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Motor unit control and force fluctuation during fatigue

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ABSTRACT

During isometric contractions, the fluctuation of the force output of muscles increases as the muscle fatigues and the contraction is sustained to exhaustion. We analyzed motor unit firing data from the Vastus Lateralis muscle to investigate which motor unit control parameters were associated with the increased force fluctuation. Subjects performed a sequence of isometric constant-force contractions sustained at 20% maximal force, each spaced by a 6 s rest period. The contractions were performed until the mean value of the force output could not be maintained at the desired level. Intramuscular EMG signals were detected with a quadrifilar fine-wire sensor. The EMG signals were decomposed to identify all the firings of several motor units by using an Artificial Intelligence based set of algorithms. We were able to follow the behavior of the same motor units as the endurance time progressed. The force output of the muscle was filtered to remove contributions from the tracking task.

The coefficient of variation of the force was found to increase with endurance time ($p<0.001$, $R^2=0.51$). We calculated the coefficient of variation of the firing rates, the synchronization of pairs of motor unit firings, the cross-correlation value of the firing rates of pairs of motor units, the cross-correlation of the firing rates of motor units and the force, and the number of motor units recruited during the contractions. Of these parameters, only the cross-correlation of the firing rates ($p<0.01$, $R^2=0.10$) and the number of recruited motor units ($p=0.042$, $R^2=0.22$) increased significantly with endurance time for grouped subjects. A significant increase ($p<0.001$, $R^2=0.16$) in the cross-correlation of the firing rates and force was also observed. It is suggested that the increase in the cross-correlation of the firing rates is likely due to a decrease in the sensitivity of the proprioceptive feedback from the spindles.

INTRODUCTION

Contracting muscles do not produce a smooth or steady force. The cause of the force fluctuation has been a topic of some interest during the past 60 years (Halliday and Redfearn, 1956, among others). It has been further reported (Gottlieb and Lippold, 1983; Furness *et al*., 1977; among others) that these fluctuations increase both during and after sustained contractions as the muscle is fatigued.

When a muscle contracts, the central nervous system regulates muscle force production by varying two main motor unit parameters: the recruitment of new motor units and the modulation of firing rates of active motor units. The firing behavior of motor units can be assessed by parameters such as the firing rate, firing variability, synchronization of motor unit firings, and the common modulation of motor unit firings. The literature contains varying reports on the behavior, influence and causality of these parameters on the increasing force fluctuation during fatigue. For instance, De Luca and Forrest (1973) and Garland *et al.* (1994) reported a decrease in the firing rate of most motor units during a short-lasting fatiguing task. Adam and De Luca (2005) later found that this initial decrease was followed by an increase as the muscle continued to contract and progress towards exhaustion. After eccentric exercise the firing rate increases (Dartnall *et al.*, 2008).

There have been contrasting reports on the changes of firing rate variability with fatigue. Variability of the firing rate was found to increase after a fatiguing exercise by Garland *et al.* (1994) in the biceps brachii muscle and by Enoka *et al.* (1989) in the first dorsal interosseus muscle (FDI). In contrast, Macefield *et al.* (2000) observed no systematic change in firing rate variability of the extensor hallucis longus muscle when fatigued during a sustained maximum voluntary contraction (MVC). A causal relationship between the firing rate variability was highlighted in a simulation study by Moritz *et al.* (2005). However, contrasting reports have been published. Firing variability was regarded as a likely contributor to the increased force fluctuations observed in elderly subjects at low forces by Tracy *et al.* (2005) and Laidlaw *et al.* (2000), but another study of some of the same authors (Galganski *et al*., 1993) reported an increase in force variability but not in firing rate variability in elderly subjects. Additionally, Semmler and Nordstrom (1998) reported that increased force variability was not accompanied by a change in firing rate variability when comparing skill-trained and strength-trained subjects.

Controversial reports can also be found for synchronization and common modulation of motor unit firings. In a simulation study, Yao *et al.* (2000) found that synchronization had a substantial effect on the amplitude of force fluctuations, and the authors suggested that it may explain some of the experimentally observed increases in the amplitude of the surface EMG (sEMG) signal, such as those which occur during fatiguing contractions. Both synchronization and low-frequency coherence of motor unit firings were found to increase after eccentric exercise by Dartnall *et al.* (2008). In contrast, Semmler and Nordstrom (1998) reported no relation between either synchronization or common modulation of firings and force fluctuations when comparing skill-trained and strength-trained subjects. Synchronization did not contribute to the increased force fluctuations during low-force isometric contractions in elderly subjects in a study by Semmler *et al.* (2000). Similarly, Nordstrom *et al.* (1990) noted no change in the strength of synchronization in the Masseter muscle during a fatiguing contraction. Interestingly, Holtermann *et al.* (2008), using a novel sEMG method, noted an increase in both synchronization and force variability, but no causal dependency between these two parameters, during a fatiguing contraction. There can be many reasons for the discrepancies among the reported observations. Some differences may be due to the measurement of the force variability; others to the analysis of grouped motor units from different contractions and/or subjects.

In this study we were interested in investigating if modifications occurred in the neural control of motor units. In our protocol we requested the subjects to use visual feedback in order to follow a ramp trajectory up to 50% MVC and then maintain a force output constant at 20% MVC for approximately 50 s. This protocol requires the subjects to track the visually displayed force output about a mean value. This tracking process *per se* introduces a force-variability due to the innate ability of the subjects to modulate the force output on the basis of the processed visual cue. We removed this tracking fluctuation from the data and focused on the force variability caused by the intrinsic force production. In this study we investigated the behavior of the control properties of motor units during fatiguing contractions sustained to exhaustion and related the behavior to the increasing force fluctuation. Our approach enabled us to follow the firings of individual motor units throughout a sequence of sustained contractions. In this fashion we could document the alterations in the firing characteristics in the motor units and did not need to rely on observations made on the group behavior of different motor unit populations.

