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Determining patterns of motor recruitment during locomotion

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Summary

Motor units are the functional units of muscle contraction in vertebrates. Each motor unit comprises muscle fibres of a particular fibre type and can be considered as fast or slow depending on its fibre-type composition. Motor units are typically recruited in a set order, from slow to fast, in response to the force requirements from the muscle. The anatomical separation of fast and slow muscle in fish permits direct recordings from these two fibre types. The frequency spectra from different slow and fast myotomal muscles were measured in the rainbow trout *Oncorhynchus mykiss*. These two muscle fibre types generated distinct low and high myoelectric frequency bands. The cat paw-shake is an activity that recruits mainly fast muscle. This study showed that the myoelectric signal from the medial gastrocnemius of the cat was concentrated in a high frequency band during paw-shake behaviour. During slow walking, the slow motor units of the medial gastrocnemius are also recruited, and this appeared as increased muscle activity within a low frequency band. Therefore, high and low frequency bands could be distinguished in the myoelectric signals from the cat medial gastrocnemius and probably corresponded, respectively, to fast and slow motor unit recruitment. Myoelectric signals are resolved into time/frequency space using wavelets to demonstrate how patterns of motor unit recruitment can be determined for a range of locomotor activities.

Key words: muscle, motor unit, electromyography, fish, cat, paw-shake, wavelet, rainbow trout, *Oncorhynchus mykiss*.

Introduction

During animal movement, the locomotor muscles are used for a variety of different functions, including producing the power required for skeletal motion, transferring that power across joints, and thus limb segments, and for fine control and support of the body. Each of these functions can place different demands on the muscles. The locomotor muscles comprise different types of muscle fibre, which can be broadly described as slow-twitch and fast-twitch (Close, 1965; Bárány, 1967), as distinguished by the rate of their myofibrillar-ATPase reaction. In some cases, the intrinsic speed of shortening does not reflect the ATPase activity, so these classes of muscle fibre are also referred to as type I and type II, respectively. Muscle fibres have been further subdivided in some animals; for instance, four distinct types have been identified in the rat (types Ia, IIa, IIx and IIb) (Schiaffino and Reggiani, 1994). The different types of muscle fibre vary in their ease of recruitment, metabolism and twitch characteristics, and it is to be expected that the fibre type used for any activity should match the demands of metabolism and twitch kinetics.

In fish, the different muscle fibre types are anatomically separated, and direct electromyographic recordings from the muscles have shown that there is an orderly recruitment of slow red through intermediate pink to fast white muscle fibres as the swimming velocity increases (Johnston et al., 1977). In higher vertebrates, the different muscle fibres are mixed within each muscle, making it more difficult to record the myoelectric activity from isolated fibre types. For mammals, it has been shown that for many activities there is a graded level of muscle recruitment that is driven by the different thresholds of the motor neurones: ‘the size principle of motor recruitment’ (Denny-Brown and Pennybacker, 1938; Henneman et al., 1965, 1974). The size principle predicts that muscle fibre recruitment is determined by force requirements, with the slow muscle fibres being activated for low-force contractions and increasingly fast muscle fibres being additionally activated to supply greater force demands.

Muscle activity patterns can be measured using electromyography, whereby the myoelectric signal generated by the motor unit action potentials is detected by electrodes. Myoelectric patterns contain information on the timing, frequency and intensity of the myoelectric signals. Deciphering this information allows particular details of the muscle activity

to be determined. For instance, the time taken to reach maximum force after activation depends on factors such as the muscle fibre type, the activation level and the contraction dynamics and, for isometric twitches in mammalian muscle, this time delay is in the range 23–73 ms (Burke et al., 1971, 1973; Gonyea et al., 1981). The intensity of the myoelectric signal indicates the activation level within the muscle. The force achieved by the muscle depends on this activation level, the number and fibre type of the muscle fibres activated, the contraction dynamics and the history of previous contractions (van Leeuwen, 1992). The frequency content of the myoelectric signal depends on, amongst other things, the shape of each action potential and the velocity with which the action potentials are conducted along the muscle fibres. Fast-twitch muscle fibres can have faster (1.5 times) conduction velocities than slow-twitch fibres even when they have similar diameters (Sadoyama et al., 1988), and the myoelectric frequency content increases with increased fibre conduction velocity (Lindström et al., 1977). The timing, activation level and motor unit recruitment patterns within a muscle all, therefore, leave characteristic features within the myoelectric signal.

Recent studies have shown that different locomotor demands result in changes in the myoelectric patterns at specific frequencies and times within a running stride in man (Wakeling et al., 2001a,b). Therefore, to obtain insight into muscle recruitment patterns for a given activity, the myoelectric signals must be resolved simultaneously into time and frequency space. However, the interpretation of such results is limited by our knowledge of the myoelectric frequency spectra for distinct fast- or slow-twitch muscle fibres and, thus, fast and slow motor units. The mean frequency within a myoelectric signal has been shown to correlate with the proportions of different fibre types between individuals (Moritani et al., 1985; Gerdle et al., 1988; Elert et al., 1992) and to increase when a greater proportion of fast muscle fibres are activated (Solomonow et al., 1990), suggesting that faster motor units generate higher-frequency components in the electromyogram (EMG). However, the frequency spectra for distinct populations of fast-twitch or slow-twitch muscle fibres have not yet been documented. Fish are peculiar among the vertebrates in that the fast- and slow-twitch muscle fibres occur in distinct anatomical compartments in most species (Bone, 1978). This peculiarity has enabled us to study the myoelectric frequency spectra from distinct fast- and slow-twitch muscles.

The aim of this study was to address two questions. (A) What is the frequency response of purely fast- and purely slow-twitch muscle fibres? Fish were used as a model to address this question because of the anatomical separation of their fast and slow muscle fibres. (B) Can the frequency spectra from distinct muscle fibre types be identified in the electromyograms from mixed muscle? A cat model was used to address this second question because it is known that the paw-shake behaviour uses a high proportion of fast muscle (Smith et al., 1980; Fowler et al., 1988). To demonstrate that the recruitment patterns of different muscle fibre types can be distinguished within the EMG, recordings are also presented from the cat walking and galloping, when it uses slow- and fast-twitch fibres in different proportions according to the demands on the muscle.

