Published in final edited form as:

Comp Exerc Physiol. 2014 ; 10(2): 75–88. doi:10.3920/CEP140001.

From maturity to old age: tasks of daily life require a different muscle use in horses

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Abstract

In vertebrates ageing is characterized by reduced viscoelasticity of the ligamentous and tendineous structures and fibre changes in muscle. Also, some vertebral joint degeneration develops with ageing. The aim of this study was to apply dynamic time warping to compare the temporal characteristics of the surface electromyography (sEMG) data and to illustrate the differences in the pattern of muscle use during tasks of daily life in old and mature horses. *In vivo* kinematics (24 skin markers) and sEMG measurements of neck extensors and flexors were taken in five mature horses (age 10 ± 2 years, half of mean life expectancy) and five old horses (age 25 ± 5 years, older than the mean life expectancy). All horses had the same level of activity in the 12 months prior to the measurement. Tasks measured were neck flexion and neck extension as well as neutral neck position. Muscle activation, minimum and maximum muscle activation were collected. Quartiles of muscle activity based on the maximum observed activity of each muscle were calculated to document the relative increase of activity level during the task. Kinematics as well as overall muscle activity patterns were similar across horses and age groups. However, in the neutral position old horses showed increased extensor activity compared to mature horses, indicating that old equine muscle requires more activity to counteract gravity. Dynamic time warping specified optimal temporal alignments of time series, and different temporal performances were identified. The age groups differed during the flexion task, while extension and neutral were more similar. The results of this study show that even in the second half of life and in the absence of muscle disuse the muscular strategy employed by horses continues to be adapted.

Keywords

ageing; muscle activity; movement solution; surface electromyography; dynamic time warping

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Introduction

In the horse, the neck is a large and heavy axial segment (Buchner *et al.*, 1996) with a characteristic role in equine locomotion (van Weeren *et al.*, 2010). The morphological features and adaptations of the neck stabilize the trunk and create leverage for fast and efficient locomotion. These functions are determined by the passive structures such as the vertebrae and skull, by the connective tissues such as the nuchal ligament and by the activity of the neck muscles (Gellman *et al.*, 2002), with the splenius muscle especially important to determine head/neck position during movement (Zsoldos *et al.*, 2010).

Reduction of the mechanical efficiency of human muscle is accompanied and partly caused by an age related loss in neuromuscular function, of both agonist muscle activation and antagonist muscle co-activation (Aagaard *et al.*, 2010). The age-related loss of spinal motoneurons leads to a decline in muscle fibre number and size (sarcopenia), causing muscle weakening; this affects the performance during everyday tasks in humans (Aagaard *et al.*, 2010). Also, postural stability is decreased with age in humans, requiring increased muscle activity during quiet standing (Laughton *et al.*, 2003).

In horses, similar to other species, reduced viscoelasticity of the ligamentous and tendineous structures, as well as osteoarthritis of the cervical joints are characteristics of the ageing process. This can lead to reduced head-neck and neck-trunk flexibility (Dunbar *et al.*, 2008). In old (aged 22 ± 0.7 years) and young horses (aged 7.3 ± 0.6 years) the differences in cytokine profiles were found to exist in muscle, adipose tissue and blood of the horse (Liburt *et al.*, 2011). Exercise training was found to improve insulin sensitivity in young horses, while old horses required higher concentration of insulin to clear glucose (Liburt *et al.*, 2011). In the gluteus medius muscle of Akhal-Teke horses the age-related decrease of the relative content of myosin heavy chain I and myosin light chain 1 slow isoforms starts older than 20 years (Alev *et al.*, 2011). It was also shown that, during training, an age-related reduced muscle growth rate is present in older horses (te Pas *et al.*, 2013).

Comparing young (aged from 20 to 35 years) and old (aged 65 years and above) human subjects, mean lumbar extensor fibre angles and the moment generation capability of the muscles were significantly decreased with age (Singh *et al.*, 2011). These effects have not yet been investigated in the spinal musculature of quadrupeds.

Surface EMG (sEMG) is currently successfully used to identify muscle activity changes in human diseases such as Parkinson's (Cecilio *et al.*, 2010) and ageing for example in masticatory muscles (Meigal *et al.*, 2009). Many of these findings are thought to be transferrable to the activity of deeper muscles, but clearly, without evidence based data, this remains an assumption prior to the measurement of the deeper muscles themselves. In adult royal Dutch sport horses (aged 9.5 ± 2.3 years), needle electromyography was used to determine normative data on quantitative motor unit action potential analysis of serratus ventralis cervicis and brachiocephalicus muscle. In the analysed six segments of each muscle higher mean amplitude, number of phases, and size were found in serratus ventralis cervicis than in brachiocephalicus muscle (Wijnberg *et al.*, 2011).