METHODS

The experiments performed to collect the data for this study have been previously reported by Adam and De Luca (2003, 2005). They are described here in brief; additional details may be obtained by referring to the previous papers.

***Subject*s --** Four healthy men reporting no known neurological disorder participated in the study. The mean ± SD for the age of the subjects was 21.25 ± 0.96 yr (range 20 - 22). An informed consent form approved by the Institutional Review Board at Boston University was administered to all subjects before participation in the study.

***Force measurement* --** Subjects were seated in a chair designed to restrain hip movement and immobilize their dominant leg at a knee angle of 60° flexion. Isometric knee extension force was measured via a load cell attached to lever arm and a pad positioned against the tibia 3 cm above the medial malleolus. Visual feedback of the knee extension force was displayed on a computer screen. The force signal was band-pass filtered from DC – 100 Hz and digitized at 2 kHz.

***EMG recording* --** Intramuscular EMG signals were recorded from the Vastus Lateralis (VL) muscle of the dominant leg by use of a quadrifilar fine wire sensor. The electrodes of the sensor were comprised of four 50 μm diameter nylon-coated Ni-Cr wires glued together and cut to expose only the cross section of the wires (De Luca and Adam, 1999). The sensor was inserted into the muscle via a 25 gauge disposable hypodermic needle, which was removed after the wires were inserted. Three combinations of pairs of wires were selected and differentially amplified to yield three separate intramuscular EMG channels. The signals were amplified, band-pass filtered (1 kHz – 10 kHz), sampled at 50 kHz, and stored on a PC for offline data analysis.

Protocol -- At the beginning of the experimental session, subjects performed three brief maximal knee extension contractions of approximately 3 s in duration. The greatest value of the three trials was chosen as the maximal voluntary contraction (MVC) force. The subjects were then asked to follow a series of force trajectories, which were displayed on a computer screen, by isometrically extending the knee joint. The tracking task was practiced a few times to ensure subjects were able to smoothly follow the trajectories. The subjects performed 7-10 contractions separated by at least 3 min of rest before proceeding to the fatigue protocol.

After the practice session, subjects proceeded to the fatigue protocol where they were asked to track repeated contractions, separated by 6 s of rest, until they could no longer maintain the target level (see Figure 1). Each contraction began with a ramp up to 50% MVC (at a rate of 10% MVC/s) and a brief hold phase; the target value was then decreased to 20% MVC and maintained at this level for 50 s. At the end of the cycle, the force level was decreased at the same rate as the initial ramp. Strong verbal encouragement was given when the force traces dipped below the 20% MVC target value by more than 1% MVC (5% of target value) and the fatigue sequence was terminated at the end of a contraction, when the dips in the force occurred at a rate of more than 2 per 10 s of constant target force.

Although the interval for analysis was the plateau region, that is, the 50 s where the force was held constant at 20% MVC, the ramp at the beginning of each cycle allowed us to observe changes in the recruitment threshold of each motor unit throughout the contraction series. The inclusion of the higher-force ramp was part of a force paradigm designed for other data collection requirements in previously published work. In this work, it proved useful for identifying the recurrence of specific motor units in separate contractions. For additional information refer to Adam and De Luca (2003).

Figure 1 near here

Data Analysis **--** Five contractions for each subject were analyzed: the first, the second, the middle, a contraction between the middle and the last, and the last contraction. A 30 s interval in the middle of the 20% MVC part of the contraction was chosen for all computations. This interval was chosen because it allowed analysis of the data in a region where many motor units were firing continuously and new ones were recruited.

The force data were analyzed after detrending the signals with a high-pass filter having a corner frequency at 0.75 Hz. The detrending was necessary to remove the low-frequency components caused by the trajectory tracking component of the force and maintain the higher-frequency components resulting from the motor unit firing behavior. The standard deviation (SD) and the coefficient of variation (CV = SD/mean value*100) of the force were computed in the same time range used for the motor unit analysis.

The intramuscular EMG signals were decomposed into their constituent motor unit action potential trains by means of the Precision Decomposition technique (LeFever and De Luca 1982a; Nawab *et al.* 2008). This is an Artificial Intelligence driven automatic technique that uses template matching, template updating and probability of firing statistics to separate and identify the individual action potentials with up to 85% accuracy. The accuracy can be improved to over 97.5% with an operator-assisted editor (Nawab *et al.* 2008). In this study, we used the technique to process three channels of intramuscular EMG signals detected via a quadrifilar fine wire sensor. The shapes of the action potentials belonging to an individual motor unit appear differently on each channel. This distinction was instrumental in identifying the occurrence of the individual firings of the individual motor units as well as enabling some of the individual motor unit action potentials to be followed amongst contractions (see also Adam and De Luca, 2003). An example of the results of the decomposition process can be seen in Figure 2a which present the timing of the individual firings of 6 motor units that were identified during the contraction that produced the force plotted in the figure. (Note that the inter-pulse intervals are plotted vertically.) Only motor units that could be identified for at least two successive contractions were considered for further analysis. The time-varying mean firing rate of each motor unit was computed by low-pass filtering the impulse train representing the time occurrence of each motor unit firing with a

Hanning window of 400 ms duration. Figure 2b shows the time-varying firing rates of the same motor units shown in Figure 2a. The firing rates were detrended to remove the slow variations by filtering the signals with a high-pass filter having a corner frequency at 0.75 Hz. An example may be seen in Figure 2c. The SD and CV (SD/mean value*100) of the mean firing rates were computed from the detrended signals.