Materials and methods

Fish electromyography

Muscle activity was measured in 16 rainbow trout [*Oncorhynchus mykiss* (Walbaum)]. Fish EMG experiments were carried out under a license from the Home Office and complied with the legal requirements of the UK. Fish were tested from each of three size groups with masses of 21.5±0.9, 99.5±6.2 and 401.8±23.2 g and lengths of 130±2, 219±4 and 325±6 mm (means ± S.E.M., N=6, N=4 and N=5 respectively). The fish were swum in a static tank with the dimensions 0.8 m×0.6 m×0.2 m (length×width×depth). The water was aerated and kept at 13 °C during the experiment.

EMG electrodes were made from 0.2 mm diameter, Teflon-coated platinum wire. The coating was removed from the end 2 mm, which was bent into a hook. Anaesthesia was initiated with a 1:5000 (w/v) solution of bicarbonate-buffered ethyl *m*-aminobenzoate (MS222) and maintained during surgery with a 1:3 dilution of the above solution. Electrodes were inserted into the fish using a 0.5 mm diameter hypodermic needle, with the wire sutured onto the leading edge of the dorsal fin. A unipolar electrode was inserted dorso-ventrally into the fast-twitch muscle on the right side of the fish, and a single electrode was inserted posterio-anteriorly into the left side. The electrodes were therefore sited 0.42±0.01 body lengths from the snout. A section of stainless-steel tubing in the water was used as a ground reference electrode. Signals were recorded using a 12-bit National Instruments DAQ-Card 1200 data-acquisition board after prior amplification using a differential amplifier (A-M Systems, WA, USA).

Initial testing showed that tail-beat frequencies of up to 16 Hz occurred for the smaller fish. Body movements cause artefacts in the signal. The magnitude of these artefacts depends partly on the muscle strain, which varies across the width of the fish (Wakeling and Johnston, 1999) and, thus, between recording sites. The low-frequency cut-off for analysis was taken at 50 Hz, which was three times the movement frequency, to minimize the bias that would be introduced from movement artefacts. Initial observations showed that power within the myoelectric signals occurred at frequencies up to 2 kHz. To measure these frequencies accurately, the recording frequency must be greater than the Nyquist frequency (which is 6–7 times the maximum signal frequency). A 20 kHz recording frequency was therefore used. The myoelectric signals were analysed within the frequency band 50–2000 Hz. It is important that the data are unfiltered before the wavelet analysis to be certain that all the myoelectric signal within the analysed range can be accounted for. The cut-off filters on the amplifier were therefore set to 10 Hz and 20 kHz so that the amplifier did not remove any of the required signal before analysis.

The superficial slow muscle fibres are used by a fish for slow-speed swimming, whereas the deeper white muscle is

used for burst swimming and fast-start manoeuvres (Bone, 1966; Blight, 1977; Kashin et al., 1979). Myoelectric signals were recorded from spontaneous slow swimming around the tank and also from fast-starts, which were initiated by thrusting a rod towards the snout of the fish. To minimize the effect of cross-talk between the different areas of muscle, the myoelectric signals for the fast-twitch white muscle were analyzed from the fast-start behaviour and the signals for the slow-twitch red muscle were analyzed from the slow swimming behaviour.

Muscle fibre diameters

At the end of the experiment, the fish were killed by a blow to the head, and transverse blocks of muscle were dissected from the EMG recording sites. The blocks were frozen in isopentane cooled in liquid nitrogen. Serial 8 μm sections were cut perpendicular to the skin and stained with haematoxylin. The cross-sectional area and diameter of 200 red and white fibres from each fish were determined using image analysis (Kontron Elektronik, Basel).

Non-parametric techniques were used to fit smoothed probability density functions to the measured fibre diameters using the kernel approach (Silverman, 1986), as previously applied to the study of fish muscle growth (Johnston et al., 1999). Values for the optimal smoothing parameter ranged from 0.054 to 0.164. The fifth, fiftieth and ninety-fifth percentiles of fibre diameter were then calculated from the smoothed distributions for each fish.

Cat force and EMG

The methods used for animal preparation, force and EMG measurements have been described in detail elsewhere (Herzog et al., 1993). The animals were humanely euthanized after the experiment. All procedures were performed according to the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Calgary. Only a brief description of these methods is provided here. Forces and EMGs from the medial gastrocnemius were measured from four outbred, adult male cats (mass 4.4±0.2 kg; mean ± S.E.M.) during walking at 0.4 m s^{-1} on a motor-driven treadmill, walking up a ramp inclined at 45°, galloping and paw-shaking. E-shaped, stainless-steel tendon force transducers were used in force measurements. The transducers were surgically implanted onto the tendon of the gastrocnemius under strictly sterile conditions with the animals deeply anaesthetized. EMGs were recorded using bipolar, indwelling wire electrodes implanted into the midbelly of the medial gastrocnemius. There was a 5 mm separation between the exposed ends of the bipolar electrodes. A common ground electrode was sutured subcutaneously around the lateral epicondyle. Leads from the force transducer and EMG electrodes were drawn subcutaneously to a back-pack connector. From this connector, signals were transmitted by a custom-made telemetry system to a computer. The animals were trained to perform the experimental behaviours for a 2-month period prior to implantation. Paw-shaking was elicited in response to placing sticky tape on the paw. Galloping was elicited by motivating the animals to chase after toys. Data were collected once the gait had returned to the pre-operative state; this was 7–10 days after implantation. For each of the locomotor behaviours, tendon forces were recorded at 500 Hz and EMGs at 2000 Hz.

EMG analysis

The intensities of the myoelectric signals were resolved into time/frequency space using wavelet analysis (von Tscharner, 2000). These methods are illustrated in the Appendix. Briefly, each wavelet acted like a bandpass filter. The intensity of the myoelectric signal was calculated to be the power of the myoelectric signal contained within the frequency band for each respective wavelet. The wavelets were nearly bi-orthogonal and were generated by non-linear scaling. The centre frequencies $f_c(k)$ for a set of wavelets are given by the relationship:

$$f_c(k) = \frac{(k + c_1)^{c_2}}{c_3}, \quad (1)$$

where c_1, c_2 and c_3 are scaling factors and k is the wavelet number. The choice of scaling factors must strike a balance between time and frequency resolution required for the analysis. c_1, c_2 and c_3 took values of 1.45, 1.959 and 0.3, respectively, and were chosen so that the time resolution for the wavelets of less than 32 ms was similar to the expected response time of a muscle activation process (von Tscharner, 2000). For the fish analysis, wavelets were used for $3 \leqslant k \leqslant 24$, with the total bandwidth of 54–1960 Hz. For the cat analysis, wavelets were used for $1 \leqslant k \leqslant 8$, with the total bandwidth of 12–304 Hz.

