Reliability of EMG determinism to detect changes in motor unit synchrony and coherence during submaximal contraction

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1. Introduction

With stronger muscle contraction more motoneurones become active and those which are already active fire at faster rates. The degree of coupling between motoneurone discharges is liable to influence the strength and fluctuation of the force output (Elble and Randall, 1976; Halliday et al., 1999; McAuley and Marsden, 2000; Christakos et al., 2006). Coordination between motoneurone discharges involves the so-called common drive which underlies the slow co-variation of the motoneurone firing frequencies within a time scale of 1–2 s (De Luca et al., 1982), as well as the synchronizing processes which are responsible for the coupling between motoneurone discharges above chance level within a much shorter time scale of ms to few tens of ms (Kirkwood and Sears, 1991). Motoneurone synchronous activity is attributed to common inputs that innervate conjointly a group of motoneurones and/or to independent inputs that are synchronized pre-synthetically (Kirkwood and Sears, 1991). In the case of uncorrelated common input firing, synchronous firings occur randomly with no specific rhythm. In the case of common inputs synchronized pre-synthetically and/or independent inputs driven by oscillatory sources, synchronous firings occur rhythmically in a correlated way. Coupling between discharges of human motor units is explored in the time domain by computing cross-correlation histograms in which firings occurring in synchrony periodically or not are grouped into central peaks (Farmer et al., 1997). The various types of periodicity generated by co-modulating processes and/or by oscillatory sources of synchronizing inputs are detected in the frequency domain by coherence analysis of motor unit discharges (Farmer et al., 1993). In view of the challenge of reliably discriminating single motor unit action potentials as more motoneurones become active, extracting an index of motor unit synchronous activity from surface EMG signals might be a good alternative to investigate the alteration of motor unit synchrony with stronger contraction. The non-linear method of recurrence quantitative analysis (RQA) has been recently introduced to quantify the amount of recurrence patterns in EMG activity using an index named determinism (Webber and Zbilut, 1994; Farina et al., 2002; Fattorini et al., 2005).

Following this track, by combining RQA applied to EMG activity and cross-correlation analysis of single motor unit discharges, Del Santo et al. (2006, 2007) recently reported the existence of a tight co-variation between EMG DET and an increase in motor unit syn-
chronous activity induced pharmacologically. These results were taken as a major argument in favour of the use of RQA to evaluate changes in motor unit synchronization. The reliability of RQA as a tool for assessing single motor unit synchronization has been recently challenged, however, in a study in which similar EMG DET values were observed in two muscles showing a striking difference in motor unit synchrony (Dideriksen et al., 2009). The purpose of the present work was to further investigate this controversial issue.

Upon testing the capability to discharge the wrist extensor motor unit pairs at different contraction levels in human subjects, we have recently shown that the occurrence of motor unit synchronous firings above-chance level was enhanced with moderate increases in contraction strength (Schmied and Descarreaux, 2010). Using this data obtained in the wrist extensor muscles originally tested by Del Santo et al. (2006), our first objective was to determine if EMG DET was enhanced in association with the increase in motor unit synchrony established by cross-correlation analyses with stronger muscle contraction (Schmied and Descarreaux, 2010).

The occurrence of recurrent patterns in EMG time series is likely to be promoted by rhythmic motor unit synchronization. Accordingly, changes in EMG DET have been observed in relation to fatigue, strength-training or Parkinsonian tremor and interpreted as reflecting an increase in the oscillatory synchronous activity of motor units (Filligoi and Felici, 1999; Ikegawa et al., 2000; Felici et al., 2001; Liu et al., 2004; Fattorini et al., 2005). Our second objective was therefore to determine if there were any relationship between EMG DET and the changes in the oscillatory components of motor unit synchronization assessed by coherence analyses at various levels of submaximal muscle contraction.

2. Materials and methods

The experimental procedure was approved by the Ethics Committee of University of Marseilles–II (CCPRPB protocol 03005). Experiments were performed on 8 healthy human subjects (males, aged 23–33 years, right-handed) who signed an informed consent form prior to the experimental procedures, as required by the Declaration of Helsinki.

