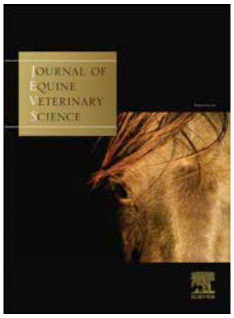

Electromyography in the horse: a useful technology?

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Review

Electromyography in the horse: a useful technology?

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Summary:

Equine performance research to date has focussed on cardiorespiratory and biomechanical assessment of the horse neglecting the role of muscles. This review considers electromyography (EMG) in the horse, with a specific focus on the role of surface electromyography (sEMG) as a tool to analyse muscle activity in the sports-horse. Three themes have been evaluated in the horse using EMG: muscle recruitment, muscle activity during exercise, and fatigue. Results support kinematic research and add to the knowledge base on how the horse moves. . sEMG is a relatively non-invasive technology requiring clipping which can be used effectively in the ridden horse. Understanding equine locomotion and how muscles responds during different exercises could inform and evaluate training practices used in the sports horse. However, issues exist for example individual variation, accuracy of sensor placement and preventing noise within the EMG signal. Therefore key concepts in research design, data acquisition and processing are explored to inform future studies and to enable reasoned judgements on the validity and reliability of sEMG as a tool to investigate muscle recruitment and activity, and subsequently assess performance in the horse. The high level of inter-subject variance observed in between subjects' designs combined with differences seen between individuals may preclude reliable comparison of muscle performance between groups of horses. Therefore within subject designs are advised for future sEMG studies. A standardised approach to data collection and analysis conforming to guidance from the human SENIAM database is recommended including consideration of the inherent challenges that present in EMG research.

1.0 What is EMG?

Electromyography is the study of motor unit action potentials (MUAPs) associated with muscle activity during movement [1]. Muscle fibres are stimulated to contract by action potentials triggering depolarisation and polarisation within individual muscle fibres of the muscle [2,3] creating electromagnetic fields which can be measured by EMG [4]. The recorded EMG signal represents the sum myoelectrical activity for a defined event in the muscle for the pick-up zone (recorded data) of the electrode used [4]. EMG electrodes have a defined pickup zone (Figure 1) which will depend upon the source of the signal and the distance from the source of the current to the electrode [5] as well as the system utilised [6,7]. Features commonly analysed within human and equine EMG research include muscle recruitment, duration of activity and activity levels [8, 9 and 10] (Figure 2).

There are two methods of EMG: indwelling EMG, subdivided into fine-wire and concentric or fine needle EMG, where electrodes are inserted into muscles of interest, and surface EMG (sEMG) where electrodes are applied to the extracellular skin surface above muscles of interest [6]. The choice of EMG method and electrode should depend on the research objectives of individual studies [11]. All techniques can fundamentally record the same muscle performance parameters, however indwelling electrodes record signals from >5cm depth, dependent on length of electrode and how deep it is inserted into the muscle, whilst the signals surface systems pick up tend to be limited to ≤5cm depth. Both surface and indwelling techniques have been used in the horse, for example: Takahashi et al. [12]: fine-wire, Wijnberg et al. [13]: needle EMG, and Robert et al. [14]: surface EMG. sEMG may possess an advantage in locomotor equine research over fine-wire and needle EMG, as sEMG electrodes have the ability to sample more motor units (MU) per electrode, making them better suited providing a representation of total activity within the large muscles of the horse. sEMG sensors provide a non-invasive method which is more ethical and acceptable for use in subjects facilitating increased access to potential subjects and wider application e.g. during ridden exercise [7, 15]. However sEMG sensors are not without their limitations. Electrodes are limited to a subject's superficial musculature and crosstalk (interference in the MUAPs recorded) can be introduced to the signal because of skin displacement associated with poor electrode attachment, due to signals from nearby muscles being integrated into the signal. These can vary between subjects owing to the depth of subcutaneous adipose tissue, which acts as a filter, between the muscle and the sensor reducing the reliability of the source of the sEMG signal detected [16, 17]. In human studies, fine wire or needle electrodes would be the method of choice to assess deep musculature and when assessing finessed movement, due to their refined topographical specificity and ability to define MUs compared to sEMG sensors [11]. For the horse, fine wire EMG has been used in a similar way for example to assess recruitment of respiratory muscles linked to stride patterns [18]. Concentric needle EMG has been successfully utilised to investigate neuromuscular dysfunction in both humans and horses due to its increased sensitivity, defined location and because it records minimal interference from other muscles increasing the accuracy of diagnosis [19]. Therefore as the scope of EMG and complementary techniques available varies [9], experimental objectives and conditions should determine the choice of EMG electrode employed within equine research [20, 21] (Table 1). An extensive review of the different characteristics of each EMG technique available is beyond the scope of this review; further details of surface, fine-wire and concentric needle EMG techniques can be found in Chowdray et al. [22], Wijnberg and Franssen [19] and Merletti and Farina [23]. It should also be noted that alternative techniques are also available to assess muscle performance. for example sonomicrometry has been used effectively in the horse to measure muscle fascicle length alongside strain gauges to measure muscle force [24]; force plate analysis combined with kinematic analysis has been used to determine workloads within the muscles and tendons associated with locomotion [25].

EMG could provide (the researcher) with a valuable tool to investigate muscle functionality. Its usefulness has been demonstrated to date in human research by the integration of the technique within sports science [26, 27]. For example: evaluating core muscle activation and performance in elite footballers [28], examining whether integrating side-step exercises into training reduces anterior cruciate ligament injury in Australian Football [29], and the influence of elbow and muscle during phasing in front-crawl swimming strokes [30]. This review investigates existing themes in equine EMG research. It also considers key aspects of research design to evaluate the potential application of sEMG as a valid and reliable methodology to examine muscle activity in the performance horse.

2.0 Themes within equine EMG research

A number of electromyography investigations have been conducted in the horse within the clinical environment investigating muscles role in pathology and the non-clinical environment, identifying and exploring muscles contribution to performance through applied research. Key areas researched include the identification of onset and offset of muscle activity and how these relate to neuromuscular dysfunction during respiration. As well as determining which muscles contribute to locomotion and exercise allowing this knowledge to be applied to inform equine performance.

2.1 Muscle recruitment

It is essential to fully understand the functional contribution of muscles during motor tasks and the coordination patterns, which occur between muscles throughout normal equine motion and within clinical assessment [6, 31]. For example, Peham et al. [32] used sEMG to assess longissimus dorsi activity during induced flexion and extension at stance in healthy horses to facilitate future comparison to horses presenting with back pain. Since fine-wire and needle EMG allow researchers to target specific and defined locations they can be useful in evaluating neuromuscular and locomotory dysfunction. Wijnberg et al. [33] who successfully used concentric needle EMG within clinical diagnosis to discriminate between normal and abnormal muscle function in horses referred for neuromuscular locomotor problems demonstrated this. Similarly, a number of fine-wire investigations have explored normal neuromuscular functionality of muscles (onset and offset of activity) associated with respiration during dynamic exercise tests to increase the understanding of how palatinus and palatopharyngeus [18], and stylopharyngeus [34] dysfunction could be indicated in respiratory disorders such as dorsal displacement of the soft palate. The increased muscle activity found during intense exercise indicates that the palatinus and palatopharyngeus muscles act to stabilise the position of the soft palate and that stylopharnygeus muscle prevents nasopharynx collapse, potentially exposing key protagonists for equine respiratory dysfunction [18, 34]. However it should be noted that the limited pickup zone of needle EMG [9] will not provide a complete representation of the workload for the entirety of large equine muscles, therefore multiple locations may need to be sampled to provide a true representation of sum muscle activity.