The level of common drive between pairs of concurrently active motor units was computed by calculating the cross-correlation function of the detrended mean firing rates of all motor unit pairs within a contraction. An example is shown in Figure 2e. The degree of common drive was obtained by measuring the maximum of the cross-correlation function in the interval of +/- 100 ms. Please see De Luca *et al.* (1982) and De Luca and Adam (1999) for details. In order to determine if the common fluctuations in the mean firing rates are also reflected in the force output of the muscle, the detrended mean firing rate of each motor unit (Figure 2c) was cross-correlated with the detrended force output (Figure 2g). The degree of cross-correlation was determined by measuring the maximum that occurred with a lag of 100 to 200 ms. An example may be seen in Figure 2f.

Synchronization between the firings of pairs of motor units was calculated according to the technique described in De Luca *et al.* (1993). The cross-interval histogram was calculated for each pair of motor units in a contraction. An example may be seen in Figure 2d. For each pair, the motor unit with the least number of firings was chosen as the reference motor unit and the other as the alternate. For each firing in the reference motor unit, the forward and backward latencies between it and the nearest firing in the alternate motor unit were accumulated in the cross-interval histogram. In order to find latencies where synchronization occurred, the count of each latency bin was compared to a statistically determined threshold, determined by using a binomial distribution and a confidence level set at 95%. The strength of synchronization was then computed for each peak in the histogram that surpasses the threshold by means of the Synch Index (SI), which represents the percentage of synchronized firings beyond that which would be expected if the two motor units were firing independently.

Figure 2 near here

RESULTS

Subjects were able to track from 6 to 10 consecutive trajectories (7.75 ± 2.06 contractions) prior to reaching the limit of their endurance capacity as measured by their ability to maintain the 20% MVC force level. The pre-fatigued knee extension MVC values measured at the beginning of the experimental session ranged from 206.01 to 220.89 N (213.12 ± 7.8 N)**.** As the contraction sequence progressed, all subjects showed a decreased proficiency in smoothly tracing the force trajectories and an increase in force fluctuations. This phenomenon is evident in Figure 3 which presents three samples of the force profile tracked by subject #2. The last contraction of subject #2 could be used only for force analysis due to a considerable degree of motor unit superposition and changes in shape.

The analyzed data from one individual subject (subject #3) are presented in Figure 4. The change in parameter values as a function of endurance time is evident and representative of the grouped patterns shown in Figure 5, which shows the behavior of all the subjects. In order to determine if the parameter values varied as a function of the contraction number, they were plotted on a normalized scale for endurance time, where the first contraction was designated as 0% endurance time and the last contraction of the series for each subject was designated as 100% endurance time. A linear regression analysis was performed on each parameter and the slope of the regression was tested for significant difference from the value 0 according to the two-tailed t-statistic using a threshold $\alpha = 0.05$. If the slope is not significantly different from 0, it would indicate that there was no influence of endurance time. Table I contains the equation of the regression line, the R^2 value, the significance level of the slope and the number of data points used in the regression.

Figures 3, 4, 5 and Table I near here

Force variability **--** The variability in the force, computed as the CV of the detrended force, increased from an average value of 0.67% ± 0.18% in the first contraction to an average value of 2.10% ± 0.99% in the last contraction prior to exhaustion. Subjects #2 and #3 showed the greatest increase in the

CV of the force. Significant positive relations were found for the CV of the force as a function of endurance time for each subject. (See Table I.) A significant positive relation was found for the CV of the force as a function of endurance time for grouped subjects. (See Table I and Figure 5.)

Firing rate variability -- The CV of the detrended mean firing rates were computed for 26 motor units throughout the sequence of contractions. The CV of the firing rates of motor units which were active in the plateau region of the first contraction did not change significantly (see Table I) as the contraction sequence progressed, whereas, the CV of the mean firing rates of motor units which began firing in the plateau region in successive contractions almost always decreased while they stabilized their firing pattern. These later recruited motor units were always characterized by a greater variability in their firing rate with respect to the previously active motor units. No significant relation between the CV of the mean firing rates of the motor units firing from the first contraction and the CV of the force was found (R^2 = 0.02, p=0.65 for subject #1; R^2 = 0.3, p=0.21 for subject #2; R^2 = 0.06, p=0.51 for subject #3; R^2 = 0.09, p=0.34 for subject #4).

Cross-correlation of firing rates -- The cross-correlation functions were computed on the firing rates in the plateau region between pairs of concurrently active motor units. Forty-two (42) pairs of motor units were followed throughout at least two, and in some cases all of the contraction sequence. All subjects showed some degree of cross-correlation of the firing rates between pairs of motor units. Regression analysis revealed a positive linear trend between the value of the common drive (computed as the maximum of the cross-correlation function in the interval of +/- 100 ms) and endurance time for three of the four subjects. (See Table I.) Only subject #4 did not show a significant increase. On average, the common drive increased from 0.25 ± 0.13 in the first contraction to 0.39 ± 0.20 in the last contraction. Subjects #2 and #3, which exhibited higher variability in the force, also showed higher common drive values than the other two subjects. When the cross-correlations of all the subjects were grouped together, the R^2 value decreased slightly, as would be expected from the inter-subject variability, but the slope value remained significant. A significant relation between the value of the common drive and the CV of the force was found for three out of four subjects (R^2 = 0.76, p<0.0001 for subject #1; R^2 = 0.14, p=0.029 for subject #2; R^2 = 0.33, p=0.01 for subject #3). Only subject #4, which did not show a significant increase of common drive with endurance time, was not characterized by a significant increase (R^2 = 0.01, p=0.74).

Cross-correlation of firing rates and force -- The same trend was found for the maximum value of the cross-correlation functions between individual motor unit firing rates and force, computed for 26 different MUs throughout the contraction sequence. The values increased as the number of performed contractions increased and a positive linear trend was found for all subjects. (See Table I.) On average, the maximum increased from 0.32 ± 0.09 in the first contraction to 0.45 ± 0.16 in the last contraction. Again, subjects #2 and #3 had the highest values. When the cross-correlations of all the subjects were grouped together, the R^2 value decreased, as would be expected from the inter-subject variability, but the slope value remained significant. A significant relation between the cross-correlation of firing rates and force and the CV of the force was found for all subjects (R^2 = 0.72, p<0.0001 for subject #1; R^2 = 0.24, p=0.028 for subject #2; R^2 = 0.66, p=0.0001 for subject #3; R^2 = 0.30, p=0.024 for subject #4).