Inherently, sEMG readings are influenced by crosstalk, which is the measurement of the electrical activity of additional, deeper muscles within the sampling area of the electrode; therefore the muscle specificity of sEMG traces is not absolute. Clearly, patterns of muscle use with sEMG can only be documented for muscles that are directly under the skin, but even for these muscles some crosstalk activity of deeper muscles may be sampled. Despite this, sEMG has led to successful identification of individual EMG patterns for example in cycling, where the patterns do not represent the locomotor strategy for pedalling (Hug *et al.*, 2010).

In a study of human finger muscle use, older adults (aged 74.5 ± 8.9 years) exhibited greater co-activation of the antagonistic muscles when they lowered their fingers and showed a lower co-activation than young adults (aged 27.4 ± 4.4 years) when lifting a light load. These findings emphasize the interaction between ageing and control and show that neuromuscular accuracy and potential efficiency of movement is impaired in older human adults (Christou and Enoka, 2011).

Muscle synergies are commonly determined as simultaneous and independent recruitment of muscles; this provides the flexibility necessary for the variability of the muscle patterns involved in a natural behaviour. This was shown e.g. in frogs (d'Avella *et al.*, 2003). The existence of such relative muscle activation (RMA) patterns has also been investigated in vertebral species, such as goats (McGuigan *et al.*, 2009) and cats (Ting and Macpherson, 2005). The existence of a similar pattern in the equine neck can be assumed. The aim of the present study is therefore to test the following hypotheses: 1) In old age, horses show reduced active and voluntary neck movements associated with reduced muscle activity of neck muscles as determined by sEMG compared to mature horses. 2) Task dependency of neck muscle use is independent of age, and the pattern of muscle use is different between flexion, extension and neutral stance.

Material and Methods

Animals

In vivo measurements were taken in ten horses without signs of neck pain or clinical neurological impairments. Horses were without history of neck or back disease, trauma or surgery. Horses underwent a detailed orthopaedic examination of the neck (including palpation, induction of passive and active movements) and a full neurological examination, with no abnormalities detected. Two age groups were investigated: the mature group included 5 horses aged 9 to 12 years (10 ± 2 years), weighing 537 ± 31 kg, and the old group included 5 horses aged 22 to 35 years (25 ± 5 years), weighing 534 ± 92 kg. The age groups were selected based on the average life expectancy of 25 years for horses (Brosnahan and Paradis, 2003). The horses were all of the same training status: over the last three years they had been kept in a free range paddock with two to three times a week intermittent trotting in hand for a total of 15 minutes. About once a month, horses were walked for 10 minutes and trotted for 5 minutes on the treadmill. Lunging of horses was carried out also on average once a month for about 10 minutes of walk and 10 minutes of trot on both hands. The horses included were of different breeds, including three Standardbreds, one Haflinger and one Warmblood in the mature group and one Standardbred, one Shagya Arab and three

Warmbloods in the old group. Three of the horses were non-pregnant and non-lactating mares, and seven were geldings.

Ethics statement

Experiments were carried out under the University of Veterinary Medicine Vienna's animal experiment licence for the horses of the university teaching herd, which was approved by the institutional ethics committee and the national authority according to § 8ff of Law for Animal Experiments (Tierversuchsgesetz – TVG, bmwf GZ 68.205/0160/II/3b/2012), more specifically the part covering non-invasive orthopaedic examinations and measurements.

Data collection: Kinematic measurements

Kinematics and surface electromyography were measured in all horses with the head and neck in a neutral stance posture as well as during active flexion and extension of the head and neck, this was triggered by holding a carrot just out of reach in the respective position. Twenty-four reflective skin markers were attached to each horse using adhesive tape. Eighteen markers were placed on left and right side on each palpable cervical vertebra (1-6), and additional markers were placed on the head, and on the withers (over the dorsal spinous process of the fifth thoracic vertebrae). Kinematic data were collected in a standard right-handed Cartesian coordinate system using ten digital high-speed cameras (Eagle Digital RealTime System, Motion Analysis Corp., Santa Rosa, California, USA) recording at 120 Hz. Data collection continued until 3 trials (each 10 seconds) had been recorded.

Data collection: EMG measurements

Before electrode placement skin resistance was reduced by thoroughly cleaning the shaved skin with slightly abrasive, roughly woven swabs. Surface electromyography activities were collected with pre-gelled bipolar AgCl electrodes (30mm in diameter) placed bilaterally on the shaved skin over the splenius (SPL) muscle at the level of the second cervical vertebra (caudal and slightly ventral to the palpable wing of the atlas), over the omotransversarius (OMO) muscle dorsolateral to the fifth cervical vertebra (over the dorsolateral transverse processes), over the cleidomastoideus (CM) muscle ventrolateral to the fourth cervical vertebra (over the dorsolateral transverse processes), and over the cleidobrachialis (CB) muscle ventrolateral to the sixth cervical vertebra (over the dorsolateral transverse processes), (Figure 1). These muscles were chosen due to their described relevance for the movement of the neck, described either as neck flexors or extensors, respectively (Orsini et al. 2005). The electrodes were secured in position by use of non-irritant adhesive at a distance of 3 cm from each other along the axis muscle fibres. A single reference electrode was placed over the right tuber coxae. Muscle tremor was evaluated similar to the investigation in quarter horses with hyperkalemic periodic paralysis by Steiss and Naylor (1986) as high frequency discharges either of variable frequency and amplitude or of constant amplitude and frequency.