A wavelet domain was defined as the time series of intensity resolved for one wavelet only. An intensity $i_{j,k}$ was calculated for each sample point j and wavelet domain k. The instantaneous mean frequency f_m of the intensity spectrum for each sample point j is given by:

$$f_m = \frac{\sum_k f_c(k) i_{j,k}}{\sum_k i_{j,k}}. \quad (2)$$

A mean frequency was calculated as the mean of the instantaneous f_m values for specific time windows for each locomotor activity. The size of this window must be sufficient to average fluctuations within the myoelectric activities but be short enough to isolate the mean frequency during times when it was likely that distinct populations of muscle fibres were being recruited. Initial analysis of the EMG data showed that appropriate durations for the time windows were 10 ms for the fish fast-start and cat paw-shake, centred on the moment of maximum intensity, and 100 ms for the fish slow swimming and cat treadmill-walking, beginning at the onset of EMG activity. These same windows were used for determining the myoelectric intensity spectra during the different activities.

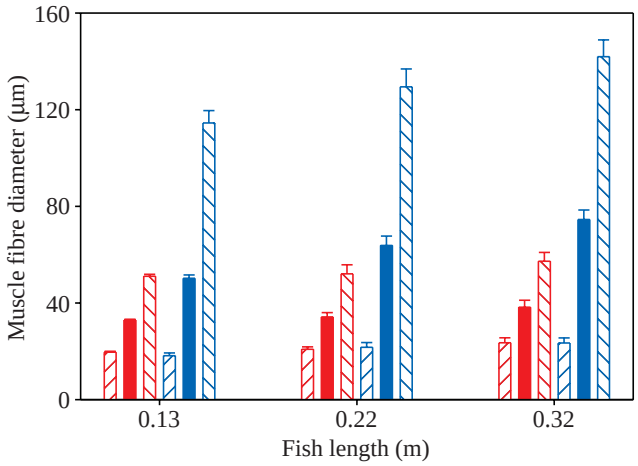

Fig. 1. Fifth, fiftieth and ninety-fifth percentiles of the muscle fibre diameters in the rainbow trout. Percentiles were calculated from the μprobability density functions of the muscle fibre diameter distribution for each fish. The columns show the mean diameter + S.E.M. for the fifth, fiftieth and ninety-fifth percentiles for each size class of fish. Red and blue columns show the percentiles for the red and white fibres, respectively. The size classes were: L=0.13±0.02 m, N=6; L=0.22±0.03 m, N=5; L=0.32±0.06 m, N=5, where L is total fish length.

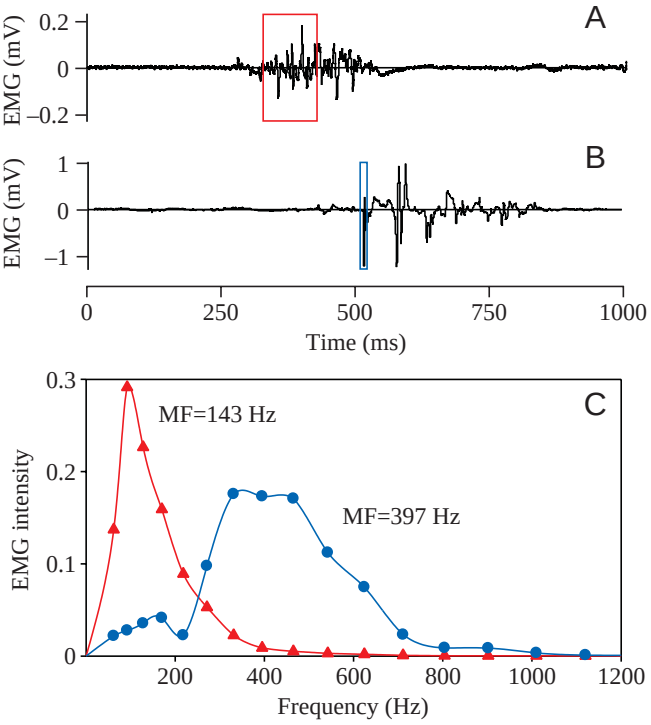

Fig. 2. Myoelectric signals and intensity spectra from fast- and slow-twitch muscle fibres. Data are taken from one fish. EMGs were recorded from red muscle (mean fibre diameter 36 μm) during slow swimming (A) and from white muscle (mean fibre diameter 74 μm) during a fast-start (B). The boxes indicate the time regions for the red fibres (red box) and white fibres (blue box) analysed for frequency content. The intensity spectra for these regions (C) show a threefold increase in mean frequency, MF, between the red (red triangles) and white (blue circles) muscle fibre types. Spectra are normalized so that total intensity equals 1.

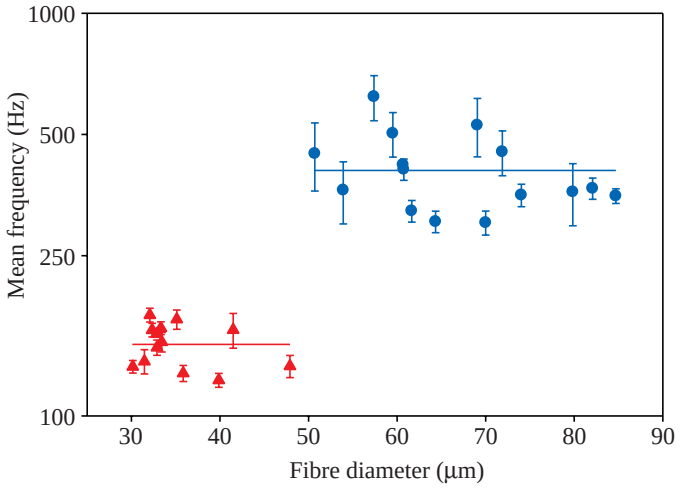

Fig. 3. Mean myoelectric frequencies for red and white muscle fibres. Each point denotes the mean ± S.E.M. (N=5–6) from one fish. White muscle fibres (blue circles) showed a significantly greater mean frequency than red muscle fibres (red triangles). There was no significant linear regression between mean frequency and mean fibre diameter for either fibre type. Solid lines indicate the average mean frequency across all fish for each muscle fibre type.

