



Review

The potential and limitations of quantitative electromyography in equine medicine

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ABSTRACT

This review discusses the scope of using (quantitative) electromyography (EMG) in diagnosing myopathies and neuropathies in equine patients. In human medicine, many EMG methods are available for the diagnosis, pathophysiological description and evaluation, monitoring, or rehabilitation of patients, and some of these techniques have also been applied to horses. EMG results are usually combined with other neurophysiological data, ultrasound, histochemistry, biochemistry of muscle biopsies, and clinical signs in order to provide a complete picture of the condition and its clinical course. EMG technology is commonly used in human medicine and has been subject to constant development and refinement since its introduction in 1929, but the usefulness of the technique in equine medicine is not yet widely acknowledged. The possibilities and limitations of some EMG applications for equine use are discussed.

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Introduction

Since the first concentric needle electrode was constructed in 1929, electromyography (EMG) has been developed extensively and used for a variety of clinical and experimental studies. The first publications on its use in humans were on myasthenia gravis and neuropathies in 1941, followed by myopathies in 1949 (Stålberg and Falck, 1997). Professor Fritz Buchthal (1907–2003) and his colleagues played an important role in the development of quantitative EMG (QEMG); these early analyses were performed manually and examined the influence of age and muscle on motor unit action potential (MUP) parameters (Fig. 1), as well as the effect of batches of needles, temperatures, and many other technical and physiological factors. In addition to the shape of an MUP, the sound produced by MUP activity and pathological spontaneous activity was evaluated (Buchthal et al., 1954; Buchthal and Rosenfalck, 1955). This pioneering group also reported on topics such as the diagnostic significance of EMG in myopathies and neuropathies (Buchthal and Pinelli, 1952, 1953; Pinelli and Buchthal, 1953a and b; Buchthal, 1970).

In the 1970s, the effect of muscle effort on MUPs in healthy, myopathic, and neuropathic patients was studied by Professor Anders Fuglsang-Frederiksen and his group at Aarhus University (Fuglsang-Frederiksen and Mansson, 1975; Fuglsang-Frederiksen

et al., 1976, 1977, 1984; Fuglsang-Frederiksen, 1981; Fuglsang-Frederiksen and Rønager, 1988). Computer technologies enabled the use of automatic analysis techniques such as peak-ratio interference pattern analysis and amplitude-turn analysis in insertional analysis (Finsterer et al., 1997; Finsterer and Fuglsang-Frederiksen, 2003). These researchers also developed the application of concentric needle EMG in healthy humans and in patients with neuromuscular disease. Fuglsang-Frederiksen is still active

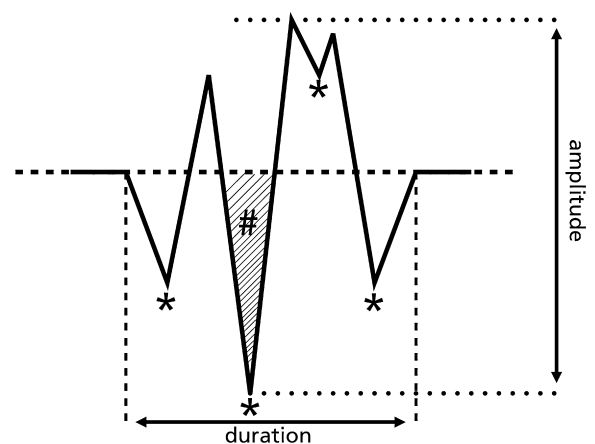


Fig. 1. # represents a phase, * represents a turn. The figure shows seven turns and five phases (for definitions see text).

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in the field, as illustrated by his recent reviews on the role of different EMG methods in evaluating myopathy (Fuglsang-Frederiksen, 2006) and on the current status of electrodiagnostic standards and guidelines in neuromuscular disorders (Fuglsang-Frederiksen and Pughdahl, 2011).

The works of Nandedkar (2008) but especially those of Professor Erik Stålberg from Uppsala University have dominated the clinical literature on applications of EMG since the 1980s and 1990s (Stålberg et al., 1996, 1999, 2000, 2013; Stålberg and Erdem, 2002; Stålberg, 2004). Stålberg developed single-fibre EMG (SFEMG) (Schwartz and Stålberg, 1975; Stålberg and Sanders, 2009; Stålberg, 2010; Tankisi et al., 2012) to study neuromuscular transmission using a needle that only records the activity of a single muscle fibre during spontaneous contraction or after electrical stimulation. In addition, SFEMG appears to be helpful in studying lower motor neuron disorders and a variety of peripheral nerve system disorders through the assessment of patterns of re-innervation (Kimura, 2001a and b).

Since the late 1970s, there have been reports from small-animal and equine medicine on topics such as EMG and nerve conduction in dogs with brachial plexus injuries (Steinberg, 1979) and neuropathies (Cooper et al., 1984). Earlier reviews have been published on EMG or nerve conduction on dogs (Farnbach, 1979, 1980) and horses (Andrews and Fenner, 1987; Wijnberg et al., 2003c; Wijnberg, 2005). Moore et al. (1988) were the first to describe EMG as a tool for evaluating laryngeal hemiplegia, which was followed by latency studies (Cook and Thalhammer, 1991; Hawe et al., 2001), but it took until 2013 for another report to appear on the use of EMG to study laryngeal musculature (Westermann and Wijnberg, 2013).

Clearly, the research and development of EMG has been very limited in equine medicine compared with human medicine. The understanding of human neuromuscular disorders has improved greatly thanks to the contribution of several EMG techniques. The marked difference in progress between the two species will be discussed in this review, focusing on quantitative needle EMG, its applications in man, and its possibilities and limitations for use in the horse (for earlier reviews, see Wijnberg et al., 2003c; Wijnberg, 2005, 2012).