For the sEMG measurements a telemetric system was used (Telemyo Mini 16, Noraxon Inc., Scottsdale, AZ, USA; sample frequency 1.2 kHz). Recordings of sEMG and kinematics were synchronized with the kinematic software to obtain simultaneous recordings. Data collection (Cortex 1.3, Motion Analysis Corp., Santa Rosa, CA, USA) continued until 3

trials (each 10 s) for each of the three tasks (neutral stance, flexion and extension) had been recorded (Figure 1).

Ultrasound investigation (TFL)

In order to minimize cross talk, electrode placement was based on ultrasound evaluation of all muscles for muscle fascicle orientation, muscle volume and confirmation that the muscle was located directly under the skin at the selected locations. Also, the absence of abnormal tissue such as scars was confirmed. A 7.5 MHz linear probe without a stand-off pad was used, and the method of using ultrasound to standardise electrode placement was adapted from Perriman *et al.* (2011).

Data processing and analysis of the kinematic data

The three dimensional coordinates of each marker during the time course of each experiment were calculated from the data by motion capture (Cortex 1.3, Motion Analysis Corp., Santa Rosa, CA, USA) software. The kinematic data was then smoothed using a Butterworth low-pass filter (cut-off frequency, 10 Hz). From the kinematics, the head position relative to the withers was calculated, in the remainder of this article this is referred to as head-trunk distance, and the changes occurring in this distance are referred to as head-trunk movement distance. The movement speed was calculated as the mean speed of the movement of the head during the whole trial, with negative speeds indicating a reduction in distance between the head and the point at two thirds of the height of the withers (flexion) and positive values indicating an increase in distance between the head and the point at two thirds of the height of the withers (extension).

Data processing and analysis of the EMG data

The EMG signal was rectified and resampled (sampling rate reduced to 120 Hz) to adapt it to the kinematic data. A Butterworth low-pass filter was applied (fifth order; cut-off frequency, 10 Hz). The mean and standard deviation of the relative muscle activation in percent (RMA%). The following equation was used for the calculation of relative muscle activation for each muscle for each time point; Muscle1 RMA

$$\% = (\text{Muscle1} + \text{Muscle2} + \text{Muscle3} + \text{Muscle4}) / \text{Muscle1} * 100$$
 The mean and standard deviation of minimum and maximum RMA%, and muscle activity quartiles based on the maximal observed muscle activities (MOA) were calculated from the sEMG. For each muscle of each horse, third quartile values (Q3) are presented, as they represent information on the spread of the muscle activity. This method was also used in previous EMG studies (Mesin *et al.*, 2011; Wijnberg *et al.*, 2003).

To compare the temporal characteristics of the EMG data the dynamic time warping (DTW) algorithm was employed (Krueger *et al.*, 2010; Mueller, 2007). The computation of temporal alignments using DTW can be divided into three basic steps first of all the computation of local distance matrix, secondly computation of a global distance matrix and at the end the search the global distance matrix for the warping path. DTW was performed for all regarded time-series at once, and this resulted in an optimal alignment of the compared time series, a so-called warping path. The warping path gave information how the time series have to be stretched or compressed to get an optimal temporal matching; where correspondences were

found within the regarded time series. These temporal deformations do not have to be linear: some segments of a time series might be stretched while others are compressed to get the optimal alignment. Based on the extracted alignment, comparisons were done on the different phases of the signals, identifying different temporal performances of the measured horses.

Statistical analysis

Normal distribution of data was confirmed using the Shapiro–Wilk test. All parametric data are provided as mean and standard deviation, and non-parametric data as median and range, interquartile range (IQR). The analysis of parametric data consisted of a one-way analysis of variance (ANOVA) with Bonferroni corrections used for post hoc comparisons (the alpha level was set at 0.05), and an independent T-test. The analysis of non-parametric data consisted of a Kruskal-Wallis test and a Mann-Whitney U test. Values of $P < 0.05$ were considered significant, while values of $P < 0.01$ were considered highly significant. Data were compared for the same tasks between the groups, as well as between the tasks within the same group. All statistical analyses were carried using statistical software (PASW Statistics, 17.0.3., SPSS Inc., Chicago, IL, USA).