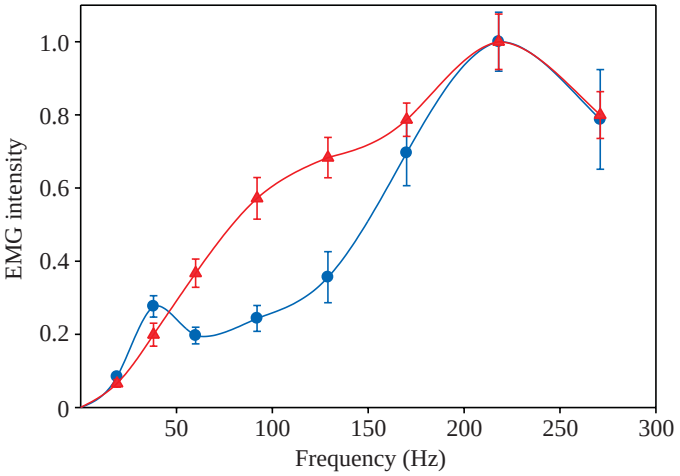

Fig. 4. Myoelectric intensity spectra for different movements in the cat. Each point shows the mean ± S.E.M. myoelectric intensity for paw-shaking (blue circles, N=24) and treadmill-walking (red triangles, N=31) at 0.4 m s^{-1} calculated from all four cats. The mean intensities for each activity were normalized to a maximum value of 1.

Results

Myoelectric spectra for distinct red and white muscle in the fish

EMGs were analysed for 154 swimming sequences from 16 fish. These swims consisted of 80 fast-starts and 74 slow-swimming manoeuvres. The fish ranged in length from 12 to 346 mm. Both red and white muscle fibre diameter increased with increasing fish length; however, the muscle from each fish spanned a range of diameters (Fig. 1). The smallest

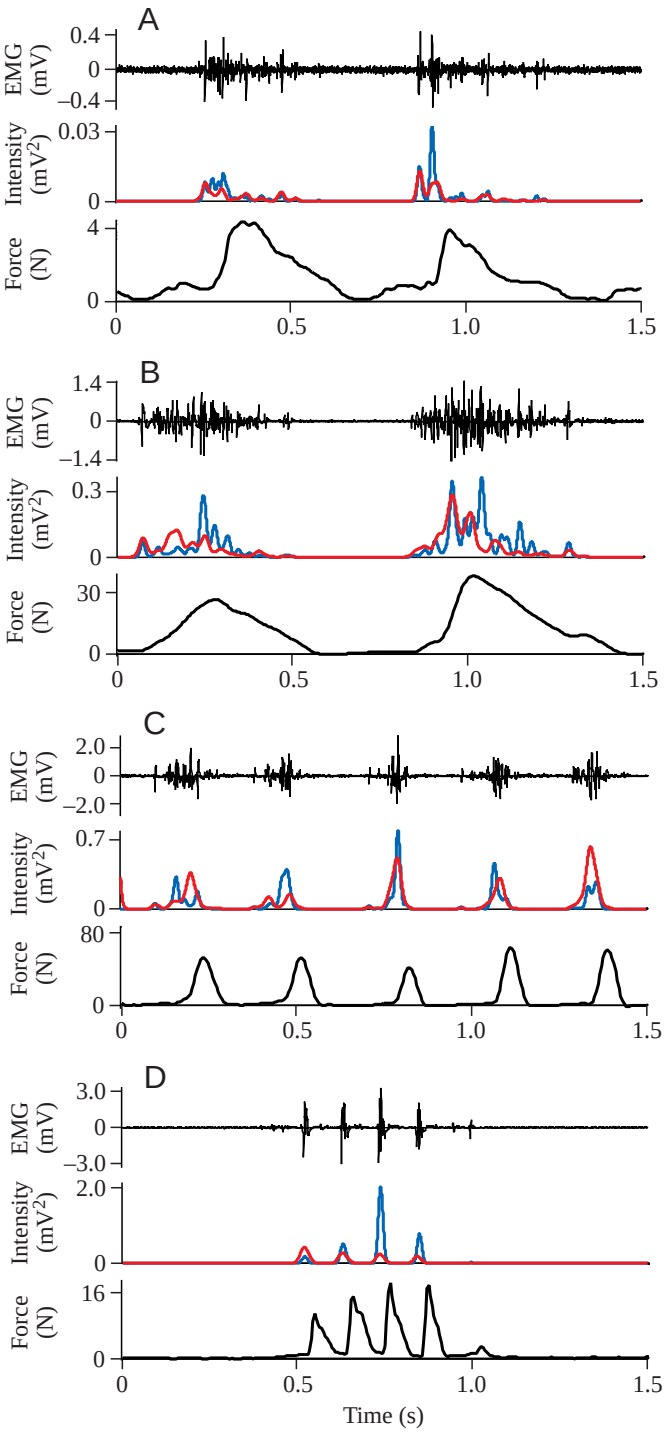

Fig. 5. EMG, intensity and tendon force for the medial gastrocnemius in the cat. Intensities are summed from wavelet domains 3 and 4 (red line, band-width 50–110 Hz) and from wavelet domains 7 and 8 (blue line, band-width 190–300 Hz). Recordings were made during treadmill-walking at 0.4 m s^{-1} (A), during walking up a 45° incline (B), during galloping (C) and during paw-shaking (D).

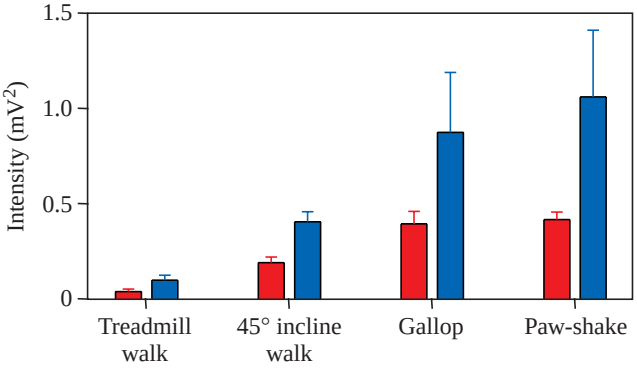

Fig. 6. Recruitment between fast and slow motor units in the medial gastrocnemius of the cat. Intensities were summed from wavelet domains 3 and 4 to represent slow motor unit activity (red columns, band-width 50–110 Hz) and from wavelet domains 7 and 8 to represent fast motor unit activity (blue columns, band-width 190–300 Hz). The maximum intensity was measured for separate strides and paw-shakes for the cat shown in Fig. 5. Values are means + S.E.M. (N=10) of these maximum intensities. Values are shown for each of the four movements.

fibres overlapped in diameter between the red and white fibre types. The fifth percentiles spanned the ranges 18–31 μm and 13–28 μm for the red and white fibre diameters, respectively. The largest white fibres had greater diameters than the largest red fibres. The ninety-fifth percentiles spanned the ranges 46–69 μm and 93–157 μm for the red and white fibre diameters, respectively. There were significant positive regressions (P<0.05) between mean muscle fibre diameter (d, μm) and total body length (L, m) for both the red and white fibres (Fig. 1). The regression equations were d=98.9L+44.5 and d=27.7L+29.0 for the red and white fibres, respectively.