The recruitment patterns for many of the key muscles associated with movement in the horse have been investigated using different EMG methodologies (Supplementary information; Table S1). For example, the response of selected forelimb, hindlimb, back and abdominal muscles to trot exercise at increasing speeds and incorporating variable inclines have been assessed using sEMG on the high speed treadmill [14, 35, 36, 37, 38, 39]. Overall the research identifies multiphasic activity in individual muscles that correspond to gait and the phases of locomotion [14, 35]. Generally, increasing velocity and the use of an inclined gradient (>6%) stimulates muscle recruitment earlier in stride cycles compared to exercise at slower speeds, accompanied by contractions of greater magnitude but reduced duration [14, 37, 38, 39]. The majority of locomotor studies use sEMG as the EMG method of choice. Surface electrodes provide a less invasive method and automatically record longer duration MUAPs due to their increased surface area compared to both types of indwelling electrode, but by their nature will not record activity beyond the superficial layers of muscle [9, 17]. In the horse, it appears that fibres in the superficial compartment of skeletal muscles are organised to facilitate short duration, rapid propulsive force production supported by a predominance of type IIA and IIX fibres (fast twitch). The deeper compartment contains fibres, which support longer duration, lower intensity activities such as postural support and constitute mainly type I fibres (slow twitch) [40, 41]. Therefore, the use of sEMG sensors could potentially create a bias for data to represent fast twitch fibre activity rather than characterise sum muscle activity, which should be considered in their interpretation.

The use of non-invasive sEMG in kinematic research could increase knowledge and understanding of muscle recruitment patterns and physiology, and also has the capacity to inform evidence-based training and performance analysis in the equine athlete [31, 42]. However it should be noted that a 0mm skin clip (i.e. no hair) is required to reduce potential noise related anomalies within sEMG data [16] but may not always be feasible in actively competing horses due to competition etiquette. Work to date has predominately used small numbers of unridden horses of varying breed, age, health, fitness and competitive status; all factors which could influence muscle activity and thus the EMG profiles obtained [31, 41]. Therefore future studies with subjects representing the broader range of equine athletes and disciplines they compete in, and in the field are warranted to establish a more pragmatic knowledge base.

2.1.1 Muscle recruitment in the ridden horse

The majority of EMG studies to date have considered the unridden horse under experimental conditions; predominately these have been conducted at walk and trot using the high speed treadmill. However, treadmills produce changes in stride characteristics and the belt contributes towards the energetic cost of locomotion [43, 44] which could influence both muscle activity and recruitment patterns. Therefore, these results cannot be directly extrapolated to horses ridden over ground [37, 45, 46]. Tokuriki and Aoki [47] and Kienapfel, [48] used fine needle and sEMG respectively to examine equine muscle during riding reporting few differences in muscle activity between the ridden and unridden conditions. Riding was only shown to increase muscle activity levels during ipsilateral hind limb contact in the biceps femoris at walk with a rider compared to without [47], perhaps reflecting the application of the aids and subsequent increased propulsion stimulated by the rider compared to the horses' free gaits on a treadmill. Similarly, no differences in EMG profiles have been reported in muscles associated with head and neck flexion when examined using draw reins compared to the same horses when ridden [48]. Unfortunately the studies do not state the experience level of riders used which could be influential [42]. Rider skill can influence collection, potentially recruiting different muscles to the horse in a free state and creating changes to muscle length and joint angles between subjects which can affect the frequency and amplitude of EMG data recorded [7]. Similarly, the rider will control impulsion and speed, factors which have been shown to increase / decrease muscle activity levels [39]. Riders control the type, duration, intensity and frequency of exercise in the horse, and therefore possess a responsibility to manage the horse appropriately to its age, health status, and experience and fitness level to prevent injury and promote career longevity. Further research is required to fully explore the influence of the rider on muscle activity and more broadly how interactions between the horse-ride dyad impact on performance and relate to acquired injuries. Studies to determine how any rider and riders of different experience levels affects horses' muscle activity and to compare equine muscle activity during riding to the unridden condition. However, few equine performance laboratories can undertake ridden work on the high speed treadmill [45] and the ability to control speed, surface and climate in the field could be limiting factors to meaningful analysis in this area.

2.2 Equine Performance: Application to training

The lack of a scientific evidence-base for equine training (fitness and skill development) and performance analysis is proposed [31, 42, 49]. Understanding how and when muscles are recruited during movement in subjects free from lameness as well as the impact of factors which can influence workload, such as speed [39] or gradient [35], has the potential to inform training practices in the competitive equine (Table 2).

sEMG research supports anecdotal training practices that fast work (increasing speed of locomotion) influences the level and duration of muscle excitation in equine muscles during locomotion generally intensifying muscle workload [14, 35, 36, 37, 38, 39]. Significant increases in sEMG intensity related to escalating velocity during trotting have been observed in the gluteus medius [35, 37, 39], biceps femoris [35] and triceps brachii [36, 39]. The raised activity has been linked to kinematic changes, specifically the reduction in stance duration which accompanies speed increases [35, 36, 37, 39]. Decreased stance duration has also been correlated with increased sEMG intensity, earlier onset of muscle recruitment and decreased duration of muscle activity in the gluteus medius [35, 37] (and triceps brachii [36, 39] during high speed exercise. Therefore in the gold-standard dynamic EMG study or during performance analysis, speed should be standardised to prevent misinterpretation of data between or within subjects across different exercise tests, unless it is in itself a research objective. The gradient horses exercise on has also been shown via sEMG to affect the timing of recruitment and muscle activity levels [35, 37, 38, 39, 50]. Generally exercise on an incline results in earlier recruitment and increased muscle activity in the hindlimb [35, 37, 39] for longissimus dorsi and rectus abdominis [14, 29] and in the forelimb [34]. An increased workload is required to retain a consistent speed during locomotion in the transition from level ground to an incline [14, 37, 51] whilst the head and neck will be extended to adapt the centre of gravity and balance the horse [14, 36, 39]. Interestingly, increased muscle activity was also observed during the decline condition with the exception of the digital extensor muscle [35]. Increased stabilisation is required for controlled locomotion down hill which necessitates a greater response in muscles associated with stability (vastus lateralis) [35] and reduced activity in muscles associated with generating forward momentum (gluteus medius) [35]. Although the transferability of the value ranges for the EMG parameters studied may be limited by the choice of breed due to genetic differences in muscle fibre profiles [52] and the variable statistical approaches employed, the research broadly supports training over undulations to condition the musculoskeletal system for locomotion over variable terrain [14, 37, 38].

The majority of equestrian disciplines require a balanced, symmetrical athlete [53]; therefore the concept of developing a balanced equine athlete is core to achieving both optimal performance and preventing injury [54]. Differences in activation of muscles on either side of the body may be related to training, acquired pathologies, an individual horse’s laterality preference (handedness) or simply be normal for the muscle under investigation due to its function. In these instances, differences between muscle workload and therefore MUAPs recorded via sEMG would be expected. sEMG has identified lateral biases exist in the horse within superficial gluteal muscles during canter exercise in racehorses [42], in the longissimus dorsii during static flexion and extension [32] and within splenius activity during walk and trot [56]. However further work is required to identify if these reflect normal muscle function related to biomechanics or indicate potential asymmetric recruitment due to poor training or acquired injury.

2.3 Equine performance: evaluation of training practices

EMG studies, especially via non-invasive and telemetric sEMG which require no wires, have the potential to contribute to the development of an evidence-base to support the use of training practices often employed in equestrianism to develop muscle mass, strength or flexibility. For example verifying the use of exercises such as gymnastic gridwork [57], riding practices such as hyperflexion [56, 57] or training aids such as the Pessoa Training Aid (59, Williams, unpublished data). In humans, EMG data have been used to quantify muscle activity within defined events to enable muscle performance and adaptation to be monitored over time within training regimens [29, 60, 61]. Williams et al. [55] used sEMG to compare muscle activity levels for consecutive runs within interval training in racehorses, reporting a high degree of variability within subjects postulated to be largely due to rider influence on the horse's workload: collection and speed. High levels of inter- and intra-subject variability in EMG data have been reported across human and equine studies, and have been attributed to athlete status (elite vs. amateur) [15], training [60], individuals' muscle fibre profile [13] and normal variation in muscle contractions [62].