Results

No tremors were noted when evaluating the sEMG signals

The contribution of each muscle to the sum of activities measured was calculated and the result of one horse is plotted representing RMA% throughout the tasks (Figure 2). Mean RMA% for the mature and old group for each task are shown in Figure 3. The overall mean RMA in the neutral position ranged from 10 to 47 % in the old group and from 11 to 54 % in the mature group, during extension RMA% ranged from 10 to 54 % in the old group and from 12 to 57 % in the mature group, during flexion RMA% ranged from 15 to 38 % in the old group and from 16 to 46 % in the mature group. Old horses showed significantly greater SPLR RMA% ($p=0.025$), OMOR RMA% ($p=0.007$) in neutral position than during flexion tasks, significantly lower CML RMA% ($p<0.001$), CMR RMA% ($p<0.001$), CBR RMA% ($p=0.033$) in neutral position than during flexion tasks, significantly greater SPLR RMA% ($p=0.04$), SPLR RMA% ($p=0.04$) during extension than flexion tasks and significantly lower CML RMA% ($p<0.001$), CMR RMA% ($p=0.001$) during extension than flexion tasks.

Mature horses showed significantly lower CML RMA% ($p=0.019$) during extension than during flexion tasks. The CBL RMA% ($p=0.045$) in the neutral position was significantly greater in the mature than in the old group, SPLR RMA% ($p=0.032$) during the extension was significantly greater in the mature than in the old group and OMOR RMA% ($p=0.044$) was significantly greater in the mature than in the old group during flexion tasks. The CML RMA% ($p=0.004$) was significantly lower in the mature than in the old group during flexion tasks.

The left and right minimum and maximum RMA% and their statistically significant differences for each muscle and each task are shown in Table 1.

Muscle activity levels in quartiles based on the maximal observed muscle activity (MOA) of each individual muscle and third quartile values (Q3) are presented in Figure 4 and their Q3 ranges are presented in Table 2.

Old horses showed significantly lower Q3 values in neutral position than during flexion tasks (CML, CBL, CBR; $p<0.001$) CMR ($p=0.003$) and significantly lower Q3 values during extension than during flexion (CML, CBL, CBR; $p<0.001$). Mature horses showed significantly lower SPLR Q3 values in neutral position than during extension tasks ($p=0.017$), significantly lower Q3 values in neutral position than during flexion tasks (SPLL $p=0.005$, CML $p=0.006$, CMR, $p=0.008$, CBL $p=0.016$, CBR; $p=0.021$) and significantly lower Q3 values during extension than during flexion tasks (CML $p=0.021$, CBL; $p=0.043$). OMOR Q3 values were significantly lower in the mature than in the old group in the neutral position ($p=0.021$).

Head-trunk movement distances (Figure 5) were compared at neutral position ranging from 8 to 23 mm in the old group and from 6 to 30 mm in the mature group. At full extension the head trunk distance ranged from 213 mm to 460 mm in the old group and 288 to 425 mm in the mature group while at flexion they ranged from 64 to 245 mm in the old group and 113 to 188 mm in the mature group. Old horses showed significantly lower head-trunk movement distance in the neutral position than during flexion task ($P<0.001$) and significantly lower head-trunk movement distance during extension than during flexion task ($p=0.001$). Mature horses showed significantly lower head-trunk movement distance in the neutral position than during extension task ($p<0.001$), significantly lower head-trunk movement distance in the neutral position than during flexion task ($P<0.001$) significantly lower head-trunk movement distance during extension than during flexion task ($p<0.001$).

Head-trunk movement speed (Figure 5) ranged from 0 to 5 mm/s in the old group and from 0 to 3 mm/s in the mature group. During extension the speed ranged from 15 to 81 mm/s in the old group and 26 to 70 mm/s in the mature group while during flexion speed ranged from -48 to -128 mm/s in the old group and -61 to -128 mm/s in the mature group. There were no statistically significant differences in speed changes of the three tasks.

Left and right DTW distances during the neutral position and during extension and flexion task based on the left and right side. The DTW distances were at neutral position left 82 ± 26 and right 78 ± 4 in the old group and left 78 ± 13 and right 76 ± 9 in the mature group, at extension left 77 ± 13 and right 79 ± 7 in the old group and left 76 ± 15 and right 77 ± 7 in the mature group, at flexion left 112 ± 37 and right 134 ± 35 in the old group and left 95 ± 23 and right 99 ± 15 in the mature group. Old horses showed significantly greater left DTW distances in the neutral position than during flexion task ($p=0.003$), and significantly lower left DTW distances during extension than during flexion tasks ($p=0.004$). Mature horses showed significantly lower right DTW distances in the neutral position than during flexion task ($p=0.0026$), and significantly lower right DTW distances during extension than during flexion tasks ($p=0.047$).