The red and white muscle fibres generated myoelectric signals with distinct frequency bands (Fig. 2). The red fibre intensity spectrum was predominant at much lower frequencies than the white fibre intensity spectrum. A t-test showed that the mean frequency of these spectra was significantly (P<0.05) lower for the red fibres (mean frequency 150.5±5.1 Hz, N=13 fish) than for the white fibres (mean frequency 407.1±23.3 Hz, N=15 fish; means ± S.E.M.). In all cases, the mean frequencies for the red fibres were lower than those for the white fibres. There was no significant regression between the mean frequency and the muscle fibre diameter for either the red or the white muscle fibres from these fish (Fig. 3).

Myoelectric spectra for mixed muscle during fast and slow activities in the cat

EMGs were analysed for 24 paw-shakes recorded from the medial gastrocnemius muscle in four cats. The intensity spectra for this activity were concentrated in a high-frequency band above 150 Hz (Fig. 4). EMGs were analysed for 31 steps during treadmill walking at 0.4 m s^{-1} from the medial gastrocnemius muscle in these same four cats. The intensity spectra for treadmill-walking showed a higher proportion of low-frequency components (50–150 Hz) than for paw-shaking (Fig. 4). The spectra from each paw-shake and treadmill step

were normalized so that the mean values at wavelet domain k=7 (f_C=218 Hz) equalled 1. *t*-tests showed that the relative proportion of these low-frequency components was significantly (P<0.05) different for wavelet domains $3 \leqslant k \leqslant 5$. Within this region, the relative intensity during treadmill-walking was twice that during paw-shakes (Fig. 4).

Myoelectric intensities in the high and low frequency bands during different types of cat locomotion

The myoelectric intensities from the medial gastrocnemius in one cat are shown for different movements in Fig. 5, which shows the sum of the intensities for wavelet domains 3–4 and also for wavelet domains 7–8. These domains cover the frequencies at which the slow and fast motor units are predominantly active and correspond to the frequency bands 50–110 Hz and 190–300 Hz, respectively. The ground contact phase corresponds loosely to the region of elevated tendon force. During slow walking on the treadmill (Fig. 5A), the myoelectric intensities are similar between the high and low frequency bands. At the start of the second ground contact, a peak in the high-frequency component occurs immediately prior to the rise in tendon force; the resulting rate of increase in force is greater than for the first step. During walking up a 45° incline (Fig. 5B), there in a general increase in the EMG amplitude and tendon force compared with level treadmill-walking. The increase in EMG amplitude corresponds to an increase in the intensity in both the high and low frequency bands. The 45° incline-walking differs from the treadmill walking in that the end of the ground contact phase contains a relatively greater proportion of the high-frequency component. There is a further increase in EMG amplitude and tendon force as the cat gallops (Fig. 5C). Bursts of muscle activity occur that are relatively synchronous between the high- and low-frequency components. Similar levels of tendon force are achieved with different proportions of intensity in the high and low frequency bands. Data from paw-shaking are shown in Fig. 5D. Note that the tendon force for the paw-shake is less than for galloping because the limb is unloaded, even though the muscle activity was greater. The myoelectric intensity occurred almost exclusively in the high frequency band for the third shake, which probably corresponded to predominant recruitment of fast motor units during this behaviour.

For each step and paw-shake, the myoelectric signal can be characterised by its maximum intensity in the low and high frequency bands from wavelet domains 3–4 and wavelet domains 7–8, respectively. Such maximum intensities were determined for 10 steps from each activity for the cat depicted in Fig. 5. These intensities increased between the locomotor behaviours from treadmill-walking, through 45° incline-walking and galloping to the paw-shake behaviour (Fig. 6). The high-exertion activities of galloping and paw-shaking resulted in a large variation in the intensity in the high-frequency band (ratio of S.E.M./mean of 35%), whilst the low-frequency component was much more consistent, with a ratio of S.E.M./mean of 13%.

Discussion

Determinants of myoelectric frequency spectra

The frequency components within the intensity spectra are caused by, amongst other things, the conduction velocity and the shape of the action potentials. Local circuit theory has been used to predict that the conduction velocity of an action potential increases with the square root of the radius of the muscle fibre (Hodgkin, 1954). This relationship has been demonstrated for nerve axons (Pumphrey and Young, 1938), but the only evidence for this in muscle is from *in vitro* preparations from the frog (Håkansson, 1956). Conduction velocity also depends on the electrical properties of the muscle cell membrane (Hodgkin, 1954), and the available evidence suggests that this is probably more important than the effect of fibre diameter. Differences in the electrical properties of the membranes between the fibre types have been recorded for fish (Stanfield, 1972), amphibians (Adrian and Peachey, 1965), reptiles (Proske and Vaughan, 1968) and mammals (Luff and Atwood, 1972), so it is to be expected that the different muscle fibre types will have different action potential conduction velocities. Indeed, Sadoyama et al. (1988) compared the conduction velocity in the vastus lateralis muscle between people with different proportions of fast- and slow-twitch fibres and showed that conduction velocity changed even when there was no significant difference in the mean diameter of the two fibre types. Extrapolation of these results shows that the fast-twitch fibres had a conduction velocity of 5.4 m s^{-1} compared with a velocity of 3.6 m s^{-1} for the slow-twitch fibres (Sadoyama et al., 1988). The mean conduction velocity within a muscle translates to frequency components within the EMG (Lindström et al., 1970), and a more recent experiment on the vastus lateralis has similarly shown that there was little correlation between the mean frequency of the EMG and the mean fibre cross-sectional areas within the muscle (Gerdle et al., 2000).