2.1. Experimental procedure

The protocol has been described in detail elsewhere (Schmied and Descarreaux, 2010). Briefly, subjects were asked to contract their wrist extensor muscles by pushing the back of their hand against a force transducer device (FT03, Grass Telefactor, W. Warwick, RI, USA). The force signal was displayed on an oscilloscope screen facing the subjects who were required to maintain the force trace on a target, without feedback of motor unit activity. The target was set at a level high enough to record the activity of two steadily discharging motor units over 1.5–3 min. Each motor unit pair was tested at various force levels during at least 2 (up to 7) recording periods, separated by 2-min resting periods. The force target was set in random order between the lowest and the highest levels of force, allowing reliable discrimination of both motor units. Transducer force output, calibrated in Newton, was averaged across each recording period.

2.2. Data recording

Surface EMG activity on the right extensor carpi radialis (ECR) muscles was recorded by two electrodes placed 2 cm apart over the belly of these muscles. The discharge patterns of two motor units were recorded concurrently by two tungsten microelectrodes (Frederick Haer & Co., Bowdoinham, ME, USA) inserted into the ECR muscles. EMG and motor unit activities were amplified and filtered (band-pass: 30 Hz to 1 kHz, 300–3000 Hz, respectively). Force, EMG and single motor unit signals were digitized (sampling rates: 1 kHz, 5 kHz and 30 kHz, respectively) and stored on a computer by means of a 1401 plus acquisition device driven by Spike 2-5 software (Cambridge Electronic Design, Cambridge, UK). The root mean square (RMS) values of ECR EMG activity were averaged across each of the recording periods. At the end of the experiment the subjects were asked to produce 3 bouts of maximal isometric contraction of the wrist extensor muscles, under strong verbal encouragements. The highest level of ECR EMG activity assessed in these bouts was subsequently used to normalize EMG as a percentage of maximal voluntary muscle contraction (% MVC).

2.3. Single motor unit firing pattern analysis

Single motor unit action potentials were discriminated off-line and their distribution was analyzed using the Spike 2-5 software. The firing behavior of each motor unit was plotted on an instantaneous frequency curve, as illustrated for the two motor units (MU1 and MU2) in Fig. 1A. The presence of abnormally low or high instantaneous frequency values was carefully monitored to ensure that no spike had been missed or erroneously included in the discrimination process. Motor unit macro-potentials were extracted by spike-triggered averaging of ECR EMG activity, using discriminated motor unit action potentials as triggers across each of the recording periods. Reproducibility of the motor unit macro-potentials, plotted on the right of the instantaneous frequency curves in Fig. 1A, was examined to ensure that the same motor units were being tested throughout the various levels of force.

The motor unit firing patterns were characterized on the basis of inter-spike interval (ISI) durations, excluding those longer than 300 ms (about 4–5 times the mean), taken to result from pauses in motor unit tonic activity. Instantaneous firing rates associated with each ISI were calculated and averaged across each recording for each motor unit (FRmean = mean (1/ISI)). The firing pattern of the motor unit pairs at each contraction level was thereafter described in terms of the instantaneous firing rates geometric mean obtained for both motor units (FRgeo = sqrt (FRmean 1 × FRmean 2)).

2.4. Analysis of motor unit synchronous activity

Motor unit synchronous activity was analyzed by cross-correlating the 2 spike trains in each of the recording periods, as shown in Fig. 1B and C. Cross-correlation histograms yielded the distribution of impulses produced by the analyzed motor units in 1-ms bins, 100 ms before and after impulses generated by the reference (trigger) motor unit. Synchronous firings formed a central peak in the cross-correlograms marked by a white bar located around zero in the examples illustrated (Fig. 1B and C). The synchronization peak was delimited on the basis of rising inflection occurring in the central region of the cumulative sum (Ellaway, 1978) computed with respect to mean count in a baseline located from −100 to −20 ms in the cross-correlograms. In the absence of clear-cut inflection around time 0, the strength of synchronization was arbitrarily calculated over a 20-ms window centered on trigger time.

