Quantitative motor unit action potential analysis of supraspinatus, infraspinatus, deltoideus and biceps femoris muscles in adult Royal Dutch sport horses

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Running title: Quantitative EMG of equine shoulder muscles

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List of abbreviations: BF = biceps femoris

DT = deltoideus

IS = Infraspinatus

IPA = interference pattern analysis

MUAP = motor unit action potential

MVA = maximal voluntary activity

PSA = pathological spontaneous activity

(Q)EMG = (quantitative)electromyography

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SI = size index

SS = Supraspinatus

Summary

Reasons for performing study: Reference values for quantitative electromyography (QEMG) in shoulder and hindlimb muscles of horses are limited.

Objective: To determine normative data on quantitative electromyography (QEMG) analysis of Supraspinatus (SS), Infraspinatus (IS), Deltoideus (DT) and Biceps femoris (BF) muscles.

Study design: Experimental observational study and retrospective case series.

Methods: Seven adult healthy Royal Dutch Sport horses underwent quantitative motor unit action potential (QMUAP) analysis of each muscle using commercial electromyography equipment. Measurements were made according to published methods. One-way ANOVA was used to compare QMUAP variables between muscles and posthoc testing according to Bonferroni, with p-value set at <0.05. QEMG and clinical information from horses with lower motor neuron disorders (n = 7) or myopathy (n = 4) were summarised retrospectively.

Results: 95% confidence intervals of duration, amplitude, phases, turns, area, and size index (SI) of QMUAP were 8.7-10.4 ms, 651-867 µV, 3.2-3.7, 3.7-4.7, 1054-1457 µV·ms, and 1.1-1.5 for SS muscle, 9.6-11.0 ms, 779-1082 µV, 3.3-3.7, 3.8-4.7, 1349-2204 µV·ms, and 1.4-1.9 for IS muscle, 6.0-9.1 ms, 370-691 µV, 2.9-3.7, 2.8-4.5, 380-1374 µV·ms, and 0.3-1.3 for DT muscle, and 5.7-7.8 ms, 265-385 µV, 2.7-3.2, 2.6-3.1, 296-484 µV·ms, and 0.2-0.5 for BF muscle, respectively. Mean duration, amplitude, number of phases and turns, area and SI were significantly (P<0.01) higher in SS and IS than DT and BF muscles. In

addition, 4 of 7 normal horses had >15% polyphasic motor unit action potentials in SS and IS muscles.

Conclusions: Differences between muscles should be taken into account when performing QEMG in order to be able to distinguish normal horses from horses with suspected neurogenic or myogenic disorders. These normal data provide the basis for objective QEMG assessment of shoulder and hindlimb muscles. QEMG appears to be helpful in diagnosing neuropathies and discriminating these from myopathies.

Introduction

Electromyography (EMG) is an ancillary diagnostic technique based on recording the electrical signal generated by the motor unit that consists of motor neuron, including its axon, neuromuscular endplate and skeletal muscle fibres during rest, voluntary muscle contraction or spontaneous activity [1,2]. Traditionally, EMG has been used in veterinary medicine to demonstrate pathological spontaneous activity (PSA) to assess certain neuromuscular disorders. As an example, detection of fibrillation potentials, positive sharp waves, myotonic discharges or complex repetitive discharges of various skeletal muscles have been described in horses with equine motor neuron disease [3], hyperkalemic periodic paralysis [4,5], myotonia [6], suprascapular nerve injury [7], experimentally induced hypocalcemia and hypomagnesemia [8], and in myopathy [9]. Quantitative EMG (QEMG) has been employed in human medicine since decades to further characterise certain neuromuscular disorders [2,10], to study effects of training or rehabilitation [11–13], and it has more recently been applied to equine medicine [14–17]. Motor unit action potentials are the result of eliciting voluntary or forced contraction of skeletal muscles. By not only evaluating the presence and type of insertional activity and pathological spontaneous

activity, but also quantifying the resultant motor unit action potentials (MUAPs) (duration, amplitude and other parameters), one may characterise a disorder as neurogenic, myogenic or not resulting from changes of the motor unit [1]. More recently, the observation of abnormal quantitative EMG patterns has been reported as a minimally invasive technique helpful in *ante mortem* diagnosis in horses suffering from equine motor neuron disease [15,16], equine grass sickness [17], equine degenerative myelopathy [15], myositis or myopathy [9,15], and botulism [18], even in the absence of spontaneous pathological changes of EMG. However, quantitative EMG patterns in humans are interpreted taking into account the patient's age and the muscle under study [1]. In horses it has been shown that age has an effect on QEMG parameters of MUAPs [19]. Similarly, normative values of quantitative MUAP analysis may vary between different muscles, as has been published initially for the subclavian, lateral vastus and triceps muscles [8,19–21]. Reference values have been published in equine muscles for descending pectoral, splenius and brachiocephalicus muscles since then [22,23].