The shape of an action potential is a function of the relative rates of membrane depolarisation to hyperpolarisation. These relative rates depend on the ion flows through the voltage-gated Na^+ and K^+ channels which, in turn, can differ between the muscle fibre types. Figures depicting the motor unit action potential waves from the rat show that different rates of hyperpolarisation relative to depolarisation do occur between muscles with predominantly different fibre types (Kupa et al., 1995). Furthermore, Kupa et al. (1995) found that, for rat extensor digitorum longus muscle, the mean frequency of the EMG showed a significant positive correlation with the mean muscle fibre cross-sectional area even when the conduction velocity showed no significant correlation with the fibre cross-sectional area. These results indicate that the shape of the action potentials is also an important determinant of the EMG frequency.

Fish growth is indeterminate, and the myotomal muscle grows by a combination of the recruitment of new muscle cells and the hypertrophy of existing ones. Recruitment is the dominant process for rainbow trout (Weatherley et al., 1980), resulting in each fish from this study having a wide range of

muscle fibre diameters (Fig. 1). The multi-terminal innervation pattern in fish myotomal muscle can result in a given motor neurone innervating muscle fibres covering the full range of diameters (Altringham and Johnston, 1981). Because of the wide range of muscle fibre diameters occurring in each fish (Fig. 1), it is unlikely that the data would be able to resolve correlations between the mean fibre diameter and the mean frequency of the EMG intensity spectra. Indeed, this was the case, with the data unable to show significant relationships between the mean frequency of the EMG intensity spectrum and the mean fibre diameter for either the red or the white myotomal muscle of the rainbow trout (Fig. 3).

In this study, the different fish sizes were selected so that the red muscle fibres in the large fish had similar mean diameters to the white fibres in the small fish. We have shown that a 2.7-fold difference in mean frequency occurred between the different fibre types in the rainbow trout, and that this was not related to the muscle fibre diameter. The main conclusion that can be drawn from this experiment is, therefore, that the fast and slow fibres have characteristic high and low frequency bands in the EMG intensity spectra irrespective of their diameter. The relative contributions of action potential shape and conduction velocity to the intensity spectra cannot be determined from this study. However, these results show that fast and slow muscle fibre types can be distinguished by their resulting myoelectric intensity spectra.

Motor unit recruitment during locomotion

Experiments using intracellular electrodes have shown that action potentials occur along both the red and white myotomal muscle of teleost fish (Eugène and Barets, 1983; Altringham and Johnston, 1988). During activity, the myotomal muscle of fish has electrical properties similar to those of twitch skeletal fibres of other vertebrates (Eugène and Barets, 1983). Therefore, it is to be expected that the different myoelectric intensity spectra generated by the different muscle fibre types in fish should also be generated by the different motor units within the mixed muscle of higher vertebrates.

In higher vertebrates, muscle growth, after embryonic differentiation, is largely by hypertrophy (Goldspink, 1980), so the muscle fibres are more homogeneous in diameter than in fish. The functional unit for neural control and muscular activity is the motor unit (Sherrington, 1929). Motor units consist of an α motor neurone and all the muscle fibres innervated by that motor neurone. These muscle fibres are distributed across the muscle so that adjacent fibres are from different motor units. Within a given motor unit, the muscle fibres have similar biochemical and twitch properties so that fast motor units comprise fast-twitch muscle fibres and slow motor units comprise slow-twitch muscle fibres. Control of muscle contraction is by the selective activation of the different motor units, and this muscle activation is commonly driven by the muscle force requirements in a process known as the size principle of motor unit recruitment (Denny-Brown and Pennybacker, 1938; Henneman et al., 1965, 1974).

Myoelectric frequencies have previously been shown to correlate to motor unit recruitment patterns in the cat gastrocnemius. In an elegant experiment, the motor neurones were stimulated to achieve graded recruitment of the gastrocnemius, and the median frequency from the resulting EMG power spectra increased with increased fast motor unit activity (Solomonow et al., 1990). Mean frequencies have also been shown to correlate positively with the proportion of fast-twitch muscle fibres in man for the gastrocnemius (Moritani et al., 1985), vastus lateralis (Gerdle et al., 1988) and trapezius (Elert et al., 1992) muscles. It is suggested from these studies that fast-twitch muscle fibres result in higher myoelectric frequencies than slow-twitch fibres, a conclusion that supports the present findings. However, past measurements of mean or median frequencies from mixed muscle have been unable to show how the different fibre types contribute different frequency bands to the resultant myoelectric signal. In a recent study, we have distinguished high and low frequency bands in four lower extremity muscles in man: the gastrocnemius, tibialis anterior, biceps femoris and rectus femoris (Wakeling et al., 2001a). During an experiment involving sustained running, there were distinct concentrations of intensity in the myoelectric intensity spectra at frequency bands of 25–75 Hz and 150–240 Hz, and changes in the intensity within these frequency bands were in opposite directions. These changes in myoelectric activity were not due to fatiguing mechanisms during the run, and it was suggested that these high and low frequency bands represent signals from different motor units (Wakeling et al., 2001a).

Cat paw-shakes are high-frequency activities with short cycle durations (55–110 ms) (Smith et al., 1980), precluding the action of the slow motor units in the soleus muscle because of their slow contraction and half-relaxation times of 79 and 92 ms, respectively (Spector et al., 1980). Indeed, electromyography has shown that the action of the slow motor units from the soleus is virtually absent during the paw-shake (Smith et al., 1980; Fowler et al., 1988). It has been suggested that paw-shaking is triggered primarily by low-threshold afferents innervating the central plantar pads and by selective recruitment of the fast extensors and inhibition of the slow extensor (Smith et al., 1980). Thus, during paw-shaking, we can expect to see mainly the action of the fast motor units. The intensity spectra in Fig. 4 show that 70 % of the power of the myoelectric signal occurred in the high frequency band of 150–300 Hz during the cat paw-shake.

Because of technical limitations on the sampling rate from the transceivers recording the cat EMG, higher frequencies could not be resolved from the signal. For this reason, it is recommended that higher sampling rates are used in future for similar studies, if practical. The myoelectric intensity had not reached zero at wavelet domain k=8 (centre frequency 271 Hz), and there was still power within the myoelectric signals at frequencies greater than 300 Hz. Thus, the value of 70 % is a conservative estimate for the high-frequency component. This frequency band of 150–300 Hz probably represents the major frequency components from the fast motor units. During slow treadmill-walking, the cat recruitment patterns probably follow

the size principle pattern, whereby the gastrocnemius force is produced mainly by the slow motor units with some force being produced by fast motor units. The myoelectric intensity spectra during walking (Fig. 4) are different from those for the paw-shake. When compared with the same maximum value, the intensity spectra for walking are significantly greater than those for the paw-shake in the frequency band 50–150 Hz. This is the low frequency band that presumably corresponds to the additional recruitment of the slow motor units.