The motor unit

The focus of interest in EMG is the motor unit (MU) and its individual constituents, such as the alpha motor neuron, its axon, the motor endplates, and concomitant muscle fibres. The summated MUPs of individual muscle fibre action potentials (APs) within the pickup area of the needle electrode contribute to the overall MUP. The size of the MU varies within muscles. In general, the smaller MUs are recruited at low force and larger MUs at higher force, meaning that smaller MUs containing slow twitch fibre types (I) are recruited before the larger MU containing fast twitch (II) fibre types, according to the so-called size principle.

Muscle fibres vary in diameter depending on the muscle involved, training status, age and gender (Stålberg and Daube, 2003). All fibres within an MU are activated synchronously, and in humans APs are propagated with a velocity of 1.5–6.5 m/s. The velocity is influenced by temperature, fatigue, and fibre diameter. In horses no data exist on the number of MUs in different muscles or on the number of muscle fibres per muscle. The variation in humans is from nine fibres per MU in the extrinsic eye muscles to 2000 in the gastrocnemius muscle (Stålberg and Falck, 1997). In neuromuscular disease, the random scattering of the MUs in a certain area is altered, leading to alterations in the EMG signals, since MU structure and electrophysiological events are closely related (Stålberg and Falck, 1997).

The shape of an MUP in mammals is characterised by amplitude, duration, number of turns, and number of phases (Fig. 1,

Appendix: Supplementary Table S1) (Cuddon, 2002; Wijnberg et al., 2002c; Stålberg, 2003; Daube and Rubin, 2009). Amplitude is determined by muscle fibre diameter, whereas duration, turns, and phases are related to the conduction time differences (temporal dispersion) between individual APs of the MU and, therefore, to muscle fibre AP velocity. Other parameters are MUP area and size index. MUP area is calculated automatically as is size index using EMG software and the formula $2 \times \log(\text{amplitude}) + \text{area}/\text{amplitude}$. A satellite potential is a late component of the MUP and can be recorded in both normal and pathological muscles (Kimura, 2001b).

Needle EMG can be used to study the MU and has the advantage over more classical methods (based on histochemical and biomechanical characteristics) to study muscle fibres in that it can assess multiple muscles and areas of the muscle in a relatively non-invasive manner. Alterations in microphysiology, muscle fibre composition, and interstitial tissue in both nerve and muscle disorders result in changes in the electrical signals generated by the MU. This allows assessment of the type of structural alterations of the MU in individual muscles and the distribution of the abnormalities (e.g., localised, multifocal, or generalised). In addition to identifying a lesion and its location, EMG has proved to be useful in following the progression and/or healing of neuromuscular disease (Stålberg et al., 1996, 2000; Stålberg and Falck, 1997; Cuddon, 2002; Stålberg and Daube, 2003; Fuglsang-Frederiksen, 2006; Lacombe and Andrews, 2008).

EMG electrodes

MU activity is generated by the alpha motor neuron, its axon, the motor endplates, and the muscle fibres that are innervated by the MU. This functional unit can be examined using different EMG methods (Stålberg and Falck, 1997). The uptake area of the electrical activity generated by the MU largely depends on the electrode type and size (pickup area). Single-fibre recordings use a specialised very small recording surface, permitting the measurement of electrical activity of one muscle fibre. Surface EMG records activity from a large part of a muscle or neighbouring muscle and is therefore used more often in anatomical studies or rehabilitation programmes than in the diagnosis of neuromuscular diseases (Franssen, 1995a, 1995b, 1995c; Stålberg and Falck, 1997; Daube and Rubin, 2009) and will therefore not be discussed in this review.

Selecting the type of electrode used in EMG depends on the indication. In needle EMG, the concentric needle is used most commonly because it records with minimum interference from surrounding muscles, has a fixed-size recording surface with the cannula serving as the reference electrode (Stålberg and Falck, 1997), and data are available as reference values, which are extensive for humans (Daube and Rubin, 2009) but limited for horses. Concentric needles are routinely used in both humans and horses for the diagnosis of neuromuscular disease. Needle size varies and with it the pickup area of the recording surface, which potentially influences reference values (Stålberg et al., 1996; Wijnberg et al., 2003c). The concentric needle electrode measures about 30–50 muscle fibres of an MU (Wijnberg et al., 2003c) and the pickup area of needles used in equine studies is 0.068 mm² (Wijnberg et al., 2002b and d). The MUP is the sum of potentials of individual muscle fibres innervated by a single lower motor neuron that are near the recording electrode.

Monopolar needles have a conical recording surface of 0.25-mm diameter, and two are needed for a recording. The pickup area is larger, and the variability in the distance between the two recording electrodes makes it less convenient. In equine medicine, monopolar needle use has been limited although they have been used to apply *Clostridium botulinum* toxin in several studies on string-halt (Wijnberg et al., 2009b), in pilot studies on botulinum toxin used

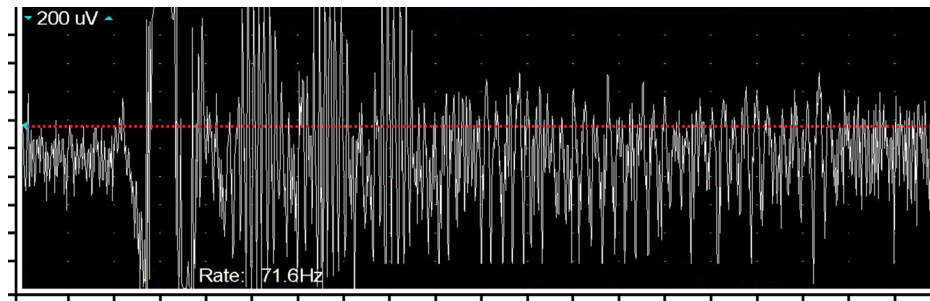


Fig. 2. Illustration of 200 μ V per division divided by dots. The red line represents the trigger line used to select a signal. Hz, Hertz.

in laminitic patients (Hardeman et al., 2013; Wijnberg et al., 2013) and in motor evoked potential studies (Nollet et al., 2003).