The aim of this study was to perform an observational QEMG analysis of the supraspinatus, infraspinatus, deltoideus and biceps femoris muscles to obtain normative data to aid the diagnosis of certain neuromuscular conditions of horses. As a secondary aim, a retrospective study of horses presented to the Equine Hospital, Faculty of Veterinary Medicine, Utrecht University for orthopaedic or neurological disorders involving the shoulder muscles was performed to illustrate the clinical use of QEMG analysis.

Materials and methods

Horses - Seven adult clinically healthy Royal Dutch Warm blood female horses were used (10.6 ± 3.9 years old, mean ± s.d. (range 7-16); 569 ± 49 kg body weight (range 485-636);

162 ± 5 cm height (range 156-171); 37.4 ± 0.5°C rectal temperature (range 36.8-38.2)) to perform an observational QEMG study of Supraspinatus (SS), Infraspinatus (IS), Deltoideus (DT) and Biceps femoris (BF) muscles. Horses belonged to the Faculty of Veterinary Medicine, Utrecht University and were kept stabled or at pasture and in regular recreational work. Horses had normal physical, lameness and neurological examination, and unremarkable cervical radiographs. Skeletal muscles under study were identified by palpation with the aid of anatomical landmarks.

EMG examination - Procedures and materials for performing QEMG have been described previously [8,20,21]. In brief, EMG examination was performed using a portable apparatus[a] and 50 mm long, 0.45 mm diameter (IS, SS, DT) and 100 mm long, 0.8 mm diameter (BF) concentric EMG needle electrodes[b]. The horse stood unsedated in stocks and a surgical pad attached to it with a girdle that connected to the preamplifier served as the ground electrode. All horses had all 4 muscles examined during a single session. Band pass was between 5 Hz and 10 kHz. Sweep speed was 10–20 ms/division. Amplifier gain was 50–100 mV for spontaneous activity and 100–500 mV for MUAP recordings.

Standard EMG examination - Insertional activity, PSA, MUAPs, and satellite potentials were recorded in each muscle. Presence or absence of PSA was examined outside the endplate region in the same regions in which MUAPs were obtained to detect any fibrillation potentials, positive sharp waves, complex repetitive discharges, or (neuro)myotonia. It was considered indicative of pathology if present in 2 or more locations.

Quantitative MUAP analysis - After insertion of the EMG needle horses were manipulated as necessary to induce moderate muscle fibre recruitment and individual MUAPs. In order to recruit SS, IS and DT muscles the horse was pushed from the contralateral shoulder in order to force the horse to bear weight on the leg being studied. Similarly, in order to recruit muscle fibres of the biceps femoris, the ipsilateral front leg was elevated and by gentle shifting of weight onto the hindleg by pulling on the withers towards the measuring site. The muscle force induced was kept at low level to avoid inducing interference patterns that would prevent analysis of individual MUAPs. At least 3 insertions and 3 directions per insertion were made per investigated muscle. The needle was redirected several times, and by selecting sharp sounding MUAPs while the needle was withdrawn with 3-mm increments, sampling was performed throughout the muscle. MUAPs were selected partly in a semiautomatic way, using a trigger line that selects identical MUAPs above chosen amplitude. The automatic MUAPs selection was corrected manually off line, where indicated, (artifacts and noise excluded). End point of MUAP duration was corrected by on screen visual assessment if not shown correctly automatically [24–26]. Amplitude, duration, number of phases, and number of turns were automatically calculated and obtained from at least 20 MUAPs per muscle, with a maximal rise time of 0.8 ms rise and identically firing at least 4 times. Percentage of polyphasic MUAPs was calculated, polyphasia being defined as ≥4 phases. MUAPs with a number of turns ≥5 were considered complex MUAPs [1,27].