Synchronization strength was evaluated in terms of both synchronous impulse probability and synchronous impulse frequency. The synchronous impulse probability index is given by peak counts above the baseline mean divided by the number of trigger spikes. The synchronous impulse frequency index is derived from the peak count above the baseline mean divided by the duration of the recording period. Statistical significance of the synchronization peak was evaluated at P < 0.05 on the basis of the z-score (Garnett et al., 1976).
2.5. Analysis of motor unit coherence

Coherence was calculated between discharges of the motor unit pairs according to the procedure described by Rosenberg et al. (1989), using the freeware toolbox developed in MatLab environment (MatWorks, Natick, MA, USA) by D.M. Halliday (University of York, York, UK). For each motor unit pair, coherence spectra were computed from 0 to 100 Hz at each of the contraction levels tested.
across non-overlapping spike train segments of 1.024 s with a frequency resolution of 0.98 Hz (Fig. 1D and E). In each spectrum, a 95% confidence level was calculated under the assumption that the 2 spike trains were independent (Rosenberg et al., 1989). Any coherence value which reached this level was considered to reflect a significant correlation between the corresponding frequency components. With each pair and at each contraction level, coherence values, whether significant or not, were pooled together and averaged within four frequency regions (0–5 Hz, 6–13 Hz, 13–25 Hz and 25–40 Hz) in keeping with previous studies (Davey et al., 1993; Kakuda et al., 1999; Marsden et al., 1999; Kim et al., 2001; Kilner et al., 2002; Myers et al., 2004; Johnston et al., 2005; Christou et al., 2007). Each band included the lower frequency limit and excluded the upper one.

2.6. Recurrence quantification analysis of EMG activity

The RQA procedure was applied to un-rectified EMG signals down-sampled at 1000 Hz, with the freeware toolbox (Cross Recurrence Plot tool, GNU General Public License) developed in MatLab environment by Marwan et al. (2007). As shown in Fig. 1F and G, RQA was performed on successive non-overlapping EMG steps of 1 s across each of the contraction levels tested for a given motor unit pair. The duration of 1 s was chosen in keeping with the 1.024 s segments used in the coherence analysis. EMG time series were unfolded without normalization in a multi-dimensional space according to the method of delayed coordinate embedding (Nieminen and Takala, 1996). The embedding parameter which is related to the number of variables underlying the system states was set at a value of 10 on the basis of results obtained by applying the method of false nearest neighbors (Kennel et al., 1992) to each of the EMG time series collected. The delay parameter was chosen in such a way as to minimize the interaction between neighboring values in the time series. A value of 3 was selected on the basis of data obtained by applying the method of mutual information analysis (Fraser and Swinney, 1986) to each of the EMG time series collected. With an embedding dimension of 10 and a delay of 3, every single EMG value was associated with a vector, including 10 of the following values selected every 3 ms in the time series.

The repeated occurrence of similar patterns in the EMG time series was detected by computing a recurrence plot (Eckmann et al., 1987) based on Euclidean differences calculated between the vectors associated with successive EMG values. Whenever the difference between vectors was less than a threshold value set as a small fraction of the maximum difference observed (Zbilut and Webber, 1992), the EMG time series values were taken as being similar, i.e., recurrent. The amount of recurrence was quantified by calculating the density of recurrent points in the recurrent plot expressed in terms of recurrence rate (Webber and Zbilut, 1994). Based on preliminary analyses with thresholds of 10, 15 and 20%, the threshold was set at 20% of the maximum absolute distance obtained in EMG phase space, to keep recurrence rate values within a range of 1–10%. The recurrence pattern present in the recurrent plot was quantified by means of the DET index (Webber and Zbilut, 1994; Marwan et al., 2007). The RQA indices (recurrence rate and DET) obtained in successive 1-s steps across each recording period are plotted in superimposition in Fig. 1F and G. The recurrence rate and DET values obtained in successive steps were averaged for each contraction level.

2.7. Statistics

Linear regression analyses were performed to evaluate the coefficients of determination between variables (mean EMG activity and force level, the motor unit firing rate geometric mean, motor unit synchronization indices, motor unit coherence, and EMG DET). The trends observed were further investigated by selecting, for each motor unit pair tested, the recording periods at minimum and maximum contraction strength in terms of EMG activity, force level and motor unit firing rate. The strength of motor unit synchronization and coherence and the amount of DET obtained at maximum and minimum contraction strength were compared by the Wilcoxon signed-rank test across the population of motor unit pairs tested.