The use of sEMG to establish progress within training is challenging as data do not accurately reflect what is happening at fibre level within the muscle and changes observed in sum amplitude may equal improvements in timing, firing synchronisation, fibre hypertrophy or increased muscle fibres being recruited [6]. Integrated EMG (iEMG) may offer researchers a tool by which sum muscle workload can be compared across events within the same subject. IEMG represents the area under a fully rectified EMG trace, to provide in essence a measure of the work done by a muscle for a defined activity period [63]. In humans, a reference state or maximum voluntary contraction (MVC) is often employed to facilitate comparison by providing a 100% effort measure which subsequent efforts can be related to [64] but this is not achievable in the horse. However a pseudo-reference state can be assigned to obtain a MVC to facilitate comparison of muscle workload across activities. The maximum contraction from within a period of dynamic exercise can be used to replicate a

MVC facilitating comparative analysis of muscle workload using iEMG [65]. For example, if a study was undertaken to compare the impact of different water heights within a water treadmill on equine hind limb muscle activity, work done at the maximum water height would be expected to represent the most demanding muscle workload. Therefore the maximum contraction obtained from this water height be could be defined as 100% activity or the equivalent of a MVC, and could be used to compare muscle workloads across water heights. EMG assessment of progress within training may have worth in the horse, but researchers will need to exercise caution in both research design and interpretation of subsequent data to ensure reliability and validity are maintained.

Normalisation of the EMG signal is used to compare activity in the same muscle between different exercise tests or on different days, or to compare activity between muscles [2]. A reference contraction, usually a MVC, the mean or peak activation for a defined sequence of muscle activity or peak-peak amplitudes can be used for normalisation [65]. However, there is no ideal normalisation method and the researcher should select the method which facilitates repeatability and best matches the muscle activity under investigation. The choice made will influence the amplitude and pattern of the subsequent EMG signal obtained. In equine sEMG, various methods of normalisation have been conducted including normalisation to the EMG signal obtained at rest [32], to mean activity across a defined number of strides [56, 90] and to a maximal contraction obtained during data collection [32]. Understanding the type of muscle activity that is being investigated will inform selection. For non-cyclic isometric contractions (no change in muscle length associated with force production) normalisation to a MVC is indicated. Whereas for evaluation of muscle activity within dynamic exercise containing cyclic contractions (a sequential pattern including shortening of the muscle: concentric contraction and lengthening of the muscle: eccentric contraction), normalisation to a peak contraction obtained from within the exercise itself or to the mean activity across the exercise event is advocated.

2.4 Fatigue

An important objective of a training regimen is to ensure the equine athlete is sufficiently fit to complete the task they are preparing for and to prevent fatigue related poor performance or injury [31]. Across all disciplines training aims to improve the aerobic capacity of the equine athlete [66] thus postponing the onset of fatigue and optimising performance. Different exercise types generate specific adaptations in muscle tissue, which can be summarised into three muscle responses, whose expression will be dependent on the individual: 1. Hypertrophy: (high intensity and strength and conditioning training), 2. Remodelling of fibre type without hypertrophy (endurance training), and 3. Remodelling of fibre type with hypertrophy (combining training and exercise methods) [67, 68]. Alongside the changes observed within muscle size, repeated muscle recruitment and practice of skills will enhance neural plasticity promoting increased synchronisation in firing rates creating pathways, which will optimise recruitment and quicken future responses [69].

Fatigue and its subsequent impact on muscle performance in humans have been examined using sEMG, for example repeated play in tennis players [70] and the influence of bike design on fatigue in cycling [71]. Plotting the mean or median frequency of MU contraction required to sustain workload over time can identify changes in a muscle's capacity for continued exercise i.e. provide a measure of fitness or fatigue [27]. In the galloping horse, the onset of fatigue is characterised kinematically by decreased stride frequency accompanied by increased stride length and suspension [31] representing physiological changes in the associated muscles of locomotion. Mechanically, fatigue is characterised by a reduction in muscle tension (force); therefore increased numbers of MUs need to be recruited to maintain activity-levels. At fibre level, fatigue generates a shift in recruitment from anaerobic (fast twitch, high frequency fibres) to aerobic (slow twitch, lower frequency) muscle fibres [8, 10]. Simultaneously fatigue changes the characteristics of MUAPs. Larger and faster Mus with short duration activity will drop out of force production first, APs will record a reduced conduction velocity and the remaining active units will synchronise to fire in bursts [2]. The consequence is a reduction in the high-frequency components of the EMG frequency spectrum, which when plotted over time consistently results in a net left shift in the mean or median frequency of the signal [4, 10, 27]. sEMG assessment of muscle fatigue has been successfully undertaken for the horse in the laboratory [10, 51]. Colborne et al. [10] assessed fatigue in the deltoid muscle of thoroughbreds during a maximal exercise test on a treadmill. A reduction in the median firing frequency of the MUAP signal over time illustrated that fatigue occurred in all horses. Cheung et al. [51] investigated fatigue onset pre- and post-training in the digital extensor muscle, demonstrating that an eight week training programme reduced the onset of fatigue within this muscle in thoroughbreds. Unfortunately here fatigue was confirmed via observation post exercise, which correlated with increased EMG amplitudes rather than by evaluating the median frequency of the MUAP signal over time which could have confirmed its presence more accurately [27].

Selecting appropriate methods to assess fatigue are critical. Takahashi et al. [12] reported trends for superficial digital flexor and deep digital flexor muscle fatigue using fine-wire EMG. However the deep location and reduced pickup zone of fine-wire electrodes limit application to the whole muscle [9, 13] and do not represent the superficial component more responsible for dynamic locomotion [41]. Only one study to date has assessed fatigue in the field [55]; using sEMG it was found that although muscle activity levels (superficial gluteal) varied between runs, no evidence presented that canter interval training (2-5 repeat runs; speed ~6m/s) in National Hunt racehorses produced fatigue regardless of subjects' fitness status. The preliminary studies suggest that the EMG signal reflects physiological changes occurring in muscle associated with fatigue or its onset, although confirmation through increased numbers of horses is needed. Performance related variables will contribute to workload, for example speed of locomotion [39] or surface gradient and type [35, 73]. The horse's breed [52], age [74], sex [75], fitness level [76], type and duration of exercise [40] and nutritional status will also influence the extent to which exercise can be sustained and contribute to the onset of fatigue [67]. Therefore it could be argued that for 'gold standard' EMG research, the use of homogenous samples where these variables are standardised is ideal. Unfortunately this is not always feasible or achievable, even in the small samples (n<10) generally used [37, 55]; the impact of confounding variables should be interpreted and reported. Recent developments in mobile telemetric sEMG systems [77] facilitate research outside of the laboratory using the ridden horse replicating or assessing real-world training and competition. These systems could be used to promote evidence-based approaches to assess fitness to compete through a standardised exercise test approach or fatigue assessment during training potentially reducing fatigue related musculoskeletal injuries. However whether fatigue in the entirety of the muscle can be truly assessed remains debatable. Therefore to fully evaluate fatigue initial studies are warranted to compare results between the superficial and deep compartments of the muscle using both indwelling and surface EMG analysis.

2.5 Lameness assessment

Musculoskeletal injury is the main reason for days lost from training or competition in the sports and race horse [78, 79, 80, 81]. Both fine needle [33] and surface EMG [32, 81] appear to have the potential as an aid to lameness diagnosis in the clinical environment. Accurate diagnosis is critical to underpin subsequent therapeutic regimes, inform prognosis and to expand the knowledge base of muscle related pathologies. Zaneb et al. [82] reported reduced activity levels (sum amplitude) and reduced minimum to mean amplitude ratios in the hamstring muscles and longissimus dorsi of lame horses at walk and trot at standardised speeds on a treadmill compared to clinically sound horses, and between the lame and non-lame limb within lame subjects, using sEMG. The results reflect the movement patterns consistent with lameness (head nod / hip hike) [83]. Wijnberg and colleagues [33, 84], have demonstrated that fine needle EMG can be used to differentiate whether lameness in the horse is related to myopathies or neuropathies. Wijnberg et al. [33] compared temporal (timing of recruitment) and spatial (amplitude) measures in the EMG signal in a range of muscles purported to be related to lameness for 104 case horses, with baseline MUAP data of 'normal' subjects. EMG evaluation influenced clinical diagnosis changing lesion location in 12% of myopathy and 37% of neuropathy cases and enabling diagnosis in 54% of idiopathic cases, demonstrating the potential of fine needle EMG to discriminate between normal and abnormal muscle function. The timing of muscle activity is considered consistent across normal human [61, 85] and equine [37] subjects. In horses where lameness is due to neurological dysfunction, differences in the amplitude profile connected with loading patterns will be present [82] but critically changes in muscle recruitment patterns will also be observed [33] distinguishing them from cases of myopathy.