In addition, other quantitative parameters recently described in horses, such as SI and MUAP area were recorded and analysed [23]. MUAP area was calculated automatically. SI was calculated automatically as 2 × log (amplitude) + area/amplitude.

IPA - The low frequency filter was set at 20 Hz, high frequency filter at 10 KHz, and sampling frequency of at least 25 Hz [24–26]. Thirty contractions at random force per segment in each muscle were recorded with standard concentric needle electrodes[b] and evaluated. Interference patterns were analysed measuring "maximal voluntary activity" (MVA) expressed by turns/second and cloud analysis [28,29]. The EMG software calculated these variables automatically. The muscle force induced was not literally maximal, but randomised muscle force induced after vigorous stimulation by pushing or pulling on the withers as described above.

Retrospective study of horses with orthopaedic or neurologic disorders of the shoulder muscles- A sample of convenience of horses presented to the Equine Hospital, Faculty of Veterinary Medicine, Utrecht University for locomotion disorders involving the shoulder muscles were retrospectively identified by searching the QEMG software database in order to compare with the normative data presented and illustrate the clinical use of QEMG. Inclusion criteria were: (1) a clinical diagnosis by a board certified specialist orthopaedics/internal medicine clinician of a disorder of the lower motor neuron system or myopathy involving at least the shoulder muscles and (2) having performed QEMG evaluation of at least the shoulder muscles. A clinical diagnosis was reached by complete orthopaedic and neurological examination, radiographs and haematology and plasma biochemistry as indicated depending on the clinical presentation. Exclusion criteria were orthopaedic disorders causing signs of lameness or other locomotion disorders.

Data analysis - Data on MUAP variables were analysed using a one-way ANOVA with repeated measures and muscle (SS, IS, ST and BF) as independent factor. Data satisfied the

assumptions of normal distribution and homoskedasticity. If the null hypothesis was rejected, post hoc testing according to Bonferroni was performed to isolate significant differences between muscles. Significance level was set at $p \leq 0.05$. The mean MUAP variables per horse were used for statistical analysis, as previously reported [17].

Data are described as mean ± standard deviation (s.d.) unless otherwise stated. A statistical software package[c] was used for all computations.

Results

In all 4 muscles under study insertional activity was not prolonged or absent and pathological spontaneous activity was not detected, only occasionally satellite potentials were identified.

QEMG – Semiquantitative analysis on line showed that MUAPs from SS and IS muscles appeared to have a higher amplitude, duration, higher number of phases and turns, than that of Biceps femoris, and DT had intermediate MUAP features (Fig 1). Mean duration was ~40% greater and mean amplitude was ~2-3 fold higher in SS and IS muscles relative to BF (Table 1). Mean duration and amplitude of DT was not statistically different from BF (Table 1). Similarly, MUAP area and size index were significantly higher (Table 1) in SS and IS relative to BF, and DT muscle presented intermediate values (Table 1). The mean ± s.d. percentage of polyphasic MUAPs was 17 ± 7, 16 ± 10, 14 ± 12 and 5 ± 5 in SS, IS, DT and BF muscles. In addition, 4 of 7 horses had >15% polyphasic MUAPs in SS and IS muscles, 3 of 7 horses had >15% polyphasic MUAPs in DT, whereas only one of 7 horses had >15% polyphasic MUAPs in BF muscle. Complex MUAPS were occasionally recorded in SS and IS muscles.

IPA – Maximal voluntary activity differed significantly between muscles. Specifically, MVA expressed by turns/second was higher in DT than BF muscles (152 ± 59 vs. 76 ± 43, p = 0.03) and amplitude of the interference pattern measured was lower in BF (257 ± 54 μV) when compared with the other 3 muscles (mean 451-460 ± s.d. 82-158 μV, P<0.02). By projecting interference patterns recordings of 30 muscle contractions per muscle, expressed as amplitude/turns plotted against turns/second of all 7 horses per muscle on top of each other, a cloud of measuring points was graphed as documented previously [23]. Each cloud was located in the lower left corner of the figure (Fig 2). The centre of gravity of measuring points was subjectively different between muscles. The normal limit in humans is set at less than 15% of the total number of points outside the normal boundaries [28].