Discussion

In this study a comprehensive comparison of muscle use and coordination patterns during different tasks was undertaken using a combination of several established methods such as sEMG (Fischer *et al.*, 2013) and emerging methods such as task dependency (Thibaudier *et al.*, 2013) and DTW (Pogorelc and Gams, 2013). The purpose of the study was to test the hypothesis that old horses show reduced voluntary neck movements associated with reduced muscle activity of neck muscles as determined by sEMG compared to mature horses. The first hypothesis has been rejected, as old horses showed overall increased muscle activity at the neutral position compared to mature horses. The results showed that the pattern of muscle use was task dependent but independent of age, therefore second hypothesis has been accepted.

Changes in kinematics with ageing

Dunbar et al. (2008) investigated coordination of head pitch and vertical displacements in the horses during overground locomotion in one old horse (24 years old) and in two younger horses (9 and 10 years old) where linear variables such as vertical displacement and velocity of the head, neck and trunk were recorded. Notable differences were detected during canter and trot; young individuals showed head pitch and vertical displacement largely out of phase while this was more in-phase in the old individual, although the overall locomotory patterns were not different (Dunbar *et al.*, 2008). In the present study head-trunk movement distances were recorded and showed differences between neutral position and extension and flexion tasks as expected, but no statistically significant differences between the age groups were found. In the horses used in the present study, no tremor was observed or measured. In elderly people, tremor is commonly seen together with instability. Morrison and Sosnoff (2009) predicted alteration in the tremor or electromyographic (EMG) output of human forearm muscles in two age groups. In elderly people (aged 72.3 ± 4.1 years), they found increased tremor and EMG activity during holding their entire upper arm against gravity; this was interpreted as higher force demand. In horses tremor is not known as a common neurological impairment of ageing (Mayhew, 2009), and also in the horses used for this study postural requirements of ageing could be investigated without muscle tremors.

Age-related muscle activity

The results of this study document statistically significant age related differences in the sEMG pattern during different neck exercises. Previous age comparisons were carried out at the motor unit level of different muscles of the horse, however, neck muscles had not yet been investigated. As a study found that at quiet standing the motor unit action potential duration, number of phases, and number of turns in the subclavian, triceps and vastus lateralis muscles were significantly lower in adult horses (aged from 6 to 10 years) than in elderly horses (aged from 18 to 21 years) (Wijnberg et al., 2003), this is similar to the findings of the present study where sEMG activity at quiet stance was found to be smaller in mature horses compared to old horses. Additional, future comparisons including foals might show how muscle synergies and movement patterns are established during musculoskeletal development and motor learning. However the three trials documented in the present study were not sufficient to document learning or short-term adaptation. Within a given muscle,

slow twitch muscle units are recruited for quiet standing and faster twitch units are recruited for the rapid response in humans (Krishnamoorthy *et al.*, 2003). A well-known effect of human ageing is that the number of fast twitch fibres are reduced (Watanabe *et al.*, 2012; Deschenes, 2004), however the technique of sEMG did not allow identification of such differences in the present study. Muscle morphology in horses also changes with age (Alev *et al.*, 2011). An investigation including motor unit level assessments might be necessary to identify whether this effect is present in the ageing horses. Left and right-sided muscles were measured separately and presented separately to exclude handedness of the horses (Lucidi *et al.*, 2013).

Task dependency

Adaptation to perturbation was shown to focus on the hindlimb extensor muscles in cats with higher activities measured in the extensors than in the flexors (Ting *et al.*, 2004). This adaptation strategy is based on the nervous system incorporating the force of gravity by reducing the drive to the flexor muscles. In the present study, where postural anti-gravity control was investigated, extensor muscle activity (Q3) predominated over flexor muscle activity during the neutral position and extended position primarily in the subjects of the old group. Krishnamoorthy *et al.* (2003) showed in humans that postural muscle synergies are task specific, and that extensor synergy assumes the primary role for antigravity weight support during quiet stance. In the present study Q3 values showed no statistical differences during extension task and in neutral position in old horses, but statistical differences were found in mature horses. This lack of difference between extension and neutral position in old horses also points to a higher need for anti-gravity muscle activity even without movement.

In cats functional muscle synergies were found to be similar in relation to muscle synergy number, synergy activation patterns, and synergy force vectors (Torres-Oviedo *et al.*, 2006). The robustness of synergy organization across perturbation types, postures, and animals, suggests that the control of motor and postural tasks by muscle synergies are a general construct used by the central nervous system for balance control. Similarly, the same muscle synergies were consistently extracted from individual frogs during kicking (d'Avella *et al.*, 2003). Although, the tasks investigated in the present study have a limited comparability to these movements, in general muscle synergy control seems to simplify movement, decreasing the number of output patterns that the nervous system must specify for a large number of muscles, while allowing flexibility in the final expression of muscle activation. In the present study the DTW distances showed no statistically significant differences between the patterns. Similarities in the activation pattern of the muscles of the mature and old group detected in the present study are therefore probably the consequence of the described general movement simplification.