Synchronization of motor units -- A total of 100 motor unit pairs were analyzed. They were obtained from all the contractions of all the subjects. Most of them (73 out of 100) showed some minor degree (average SI < 4%) of synchronization and most of the synchronized pairs (69 out of 73) presented long-term synchronization (time lag > 6 ms), while a smaller group (34 out of 73) presented short-term synchronization (time lag ≤ 6 ms). The average Synch Index was always in the range between 2 to 4% in all contractions and for all subjects. This indicates that when synchronization of motor unit firings was noted, only 2 to 4% of the firings were synchronized beyond that expected by random chance. Table I indicates that there is no clear trend suggesting that the Synch Index varies systematically as a function of contraction sequence (endurance time). Also, no trend was found for the number of synchronized motor unit pairs as a function of the contraction sequence. When the subjects were grouped, the SI and the number of synchronized pairs were statistically independent of the endurance time. (See Table I for details.)

Number of newly recruited motor units -- As it was previously noted by Adam and De Luca (2005), motor units were recruited during the successive contractions to partially compensate for the decrease in the amplitude of the force twitches of the active motor units. For each subject there was a trend for the number of observed recruited motor units to increase during the contraction sequence. In subjects #2 and #3 the trend was significant, whereas for subjects #1 and #4 it was not. (See Table I.) Nonetheless, when all subjects were grouped, the increasing trend was significant. A significant relation between the number of newly recruited motor units and the CV of the force was found only for subject #2 (R^2 = 0.96, p= 0.018). No significant relation was found for the other subjects (R^2 = 0, p=0.91 for subject #1; R^2 = 0.67, p=0.092 for subject #3; R^2 = 0.50, p=0.18 for subject #4).

DISCUSSION

A muscle does not produce a smooth or constant force, even when it is attempted to do so. In our earlier work we have shown that the firing rates of motor units are not constant and that fluctuations in the firing rates are correlated with the fluctuations in the force output of the muscle (De Luca *et al*., 1982). The question raised in this work is why the force fluctuation increases during a fatiguing contraction, as it has been reported by Furness *et al*. (1977), among others. In this study we considered only the intrinsic force fluctuations, that is, those that were caused by the motor unit firing behavior. We did so, by filtering the force and removing any influence of force corrections resulting from attempts at maintaining the force constant.

We investigated the behavior of the motor unit control parameters during constant-force isometric contractions and found only one that presented a significant relationship (in 3 out of 4 subjects) with the observed increase in the force fluctuation. It was the Common Drive derived from the cross-correlation value of the firing rates of motor units. The number of motor units that were recruited tended to increase with endurance time, even if the increase was not significant for each of the subjects, but was significant when the subjects were grouped. The relation between the number of newly recruited motor units and the coefficient of variation (CV) of the force was not significant for all subjects. The lack of significance may be due to the limited number of motor units that we were able to track.

The firing rate variability remained unaltered for all the motor units which were recruited during the first contraction and could be followed throughout subsequent contractions. Most of the motor units that were recruited during subsequent contractions decreased their CV as their firing rate increased and stabilized; as is typical of newly recruited motor units. With the minor exception of the short-term contribution of the unstable firing rates of newly recruited motor units which is overwhelmed by the unaltered CV of the rest of the active motor units, it does not seem possible for firing rate variability to cause the increase in the force variability. Our finding differs from those of other authors, who relate the force variability, during an isometric contraction, mainly to the variability in the firing rates of the active motor units. Moritz *et al.* (2005) was able to improve the performance of a motor unit model to predict force variability by acting on the firing rate variability, suggesting that this is a major determinant of the fluctuation in isometric force. This observation may be so, but the fact remains that in reality we found a significant increase in force variability without any significant increase in the firing variability throughout the endurance time that fatigued the muscle to exhaustion. Laidlaw *et al.* (2000) compared the firing behavior in the FDI muscle between young and old subjects, and found that firing variability has a role in steadiness. However, that finding only held for the lowest force levels contractions (2.5% and 5% MVC) and not for higher force levels (7.5% and 10% MVC). This finding is not unexpected because at force levels below 5% MVC, motor units have firing rates typically less than 10 pulses per second and in the absence of many other motor units the individual pulses and associated force twitches can influence the variability of the force output. Their finding would only apply to fatiguing contractions if the firing rate decreased to the low values associated with a 5% MVC contraction. Such a decrease in the firing rates, however, was not observed in VL motor units during repeated, submaximal contractions according to the fatigue protocol of this study. Instead, our findings are consistent with those of Semmler and Nordstrom (1998), who found no difference in the firing variability of motor units in the FDI muscle of skilled-trained subjects compared to strength-trained subjects, even though the skilled subjects produced lower force variability. And, those of Macefield *et al.* (2000) reported no change in firing variability of motor units in the extensor hallucis longus during a sustained MVC. As well as those of Galganski *et al.* (1993), who found no difference in the firing variability of motor units in the FDI muscle of young and elderly subjects, despite an increased force variability in elderly subjects.