Clinical cases with neuropathy or myopathy of the shoulder muscles or biceps femoris

Horses with neurological disorders involving the lower motor neurons had increased values of one or more of the following QEMG parameters: amplitude, duration, phases, turns, size index and % of polyphasia (Table 2) relative to that in normal horses (Table 1). These included horses with clinical evidence of suprascular nerve injury, locomotion disturbances characterised by decreased protraction of one or both forelimbs not caused by orthopaedic lesions, and equine motor neuron disease cases. Horses with myopathy had lower values of one or more of the following QEMG parameters: amplitude, duration and size index (Table 2) when compared to that in normal horses (Table 1).

Discussion

Our main conclusions are: (1) SS and IS muscles have similar quantitative EMG features compared to previously published data from the Triceps muscles but different from other studied skeletal muscles in horses [8,19,21,20,22], (2) the MUAP parameters of SS and IS muscles (i.e. increased MUAP duration and amplitude) observed in these normal horses would be suggestive of neurogenic disorders if muscle-specific normal data were not available and decisions were made by comparing with values from other skeletal muscles [14], and thereby ignoring the muscle specific values, and (3) the MUAPs of the biceps femoris muscle differ greatly from the lateral vastus muscle, especially in amplitude [21], again highlighting the need for muscle specific values from healthy animals.

Electromyography (EMG) has long been in use as an ancillary diagnostic method to characterise certain neurological and muscular disorders in humans [1]. EMG examination involves assessment of insertional activity during needle placement, spontaneous activity following needle insertion without active muscle contraction and motor unit action potentials during recruitment of muscle fibres by voluntary contraction. Quantitative assessment of MUAPs has been demonstrated in humans to provide more information than simply detection of prolonged insertional activity or spontaneous pathological activity [2,10]. In horses, EMG examination has been performed for decades [30]. However, unlike in human neurophysiology, quantitative assessment of MUAPs has been used rarely [14] despite an increase in the number of publications on the use of quantitative EMG in the last 10 years [9,15–17, 22]. In horses, MUAP analysis has been shown to enable distinction between normal, neuropathy or myopathy [9,15–17], but MUAP parameters are also influenced by age [19] and type of muscle [8,21,22], as well as neuromuscular disorders [18].

In the current study, SS and IS had MUAPs characterised by high amplitude, duration and percentage of polyphasia compared to the biceps femoris, and to previously reported values for other equine muscles [21,22]. MUAP amplitude is physiologically increased by muscle fibre hypertrophy (i.e. increased muscle fibre cross sectional area) and pathologically increased by collateral reinnervation of muscle fibres in neuropathy [10,15,16]. In quadrupeds, shoulder muscles contract during the weight-bearing phase of every stride to stabilise the scapulohumeral joint. The SS and IS muscles are innervated by the suprascapular nerve and its perineural anaesthesia results in marked shoulder instability with lateral luxation of the proximal humerus during weight bearing [31]. Repetitive loading of SS and IS muscle fibres leads to hypertrophy and increased cross sectional area, which in turn results in increased MUAP amplitude. Increased muscle fibre cross sectional area in SS and IS muscles relative to other appendicular muscles has been shown in small primates [32] and dogs [33], but has not been demonstrated in horses. An alternative explanation for the increased MUAP amplitude and duration in SS and IS muscles observed in the current study might be subclinical denervation-renervation: in a study of 14 healthy draught horses, 9 had histological evidence of chronic focal neuropathy of the suprascapular nerve at the site of reflection over the cranial edge of the wing of the scapula [34]. The current study lacks morphometric data which can be considered a weakness of this study. However, subclinical neuropathy of the suprascapular nerve is unlikely in the current study because horses had normal findings on physical, lameness and neurological examination and unremarkable cervical radiographs.

Quantitative EMG parameters of DT and BF muscles are not comparable to other muscles previously described in horses. The 95% confidence interval (CI) of MUAP amplitude of

DT (370-691 μV) was numerically higher than previously reported values for subclavius (266-344μV), and descending pectoralis muscles (271-327 μV), similar to that of brachiocephalicus (412-483 μV), serratus ventralis cervicis (488-551 μV) and vastus lateralis (571-836 μV); and lower than triceps (μ701-1058 μV) [21–23]. Similarly, the 95% CI of MUAP duration of DT muscle (6.0-9.1 ms) was numerically higher than previously reported values for brachiocephalicus and serratus ventralis cervicis (4.3-4.7 ms), and pectoralis descendis muscles (5.3-5.8 ms), but similar to subclavius (6.4-7.4 ms), vastus lateralis (7.8-10.2 ms) and triceps muscles (7.6-10.0 ms) [21–23]. BF had QEMG parameters (amplitude and duration) more comparable to subclavius, pectoralis descendis, serratus ventralis and brachiocephalicus, and numerically lower to that of vastus lateralis and triceps.