Analyses were conducted with JMP 8 software (SAS Institute Inc., Cary, NC, USA). In all tests, the level of significance was set at \( P = 0.05 \). Unless explicitly stated, pooled data are expressed in terms of medians (inter-quartile deviations).

3. Results

Discharges of 36 pairs of single motor units were tested together with EMG DET at various force levels in a total of 14 experiments. Three pairs were excluded because of changes in their motor unit macro-potentials across different recording periods. Three other pairs were ruled out because of the lack of a consistent relationship between their firing rates and ECR EMG activity.

Among the 30 pairs selected, 7 were tested at 2 force levels only, whereas 15 were tested for 4–7 recording periods. A total of 118 recording sequences were analyzed with duration ranging from 90 to 254 s (median: 158 (47) s), EMG activity ranging from 1 to 16% of the MVC (median: 5.11 (3.2) %), and force ranging from 0.07 to 7.2 N (median: 2.2 (2.1) N). Single motor unit firing frequencies ranged from 7.5 to 20 imp./s (median: 12.3 (3.5) imp./s). Only 16 of the 118 recording sequences included one motor unit firing at less than 9 imp./s.

3.1. Motor unit synchrony vs. EMG DET

Central peaks were detected in all but 2 of the 118 cross-correlograms computed with the discharges of the 30 motor unit pairs tested. In these 2 cases, non-significant synchrony was observed at the lowest force level tested. Peak onsets and offsets were located – 4 (4) ms before and 3 (3) ms after trigger time. The median duration of the peaks was 7.5 (4) ms, indicating short-acting coupling between motor unit discharges. Synchronous impulse probability and frequency had median values of 0.033 (0.017) imp./trigger and 0.38 (0.20) imp./s, respectively. Upon pooling together the 118 recording sequences tested, synchronous impulse probability and frequency were found to increase with force \( r = 0.34, P = 0.0002 \) and \( r = 0.39, P < 0.0001 \), respectively, with no consistent relationship with EMG level \( r = -0.14, P = 0.14 \) and \( r = 0.15, P = 0.09 \), respectively.

Upon examining the behavior of each motor unit pair with increasing contraction strength, the changes in motor unit synchrony observed with greater EMG and force level became fully congruent. Increases in EMG activity were associated with greater synchronous impulse probability and frequency in 26 and 27 motor unit pairs (Fig. 2A and B, respectively), whereas increases in force level were associated with greater synchronous impulse probability and frequency in 25 and 26 motor unit pairs (Fig. 2D and E, respectively). The results of paired comparisons, reported in Table 1, confirmed the high significance of the increases seen with both motor unit synchrony indices in relation to larger EMG activity, stronger force output and faster motor unit discharge rate.

Across the 118 sequences of ECR EMG activity recordings tested, RQA yielded a median recurrence rate of 2.8% (1.7). The DET index ranged from 10.8 to 67.5% (median: 23% (13.3)) and showed a slight tendency to decrease with increasing EMG \( r = -0.22, P = 0.02 \). However, DET did not vary at all with force level \( r = -0.02, P = 0.8 \). Contrasting with the congruent increases observed with motor
unit synchrony (Fig. 2A, B, D, and E), the lack of consistent linkage between EMG DET and contraction strength is demonstrated in Fig. 2C and F. Among the 30 motor unit pairs tested, 16 showed a decrease in DET with greater EMG activity and force (Fig. 2C and F). The paired comparisons (Table 1) failed to detect any significant change in EMG DET related to contraction strength.

Upon pooling together the 118 recording sequences tested, only 6% and 5% of the variation in EMG DET could be explained by variation in motor unit synchronous impulse probability and frequency ($r = 0.24, P = 0.007, r = 0.22, P = 0.008$, respectively).