Another firing parameter that has been associated with increasing force variability is the synchronization of motor unit firings. In a computer simulation study, Yao *et al.* (2000) showed that motor unit firing synchronization increased the amplitude of the fluctuations in the simulated force without altering the magnitude of the average force. In another simulation study, Taylor *et al.* (2003) reported that an increasing level of short-term synchronization with excitatory drive provided the closest fit to the experimentally observed relation between the coefficient of variation of the force and the mean force. Our findings are consistent with those of Semmler *et al.* (2000) who showed that an increased force-variability in older subjects was not coupled with higher levels of motor unit firing synchronization. Admittedly, their results could be influenced by the different profile of the motor unit force twitches of the young and elderly subjects nonetheless they raise the question as to the existence of a causal relationship between synchronization and the force variability. In the present study, we found that the degree of synchronization of motor unit pairs that could be tracked across contractions was remarkably low (Synch Index between 2 and 4%, see Figure 5), a value that is consistent with that of previous reports (De Luca *et al.* (1993), Taylor and *et al.* (2003), and Semmler *et al.* (2000)). Furthermore, both the degree of synchronization and the number of synchronized motor unit pairs did not change significantly as a function of sustained contractions. (See Table I.) Consequently, synchronization cannot account for the increase in the force variability during fatigue.

A motor unit parameter that was found to be altered during fatigue is the Common Drive, defined as the maximum value of the cross-correlation function of the firing rates between pairs of concurrently active motor units. It was found to increase significantly with endurance time in 3 out of 4 subjects. The increase was seen in all motor units and in all subjects. The cross-correlation between firing rates and the force also increased. These observations are consistent with the prediction of the Lowery and Erim (2005) model. In a simulation study, they superimposed low-frequency oscillations (<5 Hz) to the input of a model that generated motor unit firings (to simulate the Common Drive) and found that both common in-phase fluctuations of mean firing rates and force variability increased, while common oscillatory inputs at frequencies close to the mean firing rate were most effective in inducing short term synchronization. The question remains as to why the Common Drive increases during sustained isometric contractions.

It has previously been proposed by De Luca *et al.* (2009) that during a sustained contraction, the cross-correlation of motor unit firing rates is influenced by motor unit recruitment via the feedback from the spindles and possibly the Golgi Tendon Organs, with the spindles being the more dominant factor. Muscle spindles respond to the mechanical excitation of the non-fused muscle fibers and provide a discordant excitation to the homonymous motoneurons. Spindles in the proximity of the contracting muscle fibers either slacken or stretch depending on their orientation with respect to the fibers (Binder and Stuart, 1980; Edin and Vallbo, 1990). Thus, Ia firings either decrease or increase until the recruited muscle fibers become fused or quasi-fused. With motor unit recruitment, some motoneurons will be facilitated and some will be disfacilitated due to the discordant afferent input. Consequently, the firing rates of the motor units will vary in a discordant manner and the amplitude of their cross-correlation will decrease. Even if the alignment of the spindles with respect to the muscle fibers was uniform, a discordant afferent input could result from inhomogeneous changes in the sensitivity of the spindles during sustained contractions. In this study, we found a relationship between the number of newly recruited motor units and the cross-correlation value of all motor unit firing rates with endurance time. Thus, it is reasonable to postulate that a decreased spindle influence would result in an increase in the cross-correlation value of the firing rates when motor units are recruited during a fatiguing contraction. We are not aware of any evidence of differential changes in the excitation of individual spindle outputs, but there is evidence for a global change in the spindle firing rates during a sustained contraction. Macefield *et al.* (1991) reported a decrease in muscle spindle firing rate during voluntary contractions sustained for 1 minute. Hill (2001) suggested that the decrease could be explained by a progressive fatigue of the intrafusal fibers induced by a prolonged γ-drive to these fibers. Additional support is provided by the work of Avela *et al.* (1999, 2001) which showed a reduction in the stretch reflex and in the H-reflex amplitude after the performance of a fatiguing repeated passive stretching exercise, and suggested that this was a consequence of a reduction in the activity of the large diameter Ia afferents, resulting from the reduced sensitivity of muscle spindles.

The increasing number of motor units that were recruited during the successive contractions would also provide an increasing force-variability. As the new motor units are recruited they fire with lower firing rates, are not fused, and the individual force twitches increase the force variability. The data would suggest that there is such an influence, but the relationship is significant only for grouped subjects. Perhaps with improved technology, it might be possible to observe more recruited units and provide a data set that could establish significance for the individual subjects as well.

In conclusion, we found that during a sequence of sustained isometric force contractions performed at 20% MVC and repeated until the targeted level could no longer be maintained, the fluctuation of the force about the targeted value increased progressively. The behavior of the force was found to be correlated to the Common Drive of the motor units which increased in progressive contractions. The increasing number of newly recruited motor units is also likely to produce the increasing force-fluctuation. The coefficient of variation of the firing rates and the synchronization of the motor unit firings were not found to alter as a function of endurance time, and consequently could not account for the increase in variability of the force during fatigue.

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CAPTIONS

F*igure 1* -- Fatiguing protocol. Successive isometric contractions were tracked to exhaustion, separated by 6 s of rest. Each contraction started with a ramp up to 50% MVC (at a rate of 10% MVC/s) and a brief hold phase; the target value was then decreased to 20% MVC and maintained at this level for 50 s. At the end of the cycle, the force level was decreased at the same rate as the initial ramp. (Modified from Adam and De Luca, 2003.)

Figure 2 -- A traced force trajectory is shown superimposed on the inter-pulse intervals (A) and on the mean firing rates (B) of the active motor units. The black vertical lines indicate the 30 s interval used for all data analysis. The detrended mean firing rates (C) and the detrended force (G) in this time interval are shown. From these signals, the following parameters were computed: the strength of synchronization (D), the cross-correlation (E) between the detrended firing rates of all active motor unit pairs and the cross-correlation (F) between the detrended firing rates of each motor unit and the detrended force. Note that in (A) when the inter-pulse intervals of the motor units are greater than 200 ms, a fixed value of 200 ms is displayed.

Figure 3 -- The first, middle and last traced force trajectories for subject #2 are presented in order to show the increase in the force fluctuations with the progression of fatigue.