Single-fibre electrodes have a recording area of 25 μ m with a pickup area of around 300 μ m, referenced to the low-frequency filtering shaft of the needle. The needle records from a small area of the muscle by selectively recording APs of individual muscle fibres. It is used to study the microphysiology of the MU at the level of the terminal axon branches and neuromuscular synapse by recording the electric variability in the interval between APs of successive discharges of the same single muscle fibre in the same MU ('jitter'), which enables detection of variation in MU activity. For example, muscle fibre membrane characteristics, muscle fibre propagation velocity, function of individual motor endplates, and organisation of muscle fibres in the MU territory (Stålberg, 2010) influence the magnitude of jitter.

However, the needle is expensive and the technique requires more training than concentric needle application (Daube and Rubin, 2009; Stålberg and Sanders, 2009). Its use in humans has focused on neuromuscular disease and especially neuromuscular transmission disorders, such as myasthenia gravis (Kimura, 2001a). In horses it has been applied to study the delay in transmission of MU activity as a result of different head and neck positions (Wijnberg et al., 2010). The use of the less-expensive concentric needles for jitter measurements has not uniformly been accepted in humans (Stålberg and Sanders, 2009; Stålberg, 2010; Stålberg et al., 2013) and has not been attempted in horses.

Concentric needle EMG in normal individuals

In the resting muscle, needle placement induces insertional activity by damaging the muscle fibre membrane. After cessation of needle movement, a pause of 1 s or more enables recognition of repetitive potentials that might be induced by the insertion. A decrease or absence of activity can be the result of needle positioning in fibrous tissue or in fat. Alternatively repetitive potentials may seem prolonged when myotonic discharges follow insertional activity (Fig. 2).

Endplate activity is the recording of miniature endplate potentials due to spontaneous release of acetyl choline from vesicles in the endplate. It is recognised by irregularity in the baseline at low amplification in addition to endplate spikes resulting from individual muscle fibre potentials, and a typical 'seashell' sound. Endplate activity should not be mistaken for fibrillation potentials (Fig. 3) or short-duration MUPs (Fig. 4), which both fire in a different pattern (Daube and Rubin, 2009).

The shape of the MUP (Fig. 1) is affected by factors such as needle choice, amplifiers, cables, and filter settings. Physiological factors are age, muscle involved, and temperature (Kimura, 2001b; Wijnberg et al., 2002c, 2003c; Daube and Rubin, 2009). In horses, muscle contractions can be induced only in a limited way, as opposed to humans who can be instructed to contract muscles. To a limited extent, horses

can be motivated to contract the muscle under study and by pushing, pulling, or lifting a leg, weight shifts can induce the desired level of muscle contraction. However, this requires patience in addition to some knowledge of muscle function. The moment sharp clicking sounds generated by the MUP are heard, recording can start. Correct recordings are those with a short rise time (Daube and Rubin, 2009), which in horses is defined as ≤ 0.8 ms (Wijnberg et al., 2002b).

MUPs are assessed during low or moderate contraction of the muscle, resulting in recording activity of a limited number of MUs. During strong voluntary contraction, individual MUP characteristics cannot be measured due to superimposed potentials inducing an interference pattern. However, if during stronger levels of contraction less dense patterns occur, loss of MUs, upper motor neuron disorders, or muscle weakness may be present. The latter two causes of poor recruitment require estimates of firing rates and patient efforts (Daube and Rubin, 2009), making this application more difficult to apply objectively to horses than to humans (Wijnberg et al., 2003c; Wijnberg, 2005).

In QEMG, multiple different MUPs (minimum of 20) firing at least four times identically (Wijnberg et al., 2002b and c) and recorded in different areas within one muscle are required in order to obtain a complete assessment of the integrity of the MUs of the muscle examined (Stålberg et al., 1996, 1999, 2000; Stålberg and Falck, 1997; Preston and Shapiro, 2002; Fuglsang-Frederiksen, 2006; Daube and Rubin, 2009; Rubin, 2012). The automatic analysis has to be corrected manually, and then the duration, amplitude, number of phases, turns, percentage of polyphasic MUPs, and presence of satellite potentials (Lang and Partanen, 1976) are evaluated (Fig. 1, Appendix: Supplementary Table S1).

In semi-quantitative EMG, a grid displayed on the computer screen is used to assist in estimating several EMG parameters. In

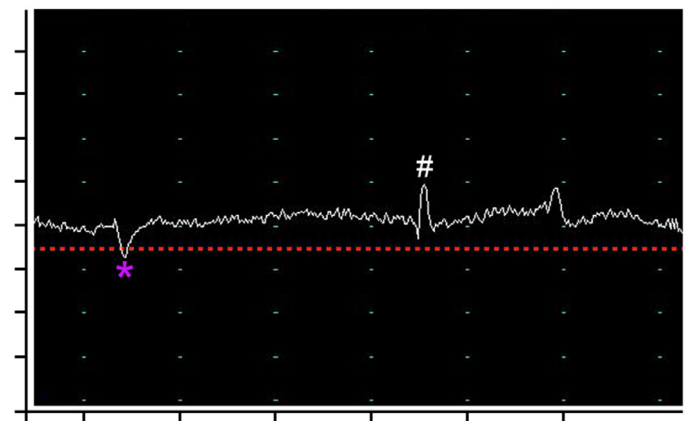


Fig. 3. Positive wave and fibrillation potential. * indicates positive wave, # indicates fibrillation potential. Scale: Y-axis, 50 V per division; X-axis, 10 ms per division.