The motor recruitment patterns for the medial gastrocnemius for one of the cats are shown in Fig. 5. During walking, the tendon forces and total EMG level from the gastrocnemius were lower for this cat than for the other three cats tested because a greater role was played by the soleus muscle (M. Kaya, unpublished observations). However, this cat was chosen for further analysis because the recordings of its galloping behaviour are unusual and help illustrate the patterns of motor recruitment. Intensity traces are shown for the low and high frequency bands that correspond to the major frequency components of the different types of motor unit. There was no overlap in the two frequency bands used, so that the intensity traces are independent of each other. During galloping and paw-shaking, the intensity recorded from the slow motor units was consistent at $0.4\,mV^2$, whereas the intensity from the fast motor units was more variable, with values ranging between 0.2 and $3.9\,mV^2$ (Fig. 6). These results satisfy predictions about motor unit recruitment according to the size principle: there is a certain level of muscle activity that is provided by the slow motor units, with extra activity coming from the additional recruitment of fast motor units. The amount of fast motor unit recruitment depends on the level of muscle activity required for the movement and this, in turn, will vary from step to step. These examples suggest that the balance of motor recruitment changes not only between activities but also within the duration of each stride. This study shows a possible approach to the determination of motor recruitment patterns during locomotion and opens up a wide range of possibilities for studies in comparative biomechanics. The examples shown here merely illustrate the technique, and the approach should be extended further to identify patterns of neuromuscular control.

Measuring motor unit recruitment

This study has shown that the activity of different muscle fibre types can be distinguished by their frequency components within a myoelectric signal. To measure motor unit recruitment patterns, the frequency bands that correspond to the different motor units must first be identified. The frequency bands that correspond to the different muscle fibres will change from muscle to muscle and from species to species as the fibre dimensions, histology and biochemistry change. The responses of different fibre types may overlap in frequency (e.g. Fig. 2), and measurements at the common frequencies would be unable to distinguish between different fibre types. Therefore, it is important that the frequency bands used for an analysis are well separated so that they identify components that are mainly from one muscle fibre type or the other. For similar reasons, it is unclear at this stage whether the myoelectric frequency bands from the different classes of fast-twitch muscle, types IIa, IIx and IIb, can be distinguished from each other. In this study, they have been discussed as one functional type, and it will require further experiments to quantify the differences in their myoelectric properties.

The actual frequencies measured at the electrodes depend on the configuration of the electrode system and the muscles being measured. Factors that affect the measured frequencies include the size and separation of the electrodes, the relative depth of the muscle fibres, attenuation through the soft tissue between the muscle and electrodes, the orientation of the electrodes relative to the fibres and the relative placement of the electrodes with respect to the innervation zones on the muscle (Roy et al., 1986; de Luca, 1997). The frequency bands that correspond to different muscle fibre types will change between measurements with different experimental configurations, and this must be remembered when comparing results from different studies. It should also be noted that attenuation of the myoelectric signals between the muscle and the electrodes depends both on the distance between the fibres and the electrodes and on the frequency of the signal. Therefore, the intensity measured at the electrodes will not equal the actual intensity on the muscle fibres, so intensities from different frequency bands should only be compared in a relative manner.

This study has shown that fast- and slow-twitch muscle fibres result in high and low frequency bands, respectively, when measured from fish myotomal muscle. High- and low-frequency components also occur in the myoelectric intensity spectra from the cat medial gastrocnemius, and the intensity within these bands corresponds to different fast and slow motor unit recruitment strategies used by the cat for a range of locomotor tasks. High and low bands of myoelectric frequency have also been recorded from man and have again been attributed to the signals from different motor units (Wakeling et al., 2001a). The relative proportion of the signal contained within these high and low frequency bands can change during the course of a stride (Fig. 5) (Wakeling et al., 2001a), and the myoelectric signals must therefore be analysed in both time and frequency space to resolve such details. The wavelet methods used here, and described previously by von Tscharner (2000), provide a suitable analysis tool that will enable greater insight to be gained into the patterns of motor unit recruitment during locomotion.

Appendix

Wavelet analysis of myoelectric signals

Myoelectric signals can be resolved into their time and frequency components simultaneously using wavelet analysis. The wavelet transform has been described “as a ‘mathematical microscope’ in which one can observe different parts of the signal by just adjusting the focus” (Karlsson et al., 2000). This Appendix serves to illustrate briefly the technique to give an understanding of the processes involved without having to

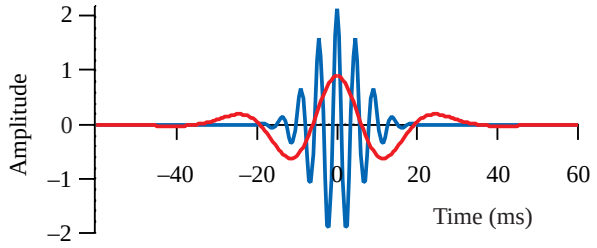

Fig. 7. Wavelets for k=2 (red) and k=7 (blue) shown in the time domain. k, wavelet number.

follow the mathematical details. The wavelet methods described here have previously been defined by von Tscharner (2000).

A wavelet is a little wave, is well-defined in both time and frequency and has a time-integral of zero. Fig. 7 shows the time domain for two wavelets used in this analysis. Because of the uncertainty principle of signal processing, the exact time and frequency of a process cannot be determined simultaneously (Kaiser, 1994), and a balance must be reached between achieving an acceptable time and frequency resolution for each wavelet. The time resolution is determined by coefficient c_3 in equation 1. Fig. 7 illustrates how the amplitude is negligible for times greater than ±30 ms for wavelet 2 and the width of the time window decreases for wavelet 7. The time windows thus have durations within a range of physiologically relevant response times for muscle contraction. Prescribing the time resolution results in bandwidths of 21 and 53 Hz for wavelets 2 and 7, respectively.