Figure 4 -- The behavior of all the analyzed variables with endurance time is presented for subject #3: the coefficient of variation (CV) of the detrended force, the Common Drive defined as the maximum value of the cross-correlation function between the detrended motor unit firing rates in the interval +/- 100 ms, the maximum valued of the cross-correlation function between the detrended motor unit firing rates and the force, the number of recruited motor units during the analyzed interval, the CV of the detrended mean firing rates, the strength of synchronization (Synch Index (SI)) (see text), and the

percentage of synchronized motor unit pairs. The first four parameters were significantly increasing with endurance time (this is indicated by the * symbol). The first plot on the right hand side shows the CV of the detrended mean firing rates as a function of endurance time for all motor units. Only motor units that were active in the first and subsequent contractions were used for the regression analysis.

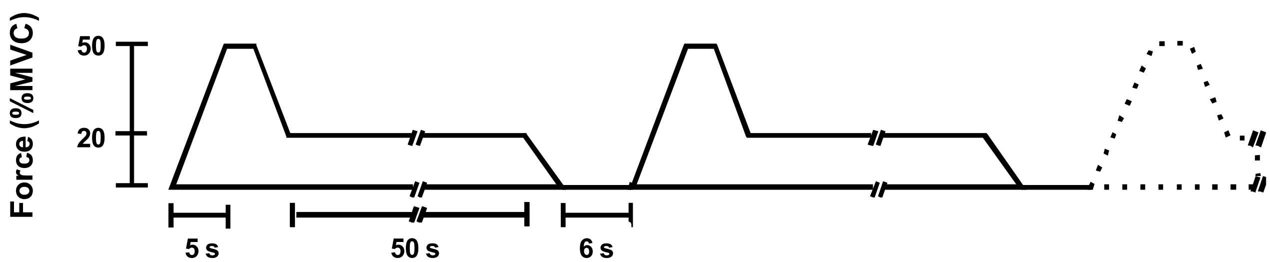
Figure 5 -- The behavior of all the analyzed variables with endurance time is presented for all subjects grouped: the CV of the detrended force, the Common Drive defined as maximum value of the cross-correlation function between the detrended motor unit firing rates in the interval +/- 100 ms, the maximum values of the cross-correlation function between the detrended motor unit firing rates and the detrended force, the number of recruited motor units during the analyzed interval, the CV of the detrended mean firing rates, the strength of synchronization, and the percentage of synchronized motor unit pairs. The first four parameters were significantly increasing with endurance time (this is indicated by the * symbol). The first plot on the right hand side shows the CV of the detrended mean firing rates as a function of endurance time for all motor units. Only motor units that were active in the first and subsequent contractions were used for the regression analysis.

Table 1 -- Statistics from the regression analysis performed on single subjects and on grouped subjects for each analyzed parameter. The equation of the regression lines, the R^2 value, the p value and the number n of data points used for the regression are reported. In each case, the independent variable x is the endurance time. In the case of the CV of the firing rates, the regression lines were drawn considering only motor units active from the first contraction.