This rather weak link was completely lost upon examining, for each motor unit pair, the EMG DET values associated to the smallest vs. the largest synchronous impulse probability (Fig. 3A) and the smallest vs. the largest synchronous impulse frequency (Fig. 3B), respectively. In both cases, EMG DET was found to either decrease (13 pairs) or increase (17 pairs) with greater motor unit synchrony. The absence of congruent co-variation between DET and motor unit synchrony was confirmed by the lack of significant change in EMG DET in paired comparisons between recordings showing the smallest vs. the largest synchronous impulse probability and frequency for each motor unit pairs ($P = 0.5$ and $P = 0.7$, respectively).

### 3.2. Motor unit coherence vs. EMG DET

As shown in Fig. 4, the moderate increases in contraction strength tested were found to affect motor unit coherence spectra. The percentage of significant coherence and the coherence median values assessed upon pooling together data obtained at lower and higher force levels (continuous and dotted lines, respectively) are plotted in Fig. 4A and D, respectively. From 0 to 5 Hz, the percentage

### Table 1

Comparison of the EMG determinism, motor unit instantaneous frequency (FRgeo mean), synchronous impulse probability and frequency (SIP, SIF), and coherence values assessed at the minimum vs. maximum levels of EMG, force and firing rate tested with each motor unit pair ($n = 30$).

<table>
<thead>
<tr>
<th></th>
<th>Min vs. max EMG activity</th>
<th>Min vs. max force</th>
<th>Min vs. max firing rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Wilcoxon test</td>
</tr>
<tr>
<td>Extension force (N)</td>
<td>1.1 (1.4)</td>
<td>3.7 (2.3)</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>ECR (%MVC)</td>
<td>4.1 (3.2)</td>
<td>6.3 (5.4)</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>EMG determinism (%)</td>
<td>26 (15)</td>
<td>24 (12)</td>
<td>NS</td>
</tr>
<tr>
<td>FRgeo mean (imp./s)</td>
<td>11.3 (1.3)</td>
<td>13.0 (2.5)</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>SIP (imp./trigger)</td>
<td>0.030 (0.015)</td>
<td>0.040 (0.01)</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>SIF (imp./s)</td>
<td>0.31 (0.19)</td>
<td>0.47 (0.25)</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>Coherence 0–4Hz</td>
<td>0.09 (0.08)</td>
<td>0.06 (0.07)</td>
<td>$P = 0.0002$</td>
</tr>
<tr>
<td>Coherence 6–12Hz</td>
<td>0.01 (0.01)</td>
<td>0.02 (0.02)</td>
<td>$P = 0.0008$</td>
</tr>
<tr>
<td>Coherence 12–25Hz</td>
<td>0.01 (0.003)</td>
<td>0.009 (0.006)</td>
<td>NS</td>
</tr>
<tr>
<td>Coherence 25–40Hz</td>
<td>0.009 (0.004)</td>
<td>0.009 (0.003)</td>
<td>NS</td>
</tr>
</tbody>
</table>
of significance and the strength of coherence were smaller at high than at low force level, whereas, conversely, between 6 and 12 Hz, coherence was greater in both incidence and strength at higher force level (Fig. 4A and D, respectively). Above 12 Hz, whatever the force level, the percentage of significant coherence and the amount of coherence dropped quite uniformly with no evidence of a peak delimiting a specific frequency band in the spectrum up to 100 Hz.

Upon grouping coherence values into 0–5 Hz, 6–12 Hz, 12–25 Hz and 25–40 Hz bands, the location of peak coherence values observed in each band were compared at lower vs. higher force levels. A significant shift was observed in the 0–5 Hz band (1.0 (0.9) vs. 1.9 (1.9) Hz, respectively, \( P < 0.0001 \)). In contrast, in the 6–12, 12–25, and 25–40 Hz bands, coherence peak values occurred at similar frequencies at lower vs. higher force levels (7.8 (3.8) vs. 8.8 (2.8) Hz, 18.5 (4.9) vs. 17.6 (4.5) Hz, and 31.2 (10.8) vs. 33.2 (7.8) Hz, respectively). Very similar data were obtained upon comparing the location of peak coherence values in each band between weaker and stronger EMG activity. In order to clarify a possible influence of motor unit firing rates on coherence in the 6–12 Hz band, a subset of 25 pairs in which both motor units fired above than 9 imp./s

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**Fig. 3.** EMG determinism in relation to synchrony level. (A and B): EMG determinism associated with the weakest vs. largest value of synchronous impulse probability (A) and frequency (B) observed with each motor unit pair. With greater synchrony, DET shows either an increase or a decrease with no predominant pattern.