The wavelets used for the cat EMG analysis form a set of partially overlapping frequency bands and are shown in Fig. 8. The degree of overlap between the wavelets is set by coefficient c_2 in equation 1 and was chosen to result in the sum across all wavelets showing a flat response in the frequency domain, as shown by the red line in Fig. 8. For each analysis, a set of wavelets was built that had centre frequencies as calculated by equation 1, and these centre frequencies occur where the wavelets have maximum amplitude in the frequency domain (Fig. 8).

Wavelets are used by convoluting them with the myoelectric signal. Convolutions are equivalent to passing the myoelectric

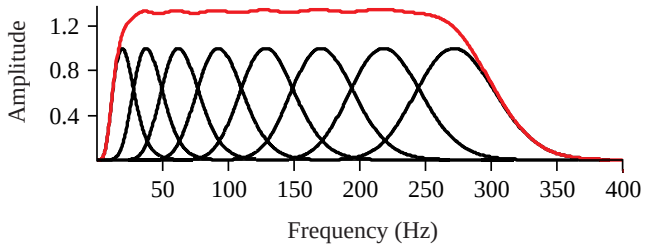

Fig. 8. Wavelets for $1 \leqslant k \leqslant 8$ (black) and the sum of those wavelets (red) in the frequency domain. k, wavelet number.

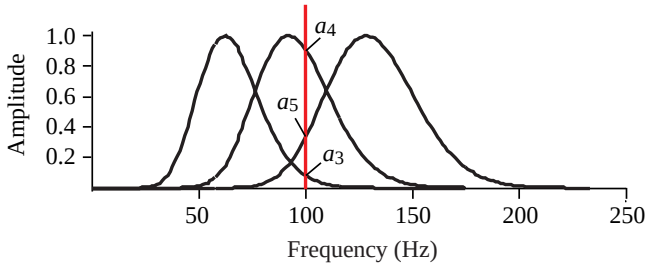

Fig. 9. Wavelets for k=3, 4 and 5 shown in the frequency domain. The vertical line at a frequency of 100 Hz (red) illustrates how one frequency within a signal can be partitioned into a_3, a_4 and a_5 amplitude components in three adjacent wavelet domains. k, wavelet number.

signal through a band-pass filter with the same frequency characteristics as the wavelet. Because of the overlap of the wavelet frequencies, a given frequency could be represented by its components in up to three adjacent wavelet domains (Fig. 9). The example in Fig. 9 shows how the amplitude A of the 100 Hz frequency component is a_3, a_4 and a_5 in the wavelet

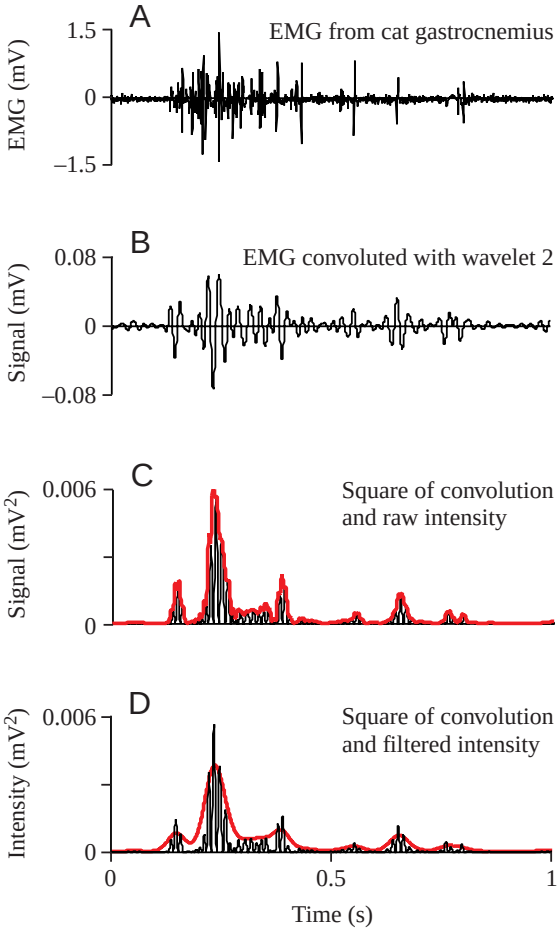

Fig. 10. Calculation of the myoelectric intensity at wavelet domain 2. The raw EMG signal (A) is convoluted with wavelet 2 (B) and squared (C). The intensity (red line) is calculated from the slope and magnitude of the square of the convolution (C) and then filtered (D).

domains 3, 4 and 5, respectively. The power of the myoelectric signal is the square of the amplitude. However:

$$A^2 = (a_3 + a_4 + a_5)^2 \neq a_3^2 + a_4^2 + a_5^2 . \quad \text{(A1)}$$

Instead:

$$A^2 = a_3^2 + a_4^2 + a_5^2 + 2d , \quad \text{(A2)}$$

where the element $2d$ represents the cross products between the wavelet components:

$$d = a_3a_4 + a_3a_5 + a_4a_5 . \quad \text{(A3)}$$

The wavelet amplitudes were scaled at each frequency so that the sum of the wavelet components at that frequency included the cross-terms and, thus, would yield the true power of the signal (von Tscharner, 2000).

The process for obtaining the myoelectric intensity from a raw EMG signal at one wavelet domain is illustrated in Fig. 10. The EMG signal is initially convoluted with the wavelet. The power within the signal at that wavelet domain is calculated at each time point from the magnitude and first time-derivative of the square of the convoluted signal. This power is termed the intensity in this wavelet analysis and forms an energy envelope around the (square) of the signal contained within this wavelet domain (von Tscharner, 2000). These computed intensities contain small oscillations due to the finite sampling rate of the EMG. These oscillations are of shorter duration than the time resolution of the wavelet and so contain no relevant information. A Gauss filter is therefore finally used to filter out this noise.

The myoelectric intensities computed using this wavelet analysis represent the power within the EMG for any given time and frequency band. EMG power spectra, which are traditionally used to assess EMG frequency content, are calculated from the square of the Fourier-transformed EMG signal (Lindström et al., 1970) and are thus also a measure of the power within the signal. The mean frequency f_m calculated in this study is comparable with the mean power frequency used to measure EMG contractions (e.g. Viitasalo and Komi, 1977); however, it can be calculated for each time point within the data. In contrast, root-mean-square analysis of EMG signal computes the amplitude, and not the power, of the signal as a function of time. The square of such a root-mean-square value is comparable with half the intensity obtained from this wavelet analysis.

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