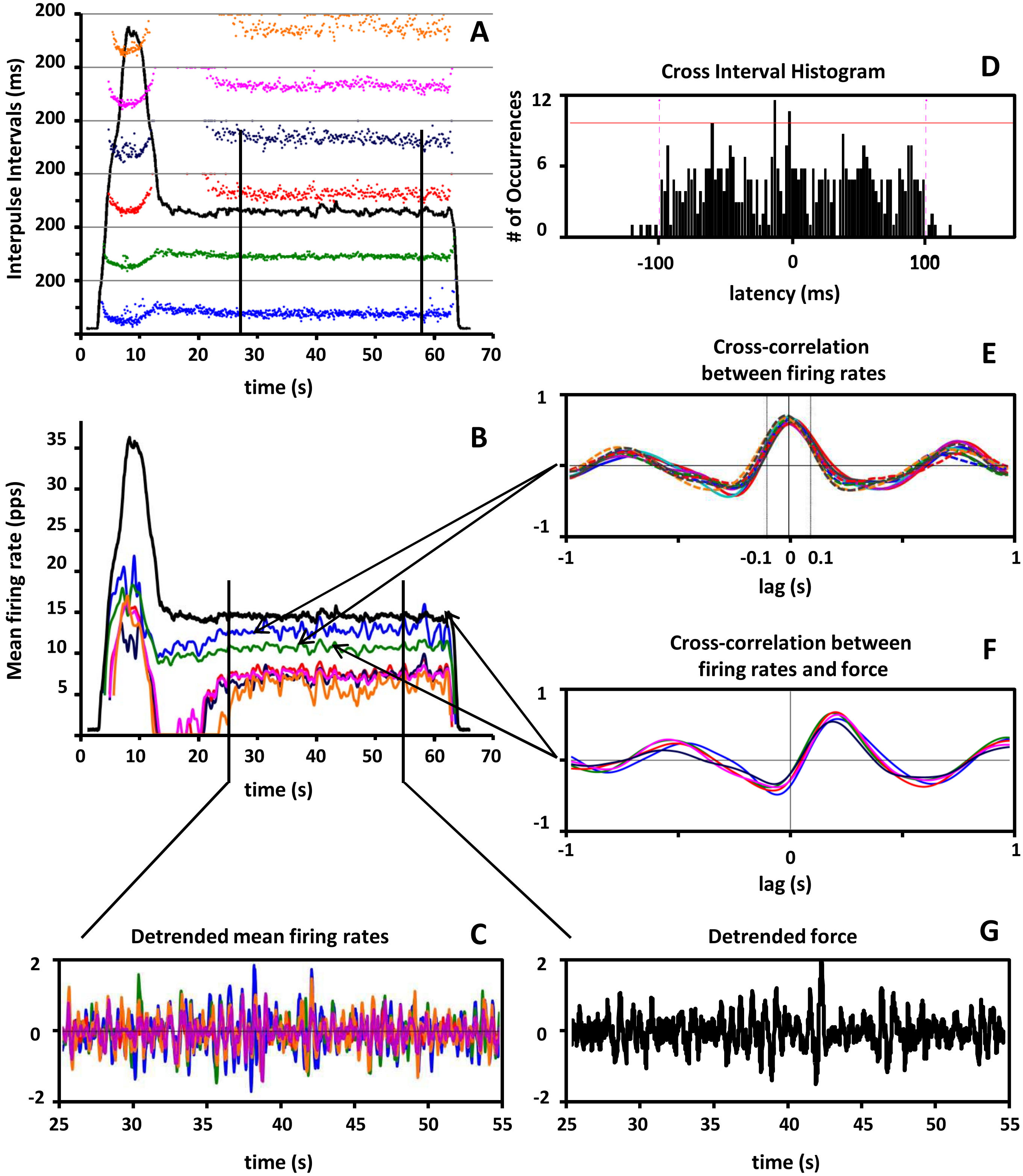
TABLES

Table I

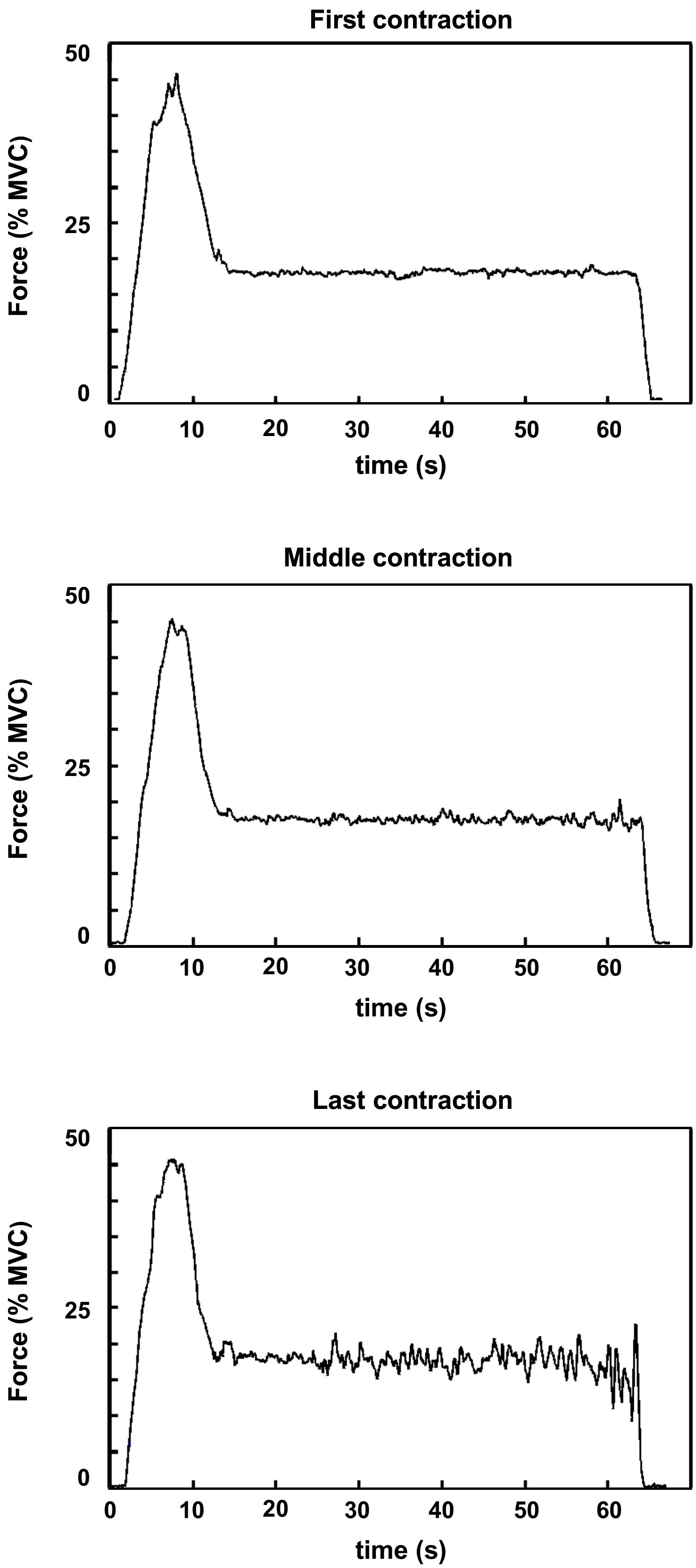
Parameters	Subject #1	Subject #2	Subject #3	Subject #4	Grouped subjects
CV force	y=0.53x+0.35 R^2=0.81 p=0.036* n=5	y=1.99x+0.51 R^2=0.80 p=0.042* n=5	y=1.53x+0.93 R^2=0.81 p=0.039* n=5	y=1.10x+0.79 R^2=0.97 p=0.002* n=5	y=1.38x+0.62 R^2=0.51 p<0.001* n=20
Cross-correlation between firing rates	y=0.27x+0.15 R^2=0.64 p<0.0001* n=25	y=0.19x+0.5 R^2=0.23 p=0.004* n=35	y=0.2x+0.45 R^2=0.27 p=0.024* n=19	y=0.22 R^2=0 p=0.92 n=21	y=0.18x+0.33 R^2=0.10 p=0.001* n=100
Cross-correlation between firing rates and force	y=0.19x+0.23 R^2=0.43 p=0.001* n=21	y=0.25x+0.51 R^2=0.33 p=0.009* n=20	y=0.26x+0.38 R^2=0.62 p<0.001* n=16	y=0.09x+0.32 R^2=0.29 p=0.026* n=17	y=0.19x+0.36 R^2=0.16 p<0.001* n=74
# Recruited MUs	y=0.32x+2.26 R^2=0.06 p=0.70 n=5	y=3.50x+5.10 R^2=0.98 p=0.01* n=4	y=5.52x+1.13 R^2=0.95 p=0.004* n=5	y=2.15x+3.97 R^2=0.54 p=0.16 n=5	y=2.51x+3.26 R^2=0.22 p=0.042* n=19
CV mean firing rate	y=-0.21x+4.47 R^2=0 p=0.93 n=11	y=2.46x+3.44 R^2=0.26 p=0.24 n=7	y=-0.73x+5.35 R^2=0.11 p=0.35 n=10	y=-1.62x+5.35 R^2=0.10 p=0.33 n=12	y=-0.26x+4.63 R^2=0 p=0.72 n=40
Synchronization between firing rates	y=0.54x+1.03 R^2=0.02 p=0.48 n=25	y=-0.51x+2.48 R^2=0.03 p=0.36 n=35	y=0.98x+1.39 R^2=0.05 p=0.38 n=19	y=0.01x+2.31 R^2=0 p=0.99 n=21	y=0.36x+1.77 R^2=0.01 p=0.34 n=100
% Synchronized MU pairs	y=0.14x+30.52 R^2=0.02 p=0.81 n=5	y=-0.12x+95.73 R^2=0.46 p=0.32 n=4	y=0.2x+66.09 R^2=0.09 p=0.62 n=5	y=0.09x+75.95 R^2=0.04 p=0.75 n=5	y=0.10x+65.39 R^2=0.01 p=0.64 n=19



