR. Normative data in electrodiagnostic medicine. *Muscle Nerve* 1997;20:4–14.

18. Mayhew IG, deLahunta A, Whitlock RH, et al. Spinal cord disease in the horse. *Cornell Vet* 1978;68(suppl):1–207.

19. Robinson JA, Naylor JM, Crichlow EC. Use of electromyography for the diagnosis of hyperkalemic periodic paresis. *Can J Vet Res* 1990;54:490–500.

20. Feinstein B, Lindegård B, Nyman E, et al. Studies on action potentials in normal human muscles. *Acta Psychiatr Neurol Scand* 1951;29:189–195.

21. Ricker K, Hertel G, Stodieck G. Increased voltage of the muscle action potential of normal subjects after local cooling. *J Neurol* 1977;216:33–38.

22. Daube JR. Electrodiagnostic studies in diagnosis and prognosis of motor neuron disease. *Neurol Clin* 1985;3:473–493.

23. van den Hoven R, Wensing TH, Breukink HJ, et al. Variation in fiber types in the triceps brachii, longissimus dorsi, gluteus medius, and biceps femoris of horses. *Am J Vet Res* 1985;46:939–941.

24. Ronéus M, Lindholm A, Åsheim A. Muscle characteristics in Thoroughbreds of different ages and sexes. *Equine Vet J* 1991;23:207–210.

25. Horák V, Dráber P, Hanák J, et al. Fibre composition and tubulin localisation in muscle of Thoroughbred sprinters and stayers. *Equine Exerc Physiol* 1991;3:262–268.

26. Buchthal F, Rosenfalck A. Action potential parameters in different human muscles. *Acta Psychiatr Neurol Scand* 1951;30: 125–131.