In previous studies, the majority of data analyses were performed in age and breed matched controls of which data were pooled and natural logarithm transformation were needed because of the skewed distribution of data [21,22]. In the current study, the mean values per horse per muscle were used for statistical analysis and the criteria for normal distribution were met in this way without transformation. The mean values per horse for each muscle were evaluated since this mostly closely resembles the clinical situation.

The results of QEMG evaluation of MUAPs observed in clinical cases diagnosed with neuromuscular conditions in the current study illustrate the characterisation of disorder as neurogenic, myogenic or not resulting from alterations of the motor unit. It must be emphasised that EMG evaluation on its own does not provide a definitive diagnosis, but

will be complementary to a thorough orthopaedic and neurological examination in selected cases [35].

The results of this study should be interpreted taking into account the relatively small number of horses although the total number of analysed MUAPs per muscle was over 140 (>20 MUAPs □ 7 horses), and for IPA this was over 210 (>30 MUAPs □ 7 horses) recordings. Only adult horses (>18 months and <18 years) were included, therefore, these normative data may not be directly extrapolated to all age categories. Breed differences and the concomitant muscle fibre composition potentially affect MUAP characteristics and it remains questionable if breed influence will affect normative data to an extent that may interfere with decision making on presence of pathology or not [9,16,17]. In common with all retrospective studies, it is possible that we missed horses with the same disorders and unremarkable QEMG findings.

In conclusion, this study provides normative QEMG values for 3 shoulder muscles and biceps femoris and more recently developed QEMG variables such as SI, MUAP area, and IPA. These normative data may be helpful in diagnosing or localising neuromuscular disorders of the caudal cervical region or proximal forearm. However, the muscle-specific data show that supraspinatus, infraspinatus and triceps muscles in normal horses have QEMG features that would be considered suggestive of neuropathy if detected in other skeletal muscles. Muscle specific normal data is essential for comparison with clinical cases suspected of having neuromuscular disorders.

Authors' declaration of interests

No competing interests have been declared.

Ethical Animal Research

The study was approved by the Committee of Animal Welfare, at the University of Utrecht. Owner informed consent for the retrospective component was not stated.

Author contributions

I. Wijnberg and myself have contributed equally to the study design, execution, data analysis and interpretation, preparation of the manuscript and final approval of the manuscript.

Manufacturers' addresses

[a]Viking Quest EMG system, Nicolet Biomedical Inc, Madison, Wisconsin, USA.

[b]Length, 50 mm; diameter 0.45 mm; sampling area 0.07mm^2, Nicolet Biomedical Inc

[c]Sigmastat 3.5 software package, Jandel Scientific, San Rafael, California, USA.

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Figure legends:

Fig 1: Representative screen captures of MUAPs of supraspinatus (A), infraspinatus (B), deltoideus (C) and biceps femoris (D) muscles. Each division represents 100 μV (vertical axis) and 5 milliseconds (horizontal axis).

Fig 2: Interference pattern presented as cloud analysis of cumulated segments in the supraspinatus (A), infraspinatus (B), deltoideus (C), and biceps femoris (D) muscles. Sec, second; Amp, amplitude; M, mean; uV, microvolt. Horizontal axis: number of turns per second; vertical axis: amplitude shown in microvolts.