3.0 Key considerations for the equine sEMG researcher

Limited research has been conducted to analyse muscle performance during exercise in the horse [31]. Factors which relate to muscle performance are commonly assessed observationally, for example fatigue, or via analysis of allied physiological variables, such as heart rate (for example: Williams and Fiander [86]). However these techniques cannot quantify recruitment, activity-levels or adaptation in muscle tissue, or account for individual variation; factors which could directly influence performance. sEMG has the potential to be a valuable tool to evaluate muscle performance in the horse, however it is not a perfect technology. Key considerations the aspiring equine sEMG researcher should address within their research framework are outlined below.

3.1 Data collection guidelines

The ideal sEMG study should aim for consistency in the acquired EMG signal and should contain no distortion [16, 87]; numerous factors can influence the quality of sEMG data collected (Table 3). Dirt or grease, interference from other equipment, skin or sensor displacement from muscle movement and activity of muscles in close proximity to the one being examined, can generate myoelectric crosstalk and generalised electrical 'noise' in sEMG [16, 88, 89]. Robust experimental design should help to eliminate such interference. Accurate electrode placement optimises data collection from the muscle of interest and limits crosstalk potential [16]. The location of EMG sensors on the muscle is important to ensure an accurate representation of muscle activity is obtained. Sensor electrodes should be aligned at a consistent distance with muscle fibre direction, placed over the maximum circumference or belly of the muscle under investigation and away from tendinous insertions and innervation zones to prevent cross-talk and artefacts in the received signal [16]. Precise replication can be achieved by refining sensor location with reference to defined anatomical landmarks, the use of experienced experimenters with equine anatomy knowledge and marker systems i.e. indelible ink. Sensors should be located over the maximum circumference of the muscle avoiding tendon insertions which can generate noise [3].Whilst suitable skin preparation, ideally a 0mm clip/shave and isopropanol alcohol scrub should be undertaken to remove dirt and prevent noise [16]. However owners and riders may not wish for this to occur in areas that are visible during riding due to competition etiquette or personal preference. Human EMG researchers benefit from globally affirmed topography for sEMG sensor placement, available via the Surface EMG for Non-Invasive Assessment of Muscles (SENIAM) guidelines [3]. Equivalent guidance unfortunately does not yet exist for the horse; however equine sEMG research should aim to conform to the SENIAM recommendations for data collection and processing (available at: http://www.seniam.org/).

3.2 Within versus between subject design

Individuality within sEMG profiles is a theme observed throughout equine research [35, 51, 55, 72]. Robert et al. [14] and Zoldos et al. [90] reported 13.3% and 67% relative variance in gluteus medius and rectus abdominis for activity-levels, respectively, between individuals. Interestingly similar patterns are observed in human EMG research; average inter-subject variance in fine needle studies is ~60% [91] although ranges vary between 16-75% [15]. Higher levels of inter-subject agreement, 23% to 88%, has been reported for sEMG research with ranges between 70 and 91% reported in more recent work [92], compared to 23% agreement seen in earlier studies, reflecting advances in technology [93]. Inter-subject variability in sEMG is postulated to relate to differences in muscle fibre type, diameter and distribution between subjects [13, 62] or actual differences in motor patterns [87]. Skin thickness, depth of subcutaneous fat and distribution of sweat glands and sweat production may also result in variability between subjects as increased quantities of these will impede the return of the EMG signal from the muscle to the sensor [16]. Differences related to participant status are also observed [15]. For instance, trained human athletes demonstrate an increased propensity for MU synchronisation with muscles containing more fast-twitch fibres than their healthy non-athletic peers [15, 94]. Differences are also reported between human sprint and endurance athletes for conduction velocity [94] whilst muscle fibre profiles can differ with training type and genetics [15, 41]. Similar patterns may well exist for the horse and their potential influence should be considered when selecting participants for EMG research.

In humans, although EMG signals are considered highly individual, research has shown that muscle activation and timing patterns during movement are consistent between subjects [61, 85, 95]; a pattern which also exists across equine EMG studies. Fundamentally, healthy and sound humans will recruit specific muscles to facilitate specific movement. Within this movement the temporal characteristics: onset and offset of recruitment and the duration of the associated contraction, will be broadly consistent [61, 85, 95], characteristics which should be replicated in the horse. For the horse, changes observed in the duration of contractions correspond to kinematic patterns in locomotion for example the reduced stance duration associated with increasing speed produces earlier onset of contraction to facilitate the increased workload required in the Gluteus medius [37]. Therefore using sEMG to compare the recruitment and activation of the same muscle/s during movement, for example at different water heights on a water treadmill should be able to be successfully conducted across subjects [72, 85]. In contrast, the spatial characteristics: type of MU recruited, synchronisation, MUAP amplitude and frequency domain, are associated with muscle 'power' or workload during contractions. Assessing the spatial parameters associated with muscle performance will produce unique physiological footprints for individual horses, which will also reflect their unique conformation, and the biomechanics associated with movement during their execution of specific exercises. For example, variation in heel strike during running in humans influences muscle performance as could the angle of the hoof-pastern-axis during cantering in horses [91, 96].

Intra-subject variability is observed within human [85, 97] and equine [72] EMG data although to a lesser extent than between subjects. In human sEMG research an average intra-subject coefficient of variance of 21.6% has been proposed for healthy muscle [97]. Numerous factors can affect the reliability of sEMG data collected and influence interpretation of results [4]; examples of which are outlined in Table 4. Researchers should also take into account how variation in work and individual status will influence results within repeated measures designs, to promote validity and reliability. Some degree of normal variation is to be expected between events on the same day and subsequent days within individuals due to differences in intrinsic and extrinsic factors which influence muscle activity and the 'workload' of the individual. For example speed may vary could influence muscle workload or electrode placement may not be matched to previous data collection periods, which would provide erroneous results. Both inter- and intra-examiner reliability have been evaluated within human sEMG research with mean reliabilities of 84% and 95% reported, respectively [95, 96]. Similarly the reliability of obtaining repeated measures on the same day and between days have been analysed. Ng et al. [100] reported 75 to 90% agreement in subject's sEMG profiles for human trunk muscle activity between days. Reliability appears to increase for repeated readings obtained on the same day with 91% agreement shown in trunk muscle profiles by Dankaerts et al. [92], suggesting sEMG is a reliable methodology to assess muscle performance over time, within subjects. Techniques can also be applied to normalise MUAP obtained to increase reliability of data or techniques such as iEMG can be adopted to analyse comparative performance to allocated baseline data [63]. However these techniques are not solutions for poor experimental design and the research team should aim through their research design to control for as many confounding variables as possible.

Researchers should carefully consider the impact of both intra- and inter-subject variability within their results and report these variables to allow the reliability of their data to be judged, and consider their impact upon subsequent inferences made. Producing repeatable sEMG recordings for muscle workload parameters such as mean MUAP or peak contraction between different groups of horses is virtually impossible due to the variability in individual muscle profiles as well as the challenges of reproducing technical and workload variables accurately. Ultimately, the high degree of inter-subject variability in both human [61, 85] and equine [55, 72, 90] sEMG profiles suggests that for examination of muscle activity, sEMG would have most value as a comparative analytical tool within individual horses (requiring a within-subjects research design), rather than comparing across different groups (between-subjects design) to produce valid and reliable datasets, and avoid wasting resources.