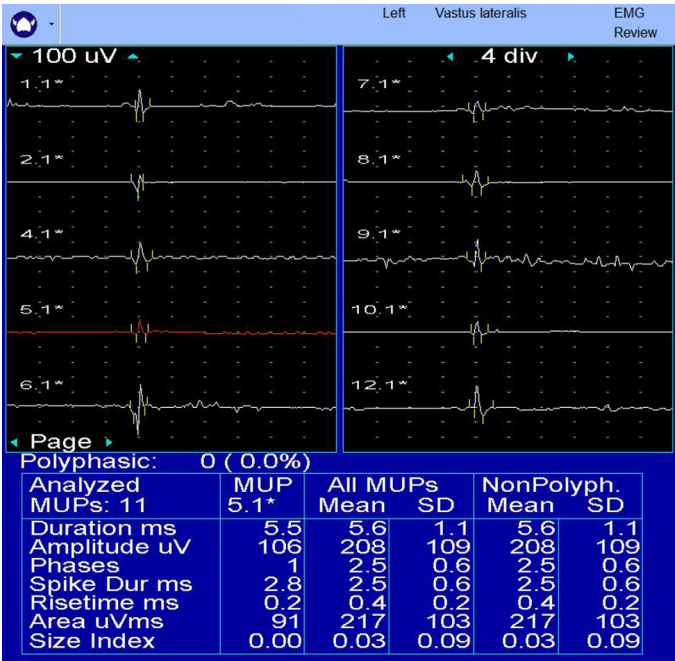


Fig. 4. Myopathic MUP in the lateral vastus muscle of a 7-year-old Royal Dutch Sport Horse gelding. One part of the review of selected MUPs is shown. Each MUP is a summation of at least four identically firing MUPs. Y-axis, 100 μ V per division; X-axis, 10 ms per division. The mean of all selected MUPs during the session is presented in the second column ‘All mean MUPs’. The individual value of MUP with number 5.1 is shown in the first column. SD, standard deviation. The normal 95% interval for this muscle in this age group is 7.8–10.2 ms duration, 571–836 V amplitude, number of phases 2.6–2.9, and number of turns 3.2–3.6. Mean percentage polyphasia is around 8.6% in a normal muscle. The low MUP values for all variables are indicative of myopathy.

humans, semi-quantitative EMG has been proven to be as valid as QEMG, and in one study in horses this was also found to be applicable (Wijnberg et al., 2009a). When the technique is mastered, the EMG physician is able to determine each parameter with an accuracy >90% (Daube and Rubin, 2009). Values are compared to normal values obtained from the same muscle and comparable age group.

Benefits of quantitative assessment of the MAP compared with subjective assessments

Quantitative EMG is considered reliable in humans and is routinely needed in questionable cases to increase the certainty of a diagnosis (Finsterer and Fuglsang-Frederiksen, 1999; Daube, 2000; Cuddon, 2002; Fuglsang-Frederiksen and Pugdahl, 2011). Physiological properties characterise the MUP and include duration, amplitude, number of phases and turns, size index, area, number of satellite potentials and % polyphasia using programmed computer algorithms.

Quantitative studies generally use 20 units to compare the findings with reference values (Kimura, 2001c). In human EMG studies, many quantitative methods are the subject of ongoing research, but in horses the number of quantitative methods used is still rather limited. However, semi-quantitative assessment of concentric needle-derived EMG signals is a fast and useful method of analysis (Wijnberg et al., 2009a; Farkas et al., 2010). An overall impression of the status of the muscle is formed by recording the MUPs across a number of needle positions and using the scale grid on the screen whilst performing the recording. This rather subjective method is dependent on the expertise and skills of the examiner and might be prone to operator bias so quantitative methods have been developed to char-

acterise MUP shapes allowing for more objectivity and reproducibility (Kimura, 2001c; Farkas et al., 2010). An experienced examiner can detect abnormalities with reasonable certainty and this can be satisfactory for the detection of unequivocal abnormalities, but might fail to diagnose less obvious deviations or mixed patterns of abnormalities. Objective measurements allow for comparison of test results between laboratories or of sequentially obtained results (Kimura, 2001c; Fuglsang-Frederiksen and Pugdahl, 2011).

Concentric needle EMG in abnormal individuals

Virtually all primary neuromuscular diseases result in changes in the electrical activity that can be recorded from muscle fibres. Objective measurements are a necessity for recognising mild diseases such as early neurogenic processes or mild myopathies (Buchthal and Pinelli, 1953; Buchthal, 1970; Wijnberg et al., 2004; Daube and Rubin, 2009). In humans and horses, EMG can be used to distinguish between neurogenic disorders (e.g., motor neuron disease or neuropathies) and myopathic disorders. Insertional activity can decrease if fibrosis is present or increase in case of membrane instability. Pathological spontaneous activity can cause abnormal spontaneous discharges such as fibrillation potentials, positive waves, myotonic discharges, complex repetitive discharges, or neuromyotonia (Vanhaesebrouck et al., 2011). MUPs can have an altered duration, amplitude, number of phases or number of turns, and recruitment patterns (Wijnberg et al., 2002d; Wijnberg, 2005; Daube and Rubin, 2009).