Muscle synergies

In humans (Krishnamoorthy *et al.*, 2003) the variation in muscle synergy composition across subjects suggests a flexibility of expression within the set of equivalent solutions present in a redundant musculoskeletal system. Because each individual has a stable muscle synergy composition across postural condition and days, the particular composition of muscle

synergies may be tuned to the body morphology and mechanics of each individual. In the present study a similar result of individuality of muscle use was found.

Co-activation

Macaluso *et al.* (2002) carried out a comparative study in young (mean age 22.8 ± 5.7 years) and old (mean age 69.5 ± 2.4 years) women on knee extensor and flexor muscles, where old women showed a lower specific tension, than the young women. The knee flexors showed a lower level of muscle strength with an increased level of co-activation during knee extension. In the present study such co-activation of flexors and extensors during the tasks was not calculated, but the RMA contribution as percentages in the different tasks (Figure 3) were compared between the age groups. Similar to the humans, the minimum and maximum percentages also showed that the old group had more significant differences between tasks, illustrating that the muscle use becomes more differentiated with age.

Pattern recognition

Kutch and Valero-Cuevas (2012) showed that low simulated EMG variety of muscle activation during voluntary muscle force production can be related to task selection in humans. It was stated that the small variety of muscle coordination patterns does not necessarily suggest a specific neural strategy. Only a small number of activation patterns is known to be controlled by the central nervous system, for the rest of the pattern details, mechanical coupling (e.g. by ligaments or fasciae) may be responsible. In the specific anatomy of the equine neck, the hypothesized mechanical coupling is most likely dominated by the substantial nuchal ligament (Gellman and Bertram, 2002a,b).

During signal processing a challenging step is to find similarities between the trials. The success of sEMG pattern recognition depends largely on the selection of features that represent raw sEMG signal for classification (Phinyomark *et al.*, 2009). Different algorithms are in use of computing feature components for classifications, for example integral of absolute value (IAV) and weighted singular value decomposition (wSVD) (Pradhan *et al.*, 2007). In the present study the technique of similarity search and an optimal alignment was used between the trials applying the method of DTW. Although the horses were of different ages, the muscle activation pattern showed easily obtainable time series for an optimal alignment. This optimal temporal matching allowed comparison of complex signals for the same tasks, even without standardization of the trials due to the animal's individual movement solutions.

Study limitations

Ten horses were divided into two groups in the present study, and this low sample size needs to be considered when extrapolating from the results of this study. Especially the within group comparisons for a group of five horses are relatively weak. Also, the health of neck of the horses selected for this study could have been investigated in more detail using e.g. laterolateral or even oblique and ventrodorsal radiographs as well as magnetic resonance imaging.

If the muscular stabilisation of the neck is to be investigated comprehensively, both more superficial and deeper muscles need to be measured. This has recently been documented for humans in a study, where both sEMG and needle EMG was used on superficial and deep neck muscles (Newell *et al.*, 2013). In the present study, however, the influence of ageing on the activity of four superficial muscles is presented, while in previous studies even single muscles were investigated using sEMG; e.g. the human quadriceps muscle (Mau-Moeller *et al.*, 2013) and the human erector spinae muscle (Tsuboi *et al.*, 2013). In horses, sEMG has not been fully validated yet, but it is used successfully for many dynamic exercise recordings (Zsoldos *et al.*, 2010, Wijnberg *et al.*, 2009).

In the present study sEMG was selected as measuring technique, as it has several advantages, including the non-invasive nature of the technique and the possibility to record multiple trials without the chance of needle failure. However needle EMGs of the deeper equine muscle together with superficial muscles would probably deliver a more descriptive synergy calculation for these task-dependency exercises; on the other hand, they could potentially also create alterations during the tasks due to mechanical strains on the needles for the deeper muscles.

Muscle synergies have previously been used to illustrate coordination of muscle use, and intra-individual adaptations of muscle use for specified tasks. Ideally synergies are calculated from the most important muscles (Hug *et al.*, 2010), determining a joint position in space, while in the present study RMA calculation was based on four surface muscles. These muscles are important for the head and neck position; however, deeper muscles, which are certainly also relevant, were not measured.

In this study genetics or breed effects as potential confounding factors could not be excluded. While Standardbred trotters are selected for their trotting speed, other breeds included are selected for other athletic endeavours such as dressage or show jumping (Warmbloods). The remaining two breeds are produced for general purpose (Haflinger and Shagya Arab).