3.3 Data analysis

No set methods are accepted as a gold standard when processing sEMG data [6, 101], however advisably some form of processing to remove noise and acquire usable data is undertaken. Common analytical approaches applied to EMG data are outlined in Table 5. Evaluation of the gross sEMG signal in real-time can provide visual information on the onset, offset, timing and duration of muscle activation [102, 103]. However raw sEMG data are recorded as a sinusoid containing negative and positive values (Plate 1); therefore data processing, such as rectification to make all data-points absolute values, is required to fully evaluate muscle responses during analysis [104]. During muscle activity, the amplitude of the sEMG signal at any given instant is stochastic and will contain multiple frequencies contributing to the force produced [6]. Within the signal, the initial MU input is a high frequency signal which ‘fires’ the subsequent muscle twitch, however the muscle fibre acts like a capacitor, therefore contractions produce lower frequency outputs [2]. Likewise, external sources of noise could contribute to the frequency domain recorded [16]. The ‘usable’ EMG signal associated with human movement is considered to exhibit frequencies between 20 and 250Hz [15, 61]; currently no equivalent frequency range has been proposed for the horse and human parameters are used. Therefore filters are usually applied to ensure only relevant frequencies that contribute to the event being assessed are analysed reducing the potential for misinterpretation of data and subsequent spurious results [2, 8]. Ideal filters have brick wall responses to cut-off frequencies (f^c) i.e. a 20Hz low-pass filter would remove all frequencies <20 Hz [105]. Many sEMG systems such as the Delsys® analysis software (Delsys® EMG Works™ Version 4.13) incorporate a hardware band-pass filter, f^c of 20 and 480 Hz respectively [77], which eliminates noise components (data <20Hz) within the signal to prevent misinterpretation of the data during subsequent analysis [16]. However, f^c only considers changes in the magnitude of the signal in relation to amplitude frequency. In reality, the magnitude the sinusoid EMG signal represents is related to both amplitude and time, therefore subsequent filters (for example linear enveloping of the signal) are often applied to integrate both frequency and phase response (timing) to accurately filter the data obtained [8, 105]. Delays in timing between two sinusoids of the same frequency results in each passing through the zero point at different times causing the resultant EMG signals to be out of phase, creating a phase-lag [8]. Therefore the phase lag of the selected filter is also important when considering timing of activity within dynamic muscle studies. The ideal filter will integrate a time delay that is independent to its frequency i.e. each frequency component within the signal will be phased in exactly the same way. Unfortunately exact-phasing is not achievable; therefore the fc is combined with a filter order, to control for phasing, to create the optimal sEMG data for evaluation [105]. It is important to note that the combined impact of the filter type, fc and order will influence the shape and magnitude of the manipulated EMG profile [8, 104] and its effect should be considered when interpreting results obtained. It should also be noted that for effective analysis of frequency components within the signal, the filtering protocol applied should only remove spurious data. It should also be noted that frequency analysis to determine fatigue requires the full frequency spectrum of data to be included in analysis, therefore minimal filtering (simple bandpass filter to remove noise) should be undertaken prior to analysis.

Comparison of the amplitude of EMG signals between events is challenging due to the variety of confounding variables which at any given point in time may vary and influence muscle activity [8, 63]. Alternative approaches to EMG data analysis can be used to compare workload between events within individuals. One approach is to normalise the EMG signal to enable comparison. For example iEMG has been successfully employed in the horse to evaluate differences (expressed as a percentage of the defined maximum workload) in the workload of brachiocephalicus and triceps brachii at walk and trot on a level surface compared to on an incline [36]. Force estimation requires normalisation to maximum voluntary contractions (MVC) values, [7, 8] which are not an option for the horse. An alternative approach within comparative evaluation is to utilise a defined reference state or the MVC from within a piece of work [64]. For example Cheung et al. [51] effectively analysed fatigue data collected in their work with baseline fatigued EMG data to assess adaptation within horses studied and Peham et al. [32] used a maximal contraction obtained during data collection to enable normalisation of subsequent related muscle activity. Another option is to compare data from related events within a defined movement cycle [56, 90] for example: the initial run on the gallops [55], the approach stride to a showjumping fence [57] and the chewing cycles pre-dentistry [72] represented valid reference states in these studies to enable subsequent assessment of muscle performance over time within subjects.

3.4 Interpretation of EMG data

Interpretation of sEMG data is acknowledged as challenging as a number of factors can influence the resultant signal [4]. Variation in signal amplitude may be attributed to an increased numbers of active MUs being recruited or to a change in the frequency of activation i.e. the firing rate has increased but the same number of MUs are being recruited [3, 106]. The subjects' muscle fibre profile will also influence the sEMG profile [8] and this can change with training or injury. An elevated amplitude of the sEMG signal could be related to an increase in fast-twitch fibre recruitment or a higher firing rate in slow-twitch fibres related to synchronised firing at the onset of muscle fatigue (both equalling more muscle effort) [7, 107]. Whilst decreased amplitudes may represent increased MU synchronisation creating the same power output with less effort i.e. coordinated muscle activity with no fatigue present associated with muscle fibre hypertrophy and enhanced neural plasticity from training [2, 7, 107] or be due to fatigue [10].

Variation in muscle length and the subsequent impact on sEMG data recorded should be considered within data analysis especially in dynamic studies. During movement, muscles perform anisometric contractions dependent upon their function. Although contraction magnitude is individualised, broad characteristics can be observed in the resultant sEMG trace [7]. Concentric contractions exhibit a larger magnitude than eccentric ones representing the greater workload associated with muscle shortening [63]. Therefore it is important that comparative events which consider kinematic patterns are selected for analysis to ensure valid conclusions are formed [15, 107], for example 5 continuous chewing cycles [72] or 10 strides of trot [14]. Synchronisation of EMG data collection with kinematic analysis increases specificity between muscle recruitment and aspects of locomotion [6] allowing the researcher to evaluate relationships between muscle activity levels and movement patterns, promoting accuracy and improving the reliability of sEMG interpretation [61].

3.5 Is a 'normal' equine EMG database feasible?

A number of barriers exist to the creation of a 'standard' EMG database in the horse. The relatively novel use of sEMG in equine research and lack of standardized methodology combined with the range of different data analysis protocols used, has led to variability especially in the interpretation of EMG data among studies [108]. For example Tessier et al. [34] reported their fine-wire data as a percentage of the EMG data obtained at HRMAX50 (iEMG) whilst Holcombe et al. [18] applied a moving average to their fine-wire data to obtain a summary profile. Data manipulation techniques such as linear enveloping using filters of different types and cut-off frequencies, are commonly applied in studies utilising sEMG [56, 72, 90] which will attenuate the EMG amplitude and change the shape of the EMG profile [6, 104, 106] preventing comparison with data values from previous work which have used different filtering protocols. Despite this, EMG is considered to be the most reliable tool available, at present, to evaluate muscle function in humans [111] and accordingly also in the horse. Future equine sEMG research should consider the body of research across humans and animal species and report upon the impact of research design and data analysis methods used, which could potentially explain variation reported, on their subsequent interpretation of the results obtained [3, 106]. Going forwards, an equine equivalent to the SENIAM system in humans would promote consistency in sensor placement and data collection protocols, thus facilitating increased comparison between studies. Similarly a consistent approach to data analysis and reporting of standardised measures in line with the human gold standard SENIAM guidelines [112] would enhance the validity and reliability of equine EMG research. It should also be remembered that some degree of skeletal muscle recruitment is ubiquitous, as it is required to maintain postural stability, therefore when evaluating muscle activity during dynamic exercise, muscle onset and offset need to be defined in context. The onset of activity related to a specific movement occurs when the corresponding AP exceeds a defined activity threshold and offset follows when the AP falls below the threshold value. More research is required to estimate if basal EMG parameters for muscle activity during different gaits and exercise conditions exist for individual muscles across horses, and within defined samples such as discipline specific breeds or equine athletes. Or whether sEMG values are specific to individual horses as reported in human athletes [61, 85] and supported by the case by the variability reported in the equine EMG research to date [10, 35, 57, 72].