Pathological spontaneous activity

Needle insertion in muscle induces a short burst of spontaneous activity, called insertional activity. This reflects the presence of living muscle fibres that depolarise as a result of mechanical irritation or damage induced by the needle. Increased insertional activity in the form of short bursts of positive sharp waves, or clear myotonic discharges, can be observed in humans and horses in various channelopathies such as hyperkalaemic periodic paralysis and in the C1CN1 mutation leading to congenital myotonia (Beech et al., 1992; Daube and Rubin, 2009; Wijnberg et al., 2012). Decreased insertional activity can occur if muscle fibres can no longer produce electrical activity, as seen in severe end-stage neuro- or myopathic disorders when fibres are replaced by fat or connective tissue, as well as in specific disorders featuring membrane dysfunction, including periodic paralysis during paralysis or in myophosphorylase deficiency during contraction (Preston and Shapiro, 2002; Daube and Rubin, 2009).

Fibrillation potentials are single-fibre APs firing as a result of muscle membrane instability in the absence of innervation. These potentials occur in the form of a brief spike with an amplitude of around 50–80 μ V in horses or as positive sharp waves (30–120 μ V) (Fig. 3, Appendix: Supplementary Table S2). Positive sharp waves originate from an injured portion of the muscle fibre, when the AP cannot propagate along the muscle fibre past the recording electrode. The amplitude of the fibrillation potential is proportional to the muscle fibre diameter, resulting in low-amplitude fibrillations in atrophied muscle fibres and larger ones from hypertrophied muscle fibres. Due to this variation, recognition can be facilitated by identifying disorders featuring the slow regular firing pattern (firing at 0.5–15 Hz) with the exception of early denervation in which the firing pattern can be irregular or intermittent.

Fibrillation potentials occur as a result of myopathic or neuro-pathic processes: for example, in muscle fibres that have lost their innervation or have been re-innervated, that have been sectioned transversely or longitudinally, or that are regenerating. In addition these potentials result from muscle fibre necrosis, fibre splitting,

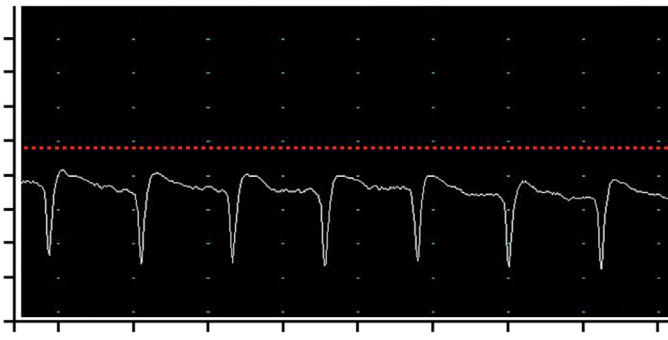


Fig. 5. Complex repetitive discharge in the subclavian muscle of an 8-month-old Royal Dutch Sport filly. Firing frequency 100 Hz. Y-axis, 200 μ V per division; X-axis, 20 ms per division.

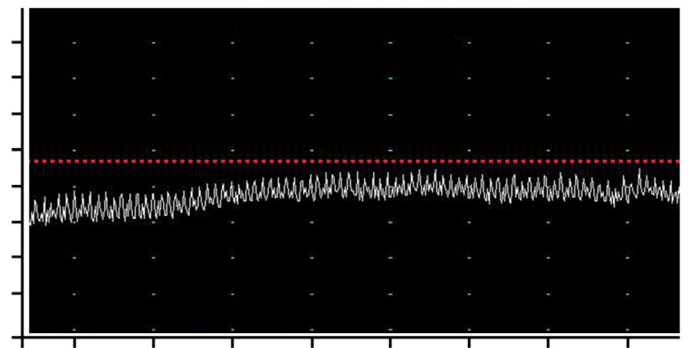


Fig. 6. Neuromyotonia in the subclavian muscle of a 205-day-old Royal Dutch Sport filly. Firing frequency 220 Hz. Y-axis, 50 μ V per division; X-axis, 50 ms per division.

or any process that separates the muscle fibre from the endplate zone, including radiculopathies, motor neuron disease, or degeneration of axons (Wijnberg et al., 2003c; Daube and Rubin, 2009; Rubin, 2012). The density of these potentials is commonly graded from 1+ to 4+ and, according to some authors (Daube and Rubin, 2009), provides a rough estimate of the number of unstable muscle fibres.

Myotonic discharges (Fig. 2) also originate from single muscle fibres with an abnormal membrane that fire spontaneously after external stimulation in a prolonged, regular, waxing and waning fashion with a frequency of 20–300 Hz. Myotonic disorders, sometimes (but not always) associated with muscle channelopathies, may induce myotonic discharges. Depending on their occurrence in relation to the electrode, they occur as brief spikes or positive waveforms (Daube and Rubin, 2009; Wijnberg et al., 2012). Also, the actual cause and whether they are induced by voluntary contraction or by external stimulation may alter their manifestation.

Complex repetitive discharges (Appendix: Supplementary Table S2, Fig. 5) are the result of spontaneous discharge of a group of muscle fibres, after initiation by a single-fibre AP leading to ephaptic spreading (i.e., through membranes instead of by means of neurotransmission across synapses) and depolarising neighbouring fibres. Complex repetitive discharges have an abrupt start and cessation. They occur in both neuro- and myopathies, such as chronic radiculopathies, peripheral neuropathies, or slowly progressive myopathies, and are found incidentally in normal biceps and iliopsoas muscles in humans (Daube and Rubin, 2009). In horses, they could also be induced by hypocalcaemia (Wijnberg et al., 2002d). Neuromyotonic discharges in horses are bursts of activity firing at high frequencies of >150 Hz (Appendix: Supplementary Table S2, Fig. 6) unrelated to voluntary activity and as a result of hyperexcitability of the nerve membranes. Channelopathies of the nerve membrane can induce neuromyotonia in both humans and horses; in man it is also associated with mechanical irritation of peripheral nerves (Wijnberg et al., 2002d; Daube and Rubin, 2009).