**Fig. 4.** Change in motor unit coherence in relation to contraction strength. (A and D): Averages of the percentage of significant coherence (A) and coherence median values (D) observed across all motor unit pairs at the lowest (median 1.1 (1.4) N, grey) vs. the highest force levels tested with each pair (median 4.2 (2.5) N, black) from 0 to 100 Hz (frequency resolution 0.98 Hz, logarithmic scale). Below 5 Hz, both the incidence and the amount of coherence decreased at higher force level. Between 6 and 12 Hz, both the incidence and the amount of coherence increased at higher force levels. Above 13 Hz, the incidence and amount of coherence remained similar at both force levels. (B, C, E, and F): Mean coherence from 0 to 5 Hz (B and C) and from 6 to 12 Hz (E and F) assessed at weaker vs. larger EMG activity (B and E), and lower vs. higher force levels (C and F). In each plot, the lines connect the values obtained with each motor unit pair in both conditions. Coherence in the 0–5 Hz band shows a predominance of decrease with greater EMG (B) and stronger force (C), whereas coherence in the 6–12 Hz band displays a predominance of increase.
was selected. Paired comparisons performed with this subset confirmed that coherence in the 6–12 Hz band peaked circa 8 Hz with no change between the lower vs. higher force and EMG levels (7.8 (3.9) Hz vs. 7.8 (1.9) Hz, \(P=0.7\) and 7.8 (3.9) Hz vs. 8.8 (2.9) Hz, \(P=0.5\), respectively). The stability of the circa 8 Hz peak coherence in the 6–12 Hz in contrast with the highly significant increase in motor unit firing rate (\(P<0.0001\)) observed in both comparisons (11.8 (3.5) vs. 13.3 (3.6) imp./s, and 11.5 (2.5) vs. 13.2 (3.7) imp./s, respectively).

The changes affecting motor unit coherence in 0–5 Hz and 6–12 Hz bands are further illustrated for each motor unit pair, by plotting the strength of coherence in these two bands in relation to the smallest vs. greatest EMG activity tested (Fig. 4B and C, respectively) and the lowest vs. strongest force output (Fig. 4C and F, respectively). Increases in EMG activity and force output were both associated with decreased coherence in the 0–5 Hz band affecting 24 pairs (Fig. 4B and C, respectively). Conversely, greater EMG activity and stronger force output were associated with increased coherence in the 6–12 Hz band in 21 motor unit pairs (Fig. 4E and F, respectively). Paired comparisons (Table 1) confirmed the statistical significance of these changes.

Upon pooling the 118 recording sequences together, no correlation was found between EMG DET and coherence in the 0–5 Hz and 12–25 Hz bands (\(r=0.13, P=0.2\), \(r=0.11, P=0.2\), respectively) whereas up to 5% and 8% of variation in EMG DET could be explained by variation in motor unit coherence in the 6–12 (\(r=0.19, P=0.04\)) and 25–40 (\(r=0.23, P=0.01\)) Hz bands, respectively.

The lack of consistent co-variation between EMG DET and motor unit coherence in the 0–5 Hz and 12–25 Hz bands is confirmed in Fig. 5 by plotting the DET values associated with the smallest vs. the largest coherence values in these 2 bands (Fig. 5A and C). The weak link observed between EMG DET and motor unit coherence in the 6–12 and 25–40 Hz bands across all recordings disappeared completely upon examining the co-variation of both parameters which each motor unit pair (Fig. 5B and D). EMG DET was found to either decrease (12 and 16 pairs) or increase (18 and 14 pairs) with greater motor unit coherence in the 6–12 Hz and 25–40 Hz bands (Fig. 5B and D, respectively). Accordingly, the changes in DET associated with greater coherence in the 0–5 Hz, 6–12 Hz, 12–25 Hz and 25–40 Hz bands did not reach significance in paired comparisons between DET values observed at lowest vs. highest coherence values in each band with each motor unit pair (\(P=0.7, P=0.7, P=0.8,\) and \(P=0.9\), respectively).