4.0 Conclusions

sEMG is non-invasive and can be used in the field or laboratory to assess muscle recruitment and activity but data are limited to the superficial compartment of the muscle. Indwelling EMG systems are invasive but fine-wire EMG can offer increased specificity and analyse the deep compartment of muscles, enabling defined areas to be assessed, whilst concentric needle EMG has value in the assessment of neuromuscular dysfunction. The different EMG types available represent valuable tools which can increase the knowledge and understanding of the functionality of equine muscle, however more research is required to validate the use of sEMG in the field, i.e. non-laboratory setting, before its full worth as a useful technology is revealed. Equine fine-wire and sEMG research has concentrated primarily on muscle recruitment, factors which influence activity levels and (use of EMG) to aid differential diagnosis in lameness cases, whilst needle EMG has been used to explore relevant muscle contribution to respiratory dysfunction. It appears that horses possess a distinct physiological footprint for muscle activity (amplitude) which corresponds to their individual physiological status and workload at specific moments in time. In contrast, consistent temporal patterns exist for muscle recruitment and the duration of muscle activity related to kinematic patterns. Therefore baseline data can only be reliably and validly obtained for timing-related parameters in the horse, although general trends in amplitude profiles have value when comparing between conditions within individual horses. Going forwards, EMG researchers and practitioners should carefully consider the impact of research design particularly 1) subject selection and 2) data analysis upon their interpretation of their results. A consistent approach across equine EMG research is needed to help to expand the evidence-base related to muscle function in the horse.

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Conflicts of interest

No conflicts of interest apply to this work.

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Table 1: Advantages and disadvantages of surface and indwelling electromyography [2, 8, 9, 11, 13, 16]

Method	Advantages	Disadvantages
Indwelling: needle and fine-wire EMG	1. Indwelling electrodes, particularly fine-wire, exhibit an increased band width, with a specific pickup area of ~50 to 200µm. 2. They are capable of testing deep muscles, depending on depth of insertion. 3. Both fine-wire and needle electrodes can isolate specific parts of large muscles, be used in small muscles or to test areas where multiple muscles converge requiring specificity to analyse the area of core interest. 4. Insertion negates the low-pass filtering effect associated with body tissues observed in surface EMG. 5. Fine needle electrodes record reduced interference from surrounding muscles, resulting in a defined and specific pick-up zone related to its location.	1. Invasive: both fine-wire and needle electrodes are inserted via a hypodermic needle. 2. Discomfort associated with insertion. 3. Potential spasticity in targeted muscles. 4. Lack of repeatability in placement. 5. Use limited to laboratory. 6. Fine-wire electrodes may break and be retained in muscle; needle electrodes are not retained in the muscle.
Surface EMG: passive and active	1. Non-invasive and therefore may be considered more ethically acceptable for use in ‘working’ or competitive horses. 2. Their non-invasive nature facilitates wider applications in dynamic studies in the field. 3. Ease of application with minimal pain. 4. Active electrodes contain integral amplifiers which reduce impedance within the skin-sensor interface, reducing the need for extensive skin preparation and electro-conductive gels. 5. Surface systems record signals from a larger cubic volume of muscle than indwelling electrodes (surface ~50mm^3 compared to ~50 to 200µm indwelling). 6. Surface systems record longer duration MUAPs due to their	1. Pickup zones for surface electrodes are limited by the distance from the muscle of interest and the detectable MUAPs being produced. 2. The pickup distance is related to the size of MU under evaluation; small MUs, ~50 fibres, are limited to ~0.5cm whilst larger units, >2500 fibres, can travel >4cm. 3. Data collected are limited to activity in proximal muscles and superficial portions of these. 4. Large pickup areas increase propensity for crosstalk within the signal. 5. Systems vary with some requiring manual placement (variable distances reported between 1-5cm) whilst other integrate fixed differential and earth electrodes. Larger distances reduce the range or frequencies picked up.

	increased surface area compared to both types of indwelling electrode.	6. Accuracy and repeatability of electrode placement can be challenging. 7. A 0mm skin clip is required for ideal skin preparation. 8. Retaining sensors in place during dynamic movement can be challenging; skin displacement can introduce data anomalies and cross-talk. 9. Subject characteristics, muscle to fat ratio, will influence the quality and quantity of signal picked up, as fat impedes the EMG signal.

Two types of electromyography (EMG) exist: surface and indwelling EMG. Table 1 summarises the advantages and disadvantages of both techniques and the subcategories within these: surface EMG (active and passive) and indwelling EMG (needle and fine-wire).

Table 2: Key objectives when training the performance horse, adapted from Ferrari *et al.* [31] and Dyson [81].

Objective 1	Preparing for competition: physiological conditioning to ensure adequate fitness and prevent fatigue
Potential application of sEMG	1. Assessment of muscle recruitment during defined exercises to target development for competition related tasks 2. Plotting the frequency of the EMG signal over time could enable fitness and fatigue to be assessed 3. Comparison of mean MUAP or sum workload via iEMG between exercise periods could aid evaluation of training progress
Objective 2	Improving performance: development of a balanced athlete and task-specific conditioning, motor skill acquisition and achievement of ‘expertise’ through improved neural plasticity
Potential application of sEMG	1. Comparison of the right and left versions of the same muscle could assess balance and contribution to workload 2. Assessment of muscle recruitment during defined exercises to target development and increase plasticity through repetition for specific motor skill tasks
Objective 3	Preventing injury and increasing career longevity: via adequate preparation of the horse and rider
Potential application of sEMG	1. Evaluation of readiness for competition through assessment of muscle physiology to support required workload 2. Assessment of fitness which could reduce fatigue related injury 3. Development of a more balanced horse could reduce overloading injuries

Three core objectives underpin training for the equine athlete: preparation for competition, improving performance and preventing injury. The potential use of surface electromyography to measure progress towards achieving these objectives is also provided.

Table 3: Factors which influence the sEMG signal [3, 4, 1, 16, 37, 68, 87, 116]

External factors	**Sources**
Inherent electrical noise	Electronic equipment
Ambient electrical noise	Electromagnetic radiation from the subject
Motion artefacts	Electrode interface Electrode cable
Inherent instability of signal	EMG amplitude is stochastic by nature, the base line motor unit firing rate at rest is not usually wanted within experimental EMG data
Causative factors (direct effect)	**Sources**
Extrinsic	Electrode structure and placement e.g. location and orientation Poor sensor to skin interface resulting in movement artefacts Temperature: muscles fire more quickly in increased temperatures compared to colder ones Rider influence: increased velocity and collection can increase muscle workload Gradient: working on an incline / decline can increase or decrease muscle activity (subject to specific function)
Intrinsic	Physiological, biochemical and anatomical factors Muscle fibre profile: number of motor units, distribution of fibre types Training: type of training will influence muscle fibre distribution; high intensity: increased fast twitch and intermediate fibres; low intensity: increased slow twitch fibres Fitness: will depend on type of training but often increased fitness produces fibre hypertrophy and improved synchronisation of firing Nutritional status: energy stores available for muscle activity Conformation: will influence muscle lengths and joint moments during locomotion affecting muscle activity Muscle fibre profiles also vary between sexes, age and breed of horse
Intermediate factors	Physical and physiological factors influenced by causative factors Cross talk can occurs due to overlapping APs from multiple muscles, motor units falling within an electrode's pickup zone or locating sensors near insertion points of the muscle The depth of the dermal layers in particular subcutaneous adipose tissue, act as a low-pass filter on the EMG signal, effectively dampening it.
Deterministic factors	Aspects influenced by intermediate factors e.g. amplitude, firing rates Exercise type will recruit different muscle fibre types which will have contribute different frequencies to the EMG signal

	Fatigue: shift from high frequency fast twitch fibre activity to lower frequency slow twitch fibre activity Dynamic locomotion causes changes in muscle length which will influence frequency and magnitude of amplitude

The surface electromyography (sEMG) signal will record all electrical activity within its defined pickup zone. The sEMG signal received will also be affected by the location of the sensor and how well it is attached to the subject as well as by internal and external factors which can influence muscle activity levels. Potential sources of interference alongside factors which can have a physiological impact on muscle function are provided in the table.