MUP amplitude and duration (see Appendix: Supplementary Tables S1 and S2)

Loss of muscle fibres due to myopathy will make the MUP smaller (Fig. 4), whereas addition of muscle fibres to the MU by re-innervation of the denervated muscle fibres will increase its size (Fig. 7). Loss of synchrony leads to a more complex configuration in humans and horses (Wijnberg et al., 2008b, 2010; Daube and Rubin, 2009). The duration of the MUPs is measured from the initial deflection of the baseline to the time of return to baseline and reflects the synchronicity of firing and the density and area of fibres within an MU.

Typically, collateral sprouting and re-innervation of an MU leads to differences in length of axons or conduction velocity over the MU and produces dispersion of endplate zones along muscle fibres, which will lead to long-duration MUPs (Fig. 7) (Benders et al., 2001; Stålberg and Erdem, 2002; Wijnberg et al., 2004, 2006; Lacombe and Andrews, 2008; Daube and Rubin, 2009). Generally, long-duration MUPs occur in chronic neurogenic disorders several weeks or months after injury in man (Daube and Rubin, 2009). They usually also have a high amplitude and show poor recruitment, but because the spike amplitude reflects only the few muscle fibres closest to the needle tip they can have a normal amplitude. Increase in duration and number of turns are considered early signs of re-innervation (Daube and Rubin, 2009).

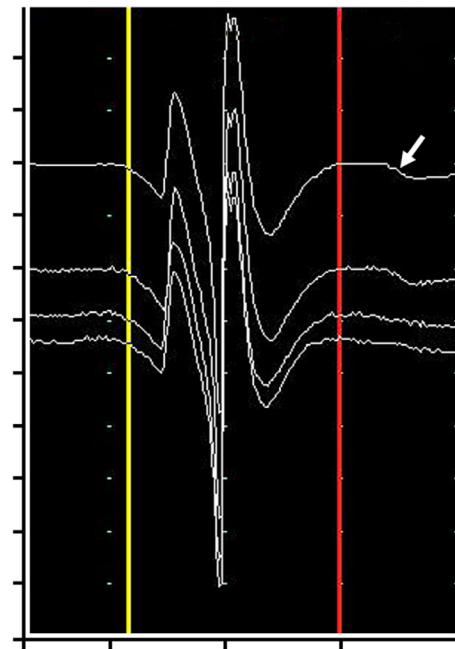


Fig. 7. MUP in the ventral cervical serratus muscle with neuropathic characteristics indicative of re-innervation in a 7-year-old Royal Dutch Sport gelding with lower motor neuron disease. The first line with the white arrow is the summed MUP of those shown below as individual firing identical MUPs. Y-axis, 100 μ V per division; X-axis, 5 ms per division. Amplitude is 764 μ V, duration is 9.1 ms, number of phases is 5, number of turns is 5, rise time is 0.25 ms, size index is 0.89. The normal mean values for this muscle in this age group is 4.3–4.6 ms duration, 488–551 μ V amplitude, number of phases 2.9–3.0, and number of turns 2.4–2.6, and 0.26–0.70 size index. Mean percent polyphasia is 7–12% in a normal muscle. The values of the MUP variables are greater than reference values for this age group and indicative of neuropathy.

In chronic myopathies a combination of short- and long-duration MUPs can be recorded. A short duration MUP is considered to be the most specific characteristic, and decreased amplitude is expected due to the loss of contributing muscle fibres (Liguori et al., 1997; Fuglsang-Frederiksen, 2006). In myopathies, fibre splitting, atrophy, and degeneration will lead to differences in conduction velocity along the muscle fibre. Necrosis or degeneration leads to the loss of muscle fibres. Short-duration MUPs occur as a result of anatomical loss or atrophy of muscle fibres from the MU. Often a low amplitude (Fig. 4) is seen; the number of innervated muscle fibres within the recording area of the electrode is decreased, thus leading to a decreased area of that MU. Severe neuromuscular junction disorders (such as botulism or newly innervated MUs following severe nerve injury) can lead to short-duration MUPs. In metabolic or endocrine disorders or mitochondrial disease, this reduction in duration can be limited (Daube and Rubin, 2009), and mitochondrial disease can lead to a mixed pattern of neurological and myopathic alterations (Mancuso et al., 2012).

Number of phases and turns (see Appendix: Supplementary Tables S1 and S2)

The 'phase' of an MU is defined as the area of an MUP on either side of the baseline and equals the number of baseline crosses plus one (Fig. 1). In both humans and horses, the majority of normal MUPs are triphasic. In most normal MUPs in both species >15% will have more than four phases (called polyphasic; Appendix: Supplementary Table S1). The number of phases reflects the degree of synchronicity of individual muscle fibre APs firing within an MU. A 'turn' is a change of direction within a phase, and the number of turns is <4 in most normal MUPs (Fig. 1). Synchronicity, and thus the number of phases and/or turns, increases due to collateral sprouting, increase in fibre density of an MU due to re-innervation, or reduced conduction of the muscle fibre membrane in myopathies. They can be of any duration and are graded by percentage of the MUPs that are polyphasic (Wijnberg et al., 2003a; Daube and Rubin, 2009). Mixed patterns of short-duration, long-duration, and polyphasic MUPs occur in chronic myositis or rapidly progressing motor neuron disease.

Recruitment patterns

Doublets, triplets, and multiplets are MUPs that fire two or more times at short intervals of 10–30 ms in a semi-rhythmic pattern under voluntary control. Hypocalcaemia, peripheral nerve hyperexcitability, and lower motor neuron disorders can induce these in both humans and horses (Wijnberg et al., 2002d, 2003c; Benders et al., 2005; van der Kolk et al., 2005; Wijnberg, 2005; Daube and Rubin, 2009).