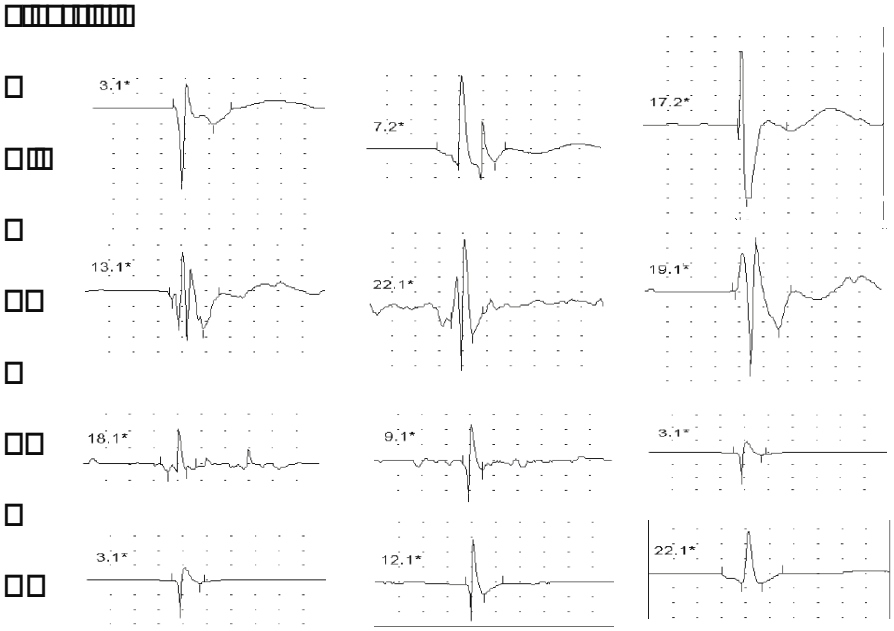
Table 1: Quantitative EMG parameters of supraspinatus, infraspinatus, deltoideus and biceps femoris muscles with significance (p-values) when compared to biceps femoris muscle.

	Muscle	Mean ± s.d.	P-value	95% CI
Amplitude	Supraspinatus	759 ± 117	<0.001	651-867
(□V)	Infraspinatus	931 ± 163	<0.001	779-1082
	Deltoideus	530 ± 173	0.1	370-691
	Biceps femoris	339 ± 81	□	265-385
Duration	Supraspinatus	9.6 ± 0.9	0.001	8.7-10.4
(ms)	Infraspinatus	10.3 ± 0.8	<0.001	9.6-11.0
	Deltoideus	7.5 ± 1.7	>0.9	6.0-9.1
	Biceps femoris	6.8 ± 1.1	□	5.7-7.8
No. Phases	Supraspinatus	3.5 ± 0.3	0.02	3.2-3.7
	Infraspinatus	3.5 ± 0.3	0.02	3.3-3.7
	Deltoideus	3.3 ± 0.4	0.2	2.9-3.7
	Biceps femoris	2.9 ± 0.3	□	2.7-3.2
No. Turns	Supraspinatus	4.2 ± 0.6	0.002	3.7-4.7
	Infraspinatus	4.3 ± 0.5	0.001	3.8-4.7
	Deltoideus	3.6 ± 0.9	0.2	2.8-4.5
	Biceps femoris	2.8 ± 0.3	□	2.6-3.1
Area	Supraspinatus	1256 ± 218	0.001	1054-1457
(□V·ms)	Infraspinatus	1777 ± 462	<0.001	1349-2204
	Deltoideus	877 ± 537	0.1	380-1374
	Biceps femoris	390 ± 102	□	296-484
Size Index	Supraspinatus	1.3 ± 0.2	<0.001	1.1-1.5
	Infraspinatus	1.7 ± 0.3	<0.001	1.4-1.9
	Deltoideus	0.8 ± 0.6	0.1	0.3-1.3
	Biceps femoris	0.4 ± 0.2	□	0.2-0.5

Table 2: Quantitative EMG parameters of supraspinatus, infraspinatus, deltoideus and biceps femoris muscles in 11 horses with suspected neurogenic or myogenic disorders of the supraspinatus, infraspinatus, deltoideus, and biceps femoris muscles. Values in bold are outside the 95% CI of reference ranges derived from 7 normal horses and given in Table 1.