The horses used in the present study were probably more different in their build, subcutaneous layer thickness and muscle volumes than the human cyclists investigated in other studies (Blake *et al.*, 2012; Sarabon *et al.*, 2012). Also, the muscles investigated in the present study are in constant use during the daily routine of the horses such as feeding and social grooming, and none of the horses were used athletically, therefore the degree of training and fine-tuning of the movements investigated was thought to be comparable between age groups. Admittedly, ultrasound imaging of muscle quality is not as precise as histology of biopsy samples or magnetic resonance imaging; these modalities might have identified much smaller differences in muscle texture. For the present study, every day movements of mature and old horses were compared, and neither additional learning effect nor major changes in tissue elasticity (especially of the *Ligamentum nuchae*) were expected.

These triggered active movements of horses depend very much on the individual's response to the trigger, and horses not interested in food will not show a suitable movement response. Timing to maximal contraction was not carried out in the two age groups, however the head

trunk movement speed was calculated and this was most likely influenced by the motivation of the horse to quickly reach the carrot and thus the required end position. Even if there is adequate motivation, as was the case in all the horses used for this study, the triggered movements cannot be standardized, and this lack of movement standardization is important for the interpretation of the results, as the lack of similarity of the movements investigated is much larger than it is for central pattern generator controlled movements such as limb movements during trot.

The speed of the movements was not different between the two ages investigated, despite the speed of fast muscle responses decreasing in elderly people together with a smaller number of motor units recruited (Edstroem *et al.*, 2007). For the present study we therefore have to assume that the voluntary movements were carried slowly without requiring such fast muscle responses.

Clinical relevance and future research perspectives

The effect of ageing on horses becomes more important for the veterinary profession as the population of geriatric horses increases (Silva and Furr, 2013; Ireland *et al.*, 2012). The results of this study show that the muscle requirements of quiet standing increase with age, and this supports the current practice of giving old horses more time for musculoskeletal rehabilitation.

Conclusion

In conclusion, the present study showed in the old horses an increased extensor activity compared to mature horses in the neutral position, indicating that old equine muscle – even in the absence of tremor - requires more activity to produce similar forces. Thus, the risk of neck muscle fatigue is high in old horses, as they are less likely to rest in recumbency, and while standing they are required to use more neck muscle activity. Additionally, the consequence of fatigue, i.e. loss of fine-tuned muscle fascicle coordination, may reduce the balance of the head and neck segment during locomotion, contributing to the commonly observed high incidence of stumbling in old horses. The individuality of movement patterns employed to achieve the tasks of daily life investigated was age independent. The results of this study show that even in the second half of life and in the absence of muscle disuse the muscular strategy employed by horses continues to be adapted.

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Figure 1. Neutral, extended and flexed position of the head and neck of a horse.

Neutral position (a), extended position (b) and flexed position (c) are shown as well as the location of the EMG surface electrodes (d) over splenius muscle (SPL), omotransversarius muscle (OMO), cleidomastoideus muscle (CM), and cleidobrachialis muscle (CB).

Figure 2. Changes in relative muscle activation throughout a 10 seconds trial.

Horse 1 during neutral position, extension and flexion with the sum of the muscle activities of all four muscles represented as 100 %. Left splenius muscle (SPL L), right splenius muscle (SPL R), left omotransversarius muscle (OMO L), right omotransversarius muscle (OMO R), left cleidomastoideus muscle (CM L), right cleidomastoideus muscle (CM R), left cleidobrachialis muscle (CB L) and right cleidobrachialis muscle (CB R).

Figure 3. Mean and standard deviation of relative muscle activation.

Relative muscle activation in percentages (RMA %) are shown in old and mature horses during neutral position, extension and flexion. Left (L) and right (R) splenius muscle (SPL), omotransversarius muscle (OMO), cleidomastoideus muscle (CM), cleidobrachialis muscle (CB) are shown.

Figure 4. Left and right third quartile muscle activities.

Left and right third quartiles of muscle activities in percent (Q3) are shown in old and mature horses in neutral position, during extension and flexion. Splenius muscle (SPL), omotransverarius muscle (OMO), cleidomastoideus muscle (CM), cleidobrachialis muscle (CB) are shown.

Figure 5. Mean movement speed and movement distance between the head and the trunk during exercises.

Mean movement distances [mm] and movement speed [mm/s] values from all horses are shown in neutral position, during extension and flexion.

Table 1

The minimum and maximum relative muscle activation percentages in neutral position and during extension and flexion with statistically significant differences of the minimum and maximum relative muscle activation percentages.