4. Discussion

4.1. Relationship between motor unit synchrony and EMG DET

The existence of a co-variation between EMG DET and motor unit synchronous activity has been reported in various muscles (Fattorini et al., 2005; Del Santo et al., 2006, 2007), and in a simulation study (Farina et al., 2002). As compared to the coefficient of determination of 70% reported in the ECR muscles by Del Santo et al. (2006), our study yielded variations in EMG DET that were only poorly explained by variation in motor unit synchronous impulse probability and frequency across the population of motor unit pairs tested (5 and 6%, respectively). In a recent study, no correlation at all was reported to occur between EMG DET and motor unit synchronous impulse frequency in two muscles showing a marked difference in synchronization strength (Dideriksen et al., 2009). It must be noted that in our study and that of Dideriksen et al. (2009), EMG DET was related to different amounts of motor unit synchrony observed in natural conditions whereas in Del Santo et al. (2006, 2007), motor unit synchrony was pharmacologically enhanced by increasing recurrent inhibition activity with \(l\)-acetylcarnitine injection. Another major methodological difference is that, in the two studies published by Del Santo et al. (2006, 2007), EMG DET values obtained across single 5-s periods were related to synchrony.
indices obtained across whole recordings lasting 2–3 min. As illustrated presently in Fig. 1F and G, EMG DET values may, however, vary greatly from one 1-s step to another. Using 5-s steps did not average this variability out (unpublished data). Taking into account this variability, in our study and that of Dideriksen et al. (2009), an average of DET values obtained in successive steps across whole recordings was used to investigate the relationship between EMG DET and synchrony indices obtained across the same period of time.

Muscle fiber conduction velocity has been shown to affect RQA with a negative effect on EMG DET (Farina et al., 2002). This effect was ruled out in the studies by Del Santo et al. (2006, 2007), showing that muscle fiber conduction velocity was unaffected by l-acetyl carnitine injection. An enhancement of conduction velocity has been reported to occur with contraction increasing up 30% of MVC (Hedayatpour et al., 2007). Within a range not exceeding 10% of MVC, however, the slight enhancement observed failed to reach the level of significance (Arendt-Nielsen et al., 1989; Caffier et al., 1993; Hedayatpour et al., 2007). Extending this observation to our study in which most of the recordings were performed within a contraction range of 1–10% of MVC (Fig. 2), it seems very unlikely that changes in conduction velocity might have affected EMG DET sufficiently to explain the poor relationship observed presently between EMG DET and motor unit synchrony.

Most importantly, EMG DET did not change consistently in the repeated comparison performed between the lowest and the highest contraction levels achieved with each motor unit pair, despite the clear increase observed in both the probability and the frequency of the motor unit synchronous firings above chance level. This clearly indicates that EMG DET is not a reliable index to evaluate the occurrence of change in motor unit synchrony in the time domain during sub-maximal contraction.

4.2. Relationship between motor unit coherence and EMG DET

Single motor unit coherence analysis identifies motoneurone synchronous discharges occurring recurrently with various forms of periodicity, whereas RQA identifies the presence of recurrent patterns in the EMG interference signal liable to result of motor unit synchronous oscillatory activity. The occurrence of recurrent patterns in EMG time series is likely to be promoted by rhythmic motor unit synchrony. Accordingly, changes in EMG DET have been observed in various muscles in relation to fatigue, strength-training or Parkinsonian tremor and interpreted as reflecting increases in the periodicity of EMG signals attributed to greater oscillatory motor unit synchrony (Filligoi and Felici, 1999; Ikegawa et al., 2000; Felici et al., 2001; Liu et al., 2004; Fattorini et al., 2005). Our study is the first to investigate the relationship between the two levels of analysis represented by EMG RQA and single motor unit coherence analysis. In order to assess the ability of RQA to detect changes in coherence, we have, firstly, compared motor unit coherence at various levels of contraction strength, and, secondly, determined if EMG DET was affected in the same way. The increases in EMG activity, force output and motor unit firing frequency tested in our study were associated with a shift of the frequency of the peak value in the 0–5 Hz band from circa 1 to 2 Hz. Moreover, mean coherence in the 0–5 Hz band was reduced by about 45% (Table 1) in keeping with data reported in the first dorsal interosseous muscle with faster motor unit firing rates (Christou et al., 2007). The complete lack of correlation observed between EMG DET and coherence in the 0–5 Hz band indicated that, in the present conditions, RQA did not detect the powerful oscillatory modulation of motor unit activity revealed by the large amount of coherence circa 1–2 Hz. This might be related to the fact that RQA was performed across 1-s steps, offering a too narrow window for detecting reliably recurrent patterns occurring with a periodicity of similar duration. Analyses using steps of longer duration are in progress to further investigate this issue.