Breed	Age	Diagnosis	Muscle	Amplitude (□V)	Duration (ms)	Phases	Turns	Size index	% polyphasia
Neuropathies									
Trackener	5 y	Peripheral neuropathy LF worse RF	Left SS	**1079**	**10.7**	**4.0**	4.8	**1.64**	**35%**
			Left IS	834	**14.1**	**4.2**	**6.3**	1.99	**60%**
			Left DT	**929**	**11.9**	3.8	**5.6**	**1.65**	19%
			Right SS	1087	11.7	**4.1**	**5.5**	1.65	**47%**
			Right DT	**998**	**12.4**	**4.2**	**5.7**	**1.97**	39%
RDSH	13 y	Suprascapular n. paralysis RF	Right SS	861	**11.7**	**4.0**	4.7	**1.62**	31%
RDSH	6 y	UMN and LMN disorder both forelimbs	Left SS	**947**	8.7	3.3	3.9	1.51	11%
			Left IS	**1871**	9.6	**4.5**	**5.5**	**2.01**	**50%**
			Left DT	**777**	7.7	3.2	3.9	1.24	8%
NRPS	13 y	Suprascapular n. paralysis LF	Left IS	796	**14.2**	3.7	5.3	**2.04**	27%
			Left DT	**733**	**10.3**	3.2	4.3	**1.54**	0%
RDSH	14 y	EMND	Left BF	331	7.9	3.0	3.4	0.18	**33%**
Hannoverian	14 y	EMND	Left BF	**805**	7.5	**5.0**	**7.2**	**0.87**	**60%**
RDSH	5 y	EMND	Left BF	381	7.2	2.6	3.2	**0.6**	**22%**
Myopathies									
Friesian	10 y	Generalised myopathy	Left BF	**151**	**4.5**	2.6	2.0	**0.00**	0 %
			Right BF	**130**	**4.9**	2.3	2.1	**0.01**	7%
--	--	Myopathy L and R	Left SS	**429**	11	3.2	3.2	**0.77**	12
			Left IS	**709**	**8.5**	2.9	3.6	**1.06**	0

RDSH: Royal Dutch Sport Horse
NRPS: Netherlands riding horse studbook
EMND: Equine motor neuron disease
LF left forelimb
RF right forelimb



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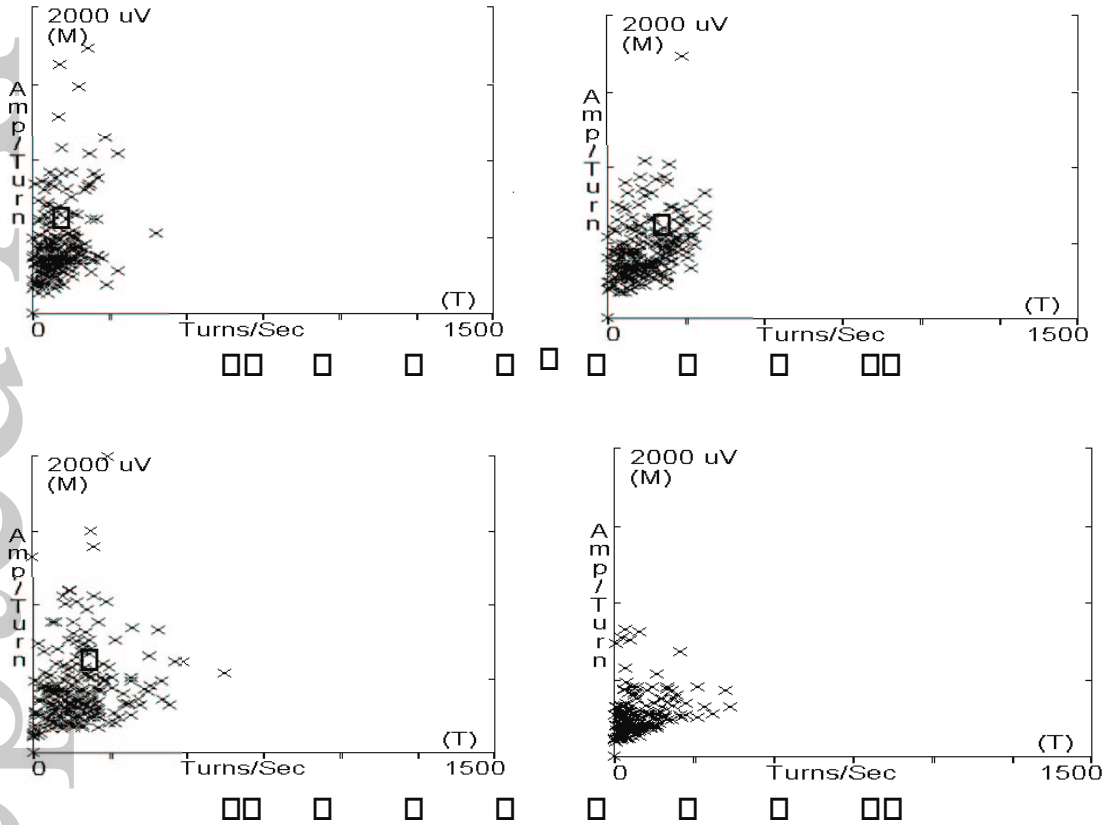

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