Firing rate will increase proportionately due to firing of additional MUs when more muscle force is requested. In neuromuscular disorders, this recruitment pattern might be altered. If MUs are lost, the firing rate of the remaining MUPs will be disproportionately high compared with the number of potentials firing, which is referred to as 'reduced recruitment'. Any process that blocks or hampers the conduction in the axons or destroys the muscle fibres of an entire MU will lead to this phenomenon. In acute axonal lesions, this may be the first alteration that is noticed. Although most commonly seen in neuropathies, it can also be present in severe end-stage myopathies such as muscular dystrophies.

Increased recruitment patterns occur when the force that an MU can generate is decreased due to loss of muscle fibres within an MU and is seen primarily in myopathies. The result is activation of more MUs than would be expected for the force exerted, thus an increase in the number of MUs recruited relative to the force (Daube and Rubin, 2009). This will generate the most typical pattern in a

myopathy, namely, a full pattern of MUPs with a low amplitude. Interference pattern analysis shows a low mean amplitude/turn value as well as a high number of turns/s in combination with a lower mean amplitude (Liguori et al., 1992, 1997; Fuglsang-Frederiksen, 2006).

Even though MUP changes can be broadly divided into neuropathic and myopathic (Appendix: Supplementary Table S2), only a thorough knowledge of different combinations of pathological spontaneous activity and changes of MUPs can lead to reliable interpretations. Abnormal patterns can be distinguished through an evaluation of recruitment, MUP shape, stability, and their changes. Also, severity, duration, and prognosis of the disease can be assessed (Preston and Shapiro, 2002; Rubin, 2012).

Clinical application of needle EMG in the horse

Reports on the use of neurophysiological techniques in horses have been published since the 1970s. A limited number of early publications have been found on the use of EMG or nerve conduction in horses (Henry et al., 1979; Henry and Diesem, 1981). In most cases, the main focus was on the detection of pathological spontaneous activity in some neurogenic disorders, such as equine lower motor neuron disease (Andrews and Fenner, 1987; Divers et al., 1994) and laryngeal hemiplegia (Moore et al., 1988), and in muscle disorders such as hyperkalaemic periodic paralysis (Beech et al., 1992). Not much attention was given to quantitative MUP analysis until 2000.

Since then, more publications have been published on data in normal horses (Wijnberg et al., 2002b and c, 2003b, 2011, 2013; Ciminaghi et al., 2004; Cunilleras and Wijnberg, 2012; Ludvikova et al., 2012), neuropathies (Benders et al., 2001, 2005; Wijnberg et al., 2004; van der Kolk et al., 2005), myopathies (Wijnberg et al., 2003a, 2004, 2008a; Ludvikova et al., 2012), and in horses with lameness problems of unknown origin (Wijnberg et al., 2004), stringhalt (Wijnberg et al., 2000, 2009b), and neck lesions (Wijnberg et al., 2002a, 2004, 2009a). Other applications of QEMG include determining the effects of some training strategies on neuromuscular function such as in (over)trained Standardbreds (Wijnberg et al., 2008b) and horses trained in different head and neck positions including the so-called low, deep, and round or 'Rollkur' positions (Wijnberg et al., 2010).

QEMG has also been proven to be useful in studying the potential effect of certain therapeutic interventions, such as the application of *Clostridium botulinum* toxin A (Botox) for its use in laminitic horses (Hardeman et al., 2013; Wijnberg et al., 2013) or phenytoin in horses with stringhalt (Wijnberg et al., 2000). The procedures are preferably performed in unsedated horses since voluntary muscle activity is required and insertion of the needle is generally accepted well, with exception of the lower leg in some horses. Individuals, especially very young horses, may require sedation in order to stand in stocks for a prolonged time; alternatively the procedure can be performed outside the stocks in a quiet stable box. Sedation will not affect the functionality of the lower motor unit itself but decreases the muscle activity so dosages should be very low (Nollet et al., 2003; Wijnberg et al., 2003b).

QEMG in horses follows the same principles as in humans (Appendix: Supplementary Table S1), by showing similar adaptations to physiological conditions, including aging and pathological processes such as myopathies and neuropathies (Daube and Rubin, 2009). Because horses can stand without muscle activity as a result of their passive 'stay apparatus', pathological spontaneous activity can be readily observed in the awake, standing horse. As in humans, muscle activation can lead to induction of pathological spontaneous activity (Wijnberg et al., 2003c).

In horses with neurological disorders, the MUP changes in much the same way as in man (Lang and Partanen, 1976; Stålberg et al., 1996; Daube, 2000; Cuddon, 2002; Stålberg and Daube, 2003;

Fuglsang-Frederiksen, 2006), as has been demonstrated in lower motor neuron disorders such as equine lower motor neuron disease (Benders et al., 2005; Swagemakers et al., 2005; van der Kolk et al., 2005) and to a lesser extent equine grass sickness (Wijnberg et al., 2006). Peripheral neuropathies also have been discovered using EMG in horses with damaged paraspinal nerves and plexus brachialis involvement due to neck problems or neuropathies of the hind legs causing lameness (Wijnberg et al., 2004). Neuropathic disorders that result in re-innervation lead to high-amplitude, long-duration MUPs with an increase in the presence of polyphasic and/or complex MUPs with or without the presence of pathological spontaneous activity (Stålberg and Falck, 1997; Daube, 2000) (Appendix: Supplementary Table S2, Fig. 7).