Contrasting with the decrease observed below 5 Hz, coherence in the 6–12 Hz band was significantly enhanced with greater EMG activity, stronger force output and faster motor unit firing rates (Table 1). Moreover, more than 30% of variation in coherence in the 6–12 Hz band could be explained by changes in the motor unit firing rate, in accordance with data obtained in the first dorsal interosseous muscle (Christou et al., 2007). In our study, the peak values in the 6–12 Hz band remained located circa 8 Hz whatever the contraction strength, and the motor unit firing frequencies which increased from about 11 to 13 Hz between the lowest and highest levels of EMG activity and force output tested. This indicates that coherence in the 6–12 Hz band is not a mere by-product of motor unit firing frequency, but rather reveals the action of a specific oscillatory drive controlling motoneurone activity with an efficiency related to contraction strength. The presence of this oscillatory drive was, however, not much apparent in EMG RQA data. Even 5% of variation in EMG DET could be explained by variation in motor unit coherence in the 6–12 Hz band. This rather weak link may partly explain why the enhancement observed in the 6–12 Hz band with stronger contraction had apparently no impact on the EMG DET index (Table 1).

In most single motor unit coherence studies, attention has been focused on a region of the spectrum varying from 10 Hz to 40 Hz (Davey et al., 1993; Farmer et al., 1993; Kim et al., 2001) with a particular emphasis on the 15–40 Hz band taken to reflect ongoing cortico-spinal activity (Farmer et al., 1993, 1997; Conway et al., 1995; Gross et al., 2002) without excluding the contribution of movement-related sensory inputs (Riddle and Baker, 2005). Upon pooling together all recordings, 8% of the variation in DET could be explained by variation in coherence in the 25–40 Hz band, corresponding to the strongest link presently observed between EMG DET and motor unit coherence. The changes in DET attributed to the changes in synchrony induced pharmacologically in Del Santo et al. (2006) study, could not, however be explained by changes in the 25–40 Hz coherence found to be unaffected in these experimental conditions (Mattei et al., 2003). In the present study, performed in the same muscle, the rather low amount of coherence observed in the 25–40 Hz remained unaffected with greater EMG activity, stronger force output and faster firing rates (Table 1). The lack of changes in DET was consistent with the lack of change in coherence in this band. In contrast with our study, it has been reported that single motor unit coherence between 15 and 30 Hz could be specifically altered in a task-dependent way without any change in the rest of the spectrum, including the 6–12 Hz (Kilner et al., 2002). Applying RQA in these conditions might help to further explore the preferential link which may exist between EMG DET and single motor unit coherence in the 25–40 Hz.

Previous studies applying RQA at more than 20% MVC, high DET values were commonly associated with the appearance of periodicity in EMG signals attributed to an increase in motoneurone synchronous activity (Filligoi and Felici, 1999; Felici et al., 2001; Fattorini et al., 2005). In the present study performed at lower contraction level using similar RQA parameters, DET was not found to co-vary consistently with the amount of synchronous impulses measured by cross-correlation analysis of single motor unit discharges, nor with the amount of oscillatory synchronous activity measured by coherence analysis. All in all, complementary to the report by Dideriksen et al. (2009), the present data speak strongly against the use of RQA to detect changes in the synchronization of motor unit discharges in both the time and frequency domains at contraction levels lower than 20% of MVC.

This does not preclude, however, the possibility that other RQA parameters, which remain to be determined, might become more
effective in extracting information about EMG recurrent patterns reflecting oscillatory synchronous activity of motoneurones.

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