Table 4: Variables which can influence the reliability or interpretation of sEMG data

Variable	**Potential impact for equine research**
Local metabolic status of muscle [87]	Selecting horses of unknown fitness for dynamic evaluation could result in anomalies in data collection related to their fatigue
Participant selection [15]	Selecting non-athletic horses would not enable comparison of the results to competitive horses
Participant selection [55]	Individual horses will present with unique muscle fibre profiles and physiology, therefore comparison of muscle performance within subjects is recommended to eliminate anomalies due to individual variation which could be present between groups of horses
Muscle temperature [13]	Temperature has been shown to affect muscle activity in humans; a 1^0C increase in temperature produces a 5-10% decrease in MUAP and inconsistent amplitude profiles
External temperature [4]	Temperature, particularly in field based training or competition studies which may include repeated bouts of exercise, can influence muscle temperature and thus performance
Acquired pathology in muscle groups under investigation 32, 11]	Pathologies could produce abnormal loading profiles or redistribution of recruitment as a compensatory adaptation resulting in misinterpretation of data

Care should be taken during experiment design to limit the impact of intrinsic and extrinsic parameters which have the potential to affect the reliability of sEMG data collected or their subsequent interpretation. Various examples of factors which could influence sEMG data are presented to illustrate their potential impact on research design.

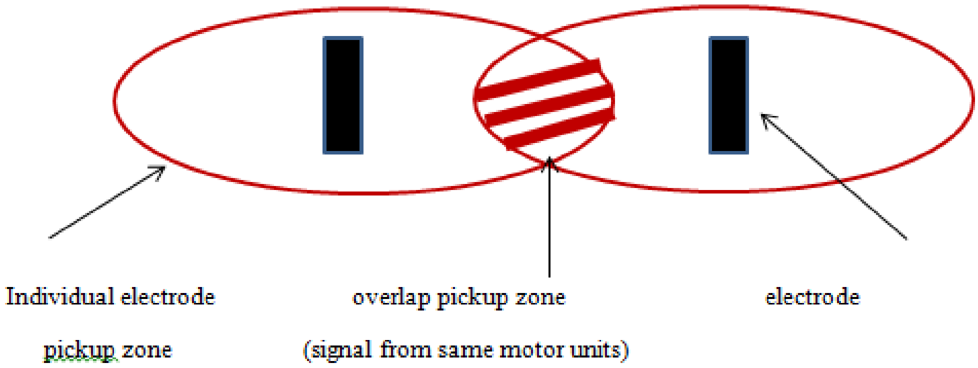
Table 5: Common analytical approached applied in the analysis of EMG data; f^c: Cut-off frequency [6, 8, 16, 2, 102, 104, 116]

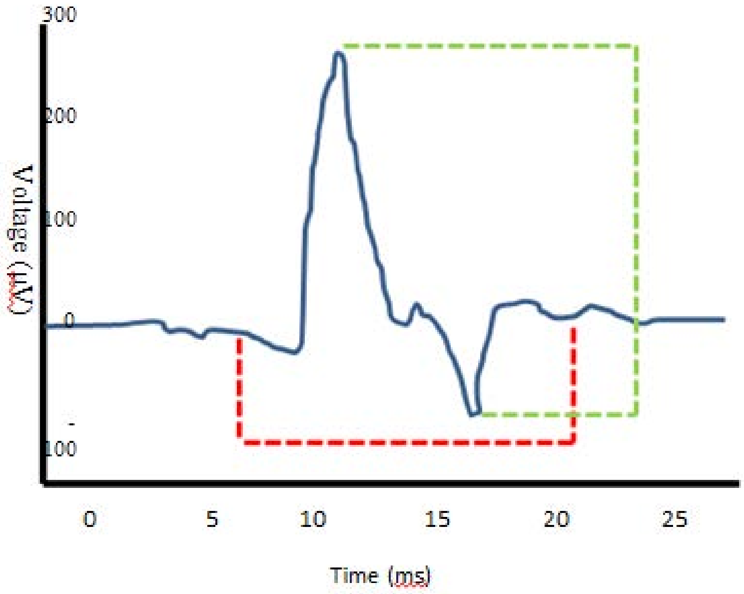
Feature	**Potential analyses that can be used**	**Equine examples**
Raw EMG signal	Plotted as a sinusoid with negative and positive values Band-pass filters can be applied to remove low and high frequency noise commonly f^c ^{o}of <20Hz and >450Hz are applied and data are viewed for a defined time period. Alternatively a low-pass or high-pass filter can be used in isolation.	Tessier *et al.* [34], fine wire EMG
Absolute value EMG signal	Data are commonly band-pass filtered to remove noise, then are converted via half or full wave rectification (absolute value, or by root mean square (relative value). The result is a positive signal, which does not cross zero and fluctuates according to the strength of motor unit action potentials (MUAPs) facilitating further quantitative analysis.	Giovagnoli *et al.* [114], sEMG
Power spectral density	Analysis of the frequencies within the signal is plotted as a histogram; frequencies can be categorised into different amplitude frequencies through the application of filtering techniques.	Williams *et al.* [72], sEMG
Shape of the EMG profile	Observation and analysis of the size and shape of the EMG profile including the numbers of turns and phases and amplitude within contractions can facilitate comparison between events.	Wijnberg *et al.* [13], fine wire EMG
Muscle recruitment: onset and offset of activity	Observation and analysis of the EMG profile (usually post band-pass filtering and rectification) to determine the activity profiles of muscles, often groups of muscles are studied synchronously.	Wijnberg *et al.*, [13], fine wire EMG Jansen et al. [103]
Muscle recruitment: threshold activation level	Power spectral density (a histogram of the different frequencies contained within the signal) and observation of the EMG profile can facilitate assessment of the action potential threshold required to stimulate contraction in the muscle under investigation.	
Muscle recruitment: timing	The duration of contractions can be measured to assess the duration of muscle workload. The timing can then be compared between events.	Jose-Cunilleras and Wijnberg, [84], fine wire EMG
Muscle activity levels: descriptive 1. Mean MUAP 2. Peak amplitude contraction (PAC)	1. Mean MUAP are calculated for a defined event or time period usually after the application of a filtering protocol. For gait studies, a linear envelope, where data are rectified and low pass filtered, are often used to expose general themes and reduce variability within the data. 2. PAC can be measured within the EMG profile again usually occurs after application of a filtering protocol.	Williams *et al.* [72], sEMG
Muscle activity levels: comparative* 1. Mean MUAP 2. PAC 3. Peak to peak /	1. Analysis of mean MUAP can be conducted between defined comparative events to assess differences and similarities in muscle performance. 2. Similarly, comparison of PAC between events can be used to assess muscle workload. 3. Comparison of minima and maxima within the EMG	1 & 2: Williams *et al.* [72], sEMG 3: Zsoldos *et al.* [56, 90] sEMG 4: Robert *et al.*

maxima to minima for amplitude of contractions 4. integrated EMG (iEMG)	signal or ratios of the minima to maxima can be conducted to assess workload between events. 4. iEMG represents the area under a fully rectified EMG trace. In essence it is the equivalent to the work done by a muscle for a defined activity period, iEMG can be undertaken over the entire period of muscle contraction, for a fixed time period, or compared to a pre-set (baseline) level as a percentage of the baseline signal.	[37]; Groesel *et al*. [115], sEMG
Fatigue	Fatigue changes the characteristics of MUAPs, the consequence is a reduction in the high-frequency components of the EMG frequency spectrum, which when plotted over time consistently results in a net left shift in the mean or median frequency of the signal. The presence of fatigue can be analysed by plotting the mean or median frequency of the EMG signal over time.	Colborne *et al*. [10], sEMG
Principle component analysis	The signal is subdivided by timing and using defined frequencies within the signal to give specific information on muscle activation related to the muscle fibre type recruited. It is also known as wavelet analysis.	Hodson-Tole, [36], sEMG
Wavelet analysis	A method used to calculate the intensity or approximation of the power of a ‘clean’ EMG signal. EMG data are low-pass filtered to remove artefacts and noise, then wavelets of defined frequencies are selected and summed, and mean and median total intensity can be calculated.	Wakeling et al. [119], sEMG

**It should be noted that for comparative analysis, the EMG signal has the potential to be complicated by ‘normal’ intra-subject variation within muscle workload, and by inter-subject variation when comparing muscle performance between subjects.*

Raw EMG data have the potential to contain spurious electrical activity therefore prior to evaluation of muscle recruitment and activity, the EMG signal is usually subjected to various analysis techniques to enable the data to be assessed effectively. A range of common features scrutinised and the analysis techniques applied to facilitate examination are provided in Table 5. Examples of studies which have used these techniques are also provided to facilitate further reading.