Aspecific myopathies (Wijnberg et al., 2003b, 2008a), ion channel disorders such as hyperkalaemic periodic paralysis (Beech et al., 1992) or congenital myotonia (Wijnberg et al., 2012), or botulism (van Nes and van der Most van Spijk, 1986; Wijnberg et al., 2013) cause a reduction of MUP amplitude and especially duration due to loss of muscle fibres contributing to the MUP, as is also seen in human myopathies (Buchthal and Pinelli, 1953; Buchthal, 1970; Fuglsang-Frederiksen, 2006; Lynch and Cohen, 2011) (Appendix: Supplementary Table S2, Fig. 4). Depending on the cause of the myopathy, pathological spontaneous activity and increased occurrence of satellite potentials can occur (Lang and Partanen, 1976; Stålberg and Falck, 1997). A limitation of EMG assessment is that although it can determine the presence of a myopathy, it will not determine the cause; histochemical analysis of muscle biopsies is needed to help in this search.

QEMG analysis, including interference pattern analysis, results in increasing the sensitivity of EMG in diagnosing myopathies (Liguori et al., 1992, 1997; Fuglsang-Frederiksen, 2006). QEMG was used in a study of 188 human myopathic patients; in five patients results were inconsistent, whereas 22 muscle biopsies showed inconsistency with the clinically diagnosed myopathy (Fuglsang-Frederiksen, 2006). Also, a site for muscle biopsy can be determined based on EMG results and the revalidation process can be monitored in an objective way.

As in humans, age- and muscle-related differences can be found within normal individual horses (Wijnberg et al., 2003b). In young individuals the muscle fibres are still developing, leading to MUPs of shorter duration and lower amplitude than in adult or older horses and these should not be misinterpreted as myopathy. With aging, the MU degenerates a little, resulting in larger, broader MUPs, which again should not be mistaken for mild neuropathy (Wijnberg et al., 2003c).

Each muscle has its own reference values (Appendix: Supplementary Table S1) (Wijnberg et al., 2002c, 2011) and, if that fact is not acknowledged, misinterpretation can easily occur. For example, the biceps femoris muscle in normal adult horses has a rather low MUP amplitude, duration, and size index and the infra- and supra-spinatus muscles show confusingly high values (Appendix: Supplementary Table S2) (Cunilleras and Wijnberg, 2012, 2014). Because temperature affects MUPs, body temperature should be taken into account if there is any deviation from the normal (Buchthal et al., 1954; Wijnberg et al., 2002b and c). Data transformation for statistical analysis results in impractical values for clinical use and not all authors provide untransformed data (Ciminaghi et al., 2004; Wijnberg et al., 2008b). It would be helpful if in the future full data sets of QEMG values were provided in a more uniform way.

Clinical implications of including EMG in the diagnostic workup

Without EMG clinicians tend to over-diagnose generalised myopathies and underdiagnose generalised neuropathies. When local

problems were noticed, the cervical area was often underestimated as a potential site for lesions and the sacroiliac area was overinterpreted as the origin of a neurogenic lesion. For example, stringhalt often appeared to be associated with cervical lesions rather than with sacroiliac abnormalities (Wijnberg et al., 2004).

In horses with unknown reasons for locomotion disturbances and/or generalised atrophy, EMG has helped in finding evidence of mostly generalised neuropathies (Wijnberg et al., 2004, 2006; van der Kolk et al., 2005; Wijnberg, 2006) and to some extent generalised myopathies (in the absence of muscle enzyme elevation) (Wijnberg et al., 2003a). It has also proved its value in helping to diagnose early stages of equine grass sickness (EGS) and equine lower motor neuron disease in stages leading to early intervention and return to (some level of) performance (Benders et al., 2001; Wijnberg, 2006). EMG can play a role in preventing surgical intervention in cases with gastrointestinal malfunction: based on EMG signs of generalised lower motor neuron disease acute EGS can be more easily diagnosed ante-mortem (Wijnberg, 2006; Wijnberg et al., 2006). This can lead to the decision *not* to perform surgery in cases with small intestinal dysfunction. Also, in some postoperative ileus cases in which gastrointestinal surgery was performed, EMG helped in diagnosing acute EGS.

With localised lesions, EMG can identify which peripheral nerves or nerve roots are involved (Wijnberg et al., 2002a, 2004, 2009a; Cunilleras and Wijnberg, 2012, 2014) for example in lame horses in which orthopaedic examination did find the cause of the lameness. In myopathies, the effect of treatment can be monitored providing an opportunity to adapt the rehabilitation programme by adjusting treatment or training methods as where intensive rehabilitation schedules do not have the desired effect, or result in decreased performance. In some of these cases EMG examination will elucidate myopathic changes, although the horses have been subjected to a rehabilitation programme for neuropathy. Based on such information training would be reduced or even stopped, leading to normalisation of the gait or performance and reappearance of normal MUPs.

In general, EMG can explain not only the origin of a lesion (neurogenic or myogenic) but also its severity and distribution. These factors help in formulating a prognosis and deciding on the most appropriate therapy that might vary from rest to limited and specific training, or targeted electrical muscle stimulation. Especially in myopathies, it can monitor recovery of the MU function during treatment.

Conclusions

In human medicine, needle EMG and nerve conduction studies are important tools in diagnosing neuromuscular disorders. In horses, the technique has been steadily used and applied to an increasing number of indications, but only by a small number of clinicians. Data collection and interpretation require a thorough understanding of MU physiology and pathogenesis, and successful application depends on controlling a variety of technical factors and mastering data-collection skills. The technique allows clinicians to draw conclusions on the type and severity of the disease as well as indicating the likely anatomical location of a lesion and disease evolution. Our understanding of equine neuromuscular disorders would greatly benefit from the wider use of QEMG to benefit the welfare of the horse.

Conflict of interest statement

Neither of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2015.07.024.

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