	NEUTRAL MINIMUM %										NEUTRAL MAXIMUM %													
	SPL			OMO			CM			CB			SPL			OMO			CM			CB		
	L	R	M±SD	L	R	M±SD	L	R	M±SD	L	R	M±SD	L	R	M±SD	L	R	M±SD	L	R	M±SD	L	R	M±SD
	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD
Mature	24 ± 7	25 ± 14	15 ± 5	11 ± 2	7 ± 2	6 ± 2	9 ± 2	7 ± 2	M±SD	M±SD	M±SD	M±SD	64 ± 8	72 ± 5	40 ± 9	42 ± 5	23 ± 7	21 ± 10	27, 1	29 ± 11	M±SD	M±SD	M±SD	M±SD
	M±SD	M±SD	M±SD	M±SD	MD, IQR	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD
Old	14 ± 8	11 ± 7	17 ± 10	12 ± 5	4, 2	4 ± 1	5 ± 2	5 ± 1	M±SD	M±SD	M±SD	M±SD	65 ± 16	71 ± 10	57 ± 16	56 ± 7	28 ± 7	27 ± 9	26 ± 6	36 ± 4	M±SD	M±SD	M±SD	M±SD
BG-N							p=0.019									p=0.009								
	EXTENSION MINIMUM %										EXTENSION MAXIMUM %													
	M±SD	M±SD	M±SD	M±SD	M±SD	MD, IQR	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	MD, IQR	M±SD	MD, IQR	M±SD
Mature	14 ± 11	8 ± 9	7 ± 3	5 ± 3	3 ± 1	3, 0	3 ± 1	3 ± 1	M±SD	M±SD	M±SD	M±SD	82 ± 5	82 ± 8	44 ± 8	45 ± 5	34 ± 7	39 ± 8	35 ± 9	40, 1	M±SD	M±SD	M±SD	M±SD
WG-N-E			p=0.019	p=0.006	p=0.004		p=0.011	p=0.014	p=0.003									p=0.047	p=0.03					
	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD
Old	14 ± 8	9 ± 3	7 ± 3	7 ± 3	3 ± 1	4 ± 1	3 ± 1	4 ± 1	M±SD	M±SD	M±SD	M±SD	81 ± 4	75 ± 3	54 ± 12	50 ± 16	30 ± 8	35 ± 9	29 ± 8	46 ± 17	M±SD	M±SD	M±SD	M±SD
WG-N-E							p=0.043																	
	FLEXION MINIMUM %										FLEXION MAXIMUM %													
	M±SD	M±SD	M±SD	M±SD	M±SD	MD, IQR	MD, IQR	MD, IQR	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	MD, IQR	M±SD	MD, IQR	M±SD	M±SD
Mature	6 ± 6	4 ± 4	5 ± 1	4 ± 1	4 ± 1	4, 3	4, 1	4 ± 2	M±SD	M±SD	M±SD	M±SD	80 ± 5	79 ± 11	49 ± 11	60 ± 10	49 ± 6	51 ± 11	36, 10	42 ± 8	M±SD	M±SD	M±SD	M±SD
WG-N-F	p=0.017	p=0.013	p=0.004	p=0.001	p=0.015		p=0.039	p=0.03	p=0.007							p=0.007	p<0.001	p=0.001						
WG-E-F																								
	M±SD	M±SD	M±SD	M±SD	M±SD	MD, IQR	MD, IQR	MD, IQR	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD
Old	5 ± 3	7 ± 8	5 ± 1	4 ± 1	4, 1	5 ± 1	3, 1	4 ± 2	M±SD	M±SD	M±SD	M±SD	76 ± 4	71 ± 9	50 ± 14	47 ± 11	57 ± 11	53 ± 10	34 ± 6	53 ± 16	M±SD	M±SD	M±SD	M±SD
WG-N-F			p=0.037	p=0.013														p<0.001	p=0.003					
WG-E-F																		p=0.001	p=0.03					

* Left (L), Right (R), Splenius muscle (SPL), omotraversarius muscle (OMO), cleidomastoideus muscle (CM), cleidobrachialis muscle (CB), Mean (M), Standard deviation (SD), Median (MD), Interquartile Range (IQR), between group comparisons (BG), within group comparison (WG), neutral (N), extension (E), flexion (F).

Table 2

Third quartile ranges based on the maximal observed muscle activities in neutral position and during extension and flexion.

Group	RANGE NEUTRAL Q3			
	SPL	OMO	CM	CB
Mature	8 – 27	5 - 27	4 - 16	6 - 23
Old	7 – 48	6 - 64	2 - 10	3 - 15
	RANGE EXTENSION Q3			
Mature	16 – 40	6 - 39	6 - 23	8 - 29
Old	8 – 43	13 - 57	4 - 13	6 – 19
	RANGE FLEXION Q3			
Mature	16 – 37	6 - 41	14 - 51	22 - 50
Old	7 – 42	16 - 40	26 - 54	14 - 49

* Third Quartile (Q3), Splenius muscle (SPL), omotraversarius muscle (OMO), cleidomastoideus muscle (CM), cleidobrachialis muscle (CB).