Individual electrode
pickup zone
overlap pickup zone
(signal from same motor units)
electrode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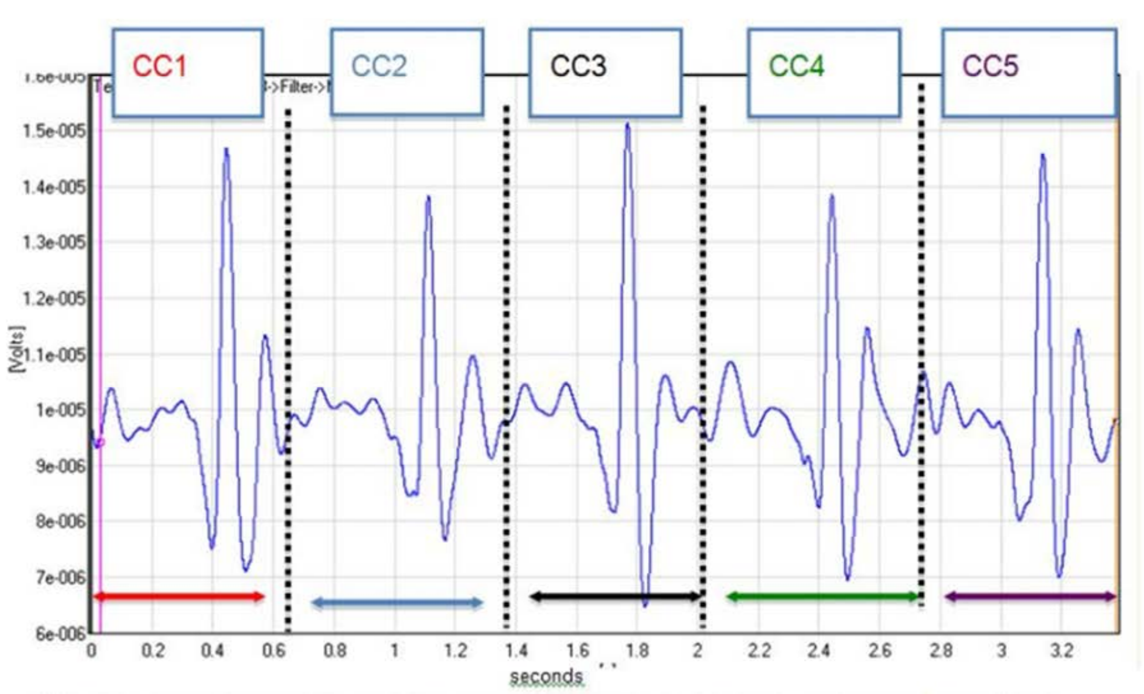
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Highlights

- Surface, fine-wire and needle electromyography have been used in equine research.
- Surface EMG is less invasive but only records superficial muscle activity.
- EMG can be applied in the horse to determine muscle recruitment and fatigue.
- An equine-adapted version of the SENIAM guidelines would improve equine EMG research.
- Grounded and strong within-subjects research designs should be used in equine EMG research.

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Supplementary Information

Table S1: Equine muscle recruitment identified through EMG; HL: hindlimb; FL: forelimb; HNP: head and neck position; sEMG: surface electromyography.

Muscle	Action	Studies
Longissimus dorsi	Walk and trot: bilateral contraction before swing Incline: contraction ends in the first third of swing Active when back flexed Walk and trot: most activity at T12, reducing in a wave caudally Walk: HL push off increases activity in ipsilateral HL Canter: continuous activity Jumping: continuous activity but reduced during suspension phase	Robert et al. [14, 37, 38, 39], sEMG Peham et al. [32], sEMG Licka and Peham [116], sEMG; Licka et al. [117], sEMG St George and Williams [57], sEMG
Rectus abdominus	Walk and trot: active HL stance Incline: active mid-stance of each HL Active when back in extension Walk and trot: unilateral activity corresponding to limb retraction	Robert et al. [14, 38, 39], sEMG Zsoldos et al. [56], sEMG
Splenius	Walk and trot: biphasic activity from start of FL stance to mid-stance Bilateral function: extend neck and elevate head Unilateral function: flex head and neck laterally Walk and trot: synchronous lateral activity Trot: increased activity in gathered HNP compared to hyperflexion	Robert et al. [14, 39], sEMG Zsoldos et al. [90], sEMG Kienapfel [48], sEMG
Gluteus medius	Walk and trot: active mid-swing to mid-stance in HL retraction	Robert et al. [37, 39], sEMG
Tenor fascia latae	Walk and trot: active mid-stance to mid-swing in HL protraction	Robert et al. [37, 39], sEMG

Brachiocephalicus	Walk and trot: active mid to late FL stance to mid to late FL swing Active when neck flexed Trot: increased activity in hyperflexed HNP compared to gathered HNP	Hodson-Tole [34], sEMG Kienapfel [46], sEMG
Triceps brachii: Long head	Walk and trot: active late swing to ground contact FL Canter: biphasic activity mid-stance to mid-swing FL Jumping: biphasic activity take-off and landing in FL	Hodson-Tole [36], sEMG St George and Williams [57], sEMG
Triceps brachii: Short head	Walk and trot: active late swing to early stance FL	Hodson-Tole [36] sEMG
Biceps femoris	Trot and canter: active mid to late HL swing until early to mid-stance in HL	Tokuriki and Aoki [47], needle EMG
External oblique abdominals	Walk and trot: bilateral activity, most active during retraction	Zsoldos et al. [56], sEMG
Superficial gluteal	Canter: biphasic activity mid-stance to mid-swing HL Jumping: biphasic activity take-off and landing in HL	St George and Williams [57], sEMG
Trapezius	Trot: increased activity in gathered HNP compared to hyperflexion	Kienapfel [48], sEMG
Extensor carpi radialis	Walk: biphasic activity, initial peak between onset and mid-swing phase; further peak in second half of swing phase	Jansen et al. [103], sEMG
Common digital extensor	Walk: highest peak just prior to ground contact at the end of the swing phase; moderate activity at onset of swing	Jansen et al. [103], sEMG
Ulnar lateralis	Walk: short burst of high amplitude activity from just before ground contact until the first part of stance	Jansen et al. [103], sEMG
Flexor carpi radialis	Walk: biphasic activity highest peak during first part of the swing phase, with second burst of activity from the end of swing until the beginning of stance	Jansen et al. [103], sEMG
Flexor carpi ulnaris	Walk: biphasic activity initial low peak at the onset of swing with a second peak of greater magnitude at the end of the swing phase	

Long digital extensor HL	Walk: active from just after mid-stance until the start of stance with multiple peaks of variable magnitude	Jansen et al. [103], sEMG
Deep digital flexor HL	Walk: biphasic activity in deep head during stance and also biphasic activity in swing	Jansen et al. [103], sEMG
Gastrocnemius	Walk: tri-phasic activity, highest peak at the end of swing phase until first part of stance, second peak observed in second half of stance and a third peak at the start of the swing phase	Jansen et al. [103], sEMG
Stylopharyngeus muscle	Phasic activity associated with respiration, increased expiration, increases with speed of locomotion	Tessier et al. [34], fine wire EMG
Palatopharnygeus and Palatinus	Continuous activity during respiration, not linked to stride frequency Most active in expiratory phase and increases with speed	Holcombe et al. [18], fine wire EMG
Omotransverarious	HNP: consistent activity of relatively low magnitude in neutral, flexed and extended positions; flexed maxima increased in older compared to mature horses	Zsoldos et al. [118], sEMG
Cleidomastoidius	HNP: consistent activation which demonstrates increasing from low to moderate magnitude through neutral, flexion and extension	Zsoldos et al. [118], sEMG
Cleidiobrachialis	HNP: consistent low activation through neutral position increasing marginally in both flexion and extension	Zsoldos et al. [118], sEMG
Splenious	HNP: active during neutral position with increased activation in flexion and extension	Zsoldos et al. [118], sEMG

Various EMG studies have used indwelling and surface EMG to investigate the timing of recruitment and activity levels in muscles associated with locomotion in the horse. A summary of their findings are provided in the table.