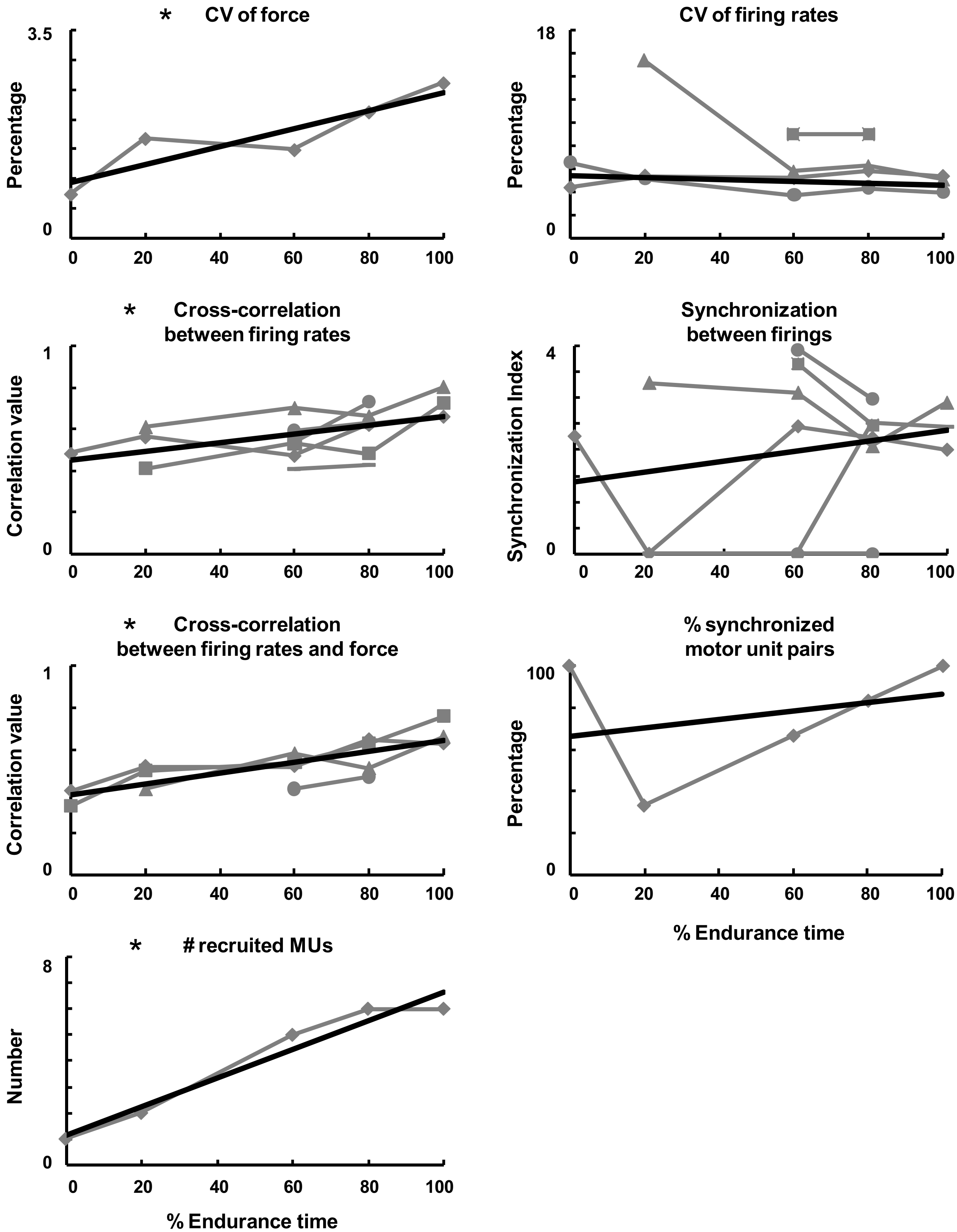
Force (%MVC)
50
20
5 s
50 s
6 s



A
Interpulse Intervals (ms)
200
200
200
200
200
200
0 10 20 30 40 50 60 70
time (s)
B
Mean firing rate (pps)
35
30
25
20
15
10
5
0 10 20 30 40 50 60 70
time (s)
C
Detrended mean firing rates
2
0
-2
25 30 35 40 45 50 55
time (s)
D
Cross Interval Histogram
# of Occurrences
12
6
0
-100 0 100
latency (ms)
E
Cross-correlation
between firing rates
1
-1
-1 -0.1 0 0.1 1
lag (s)
F
Cross-correlation between
firing rates and force
1
-1
-1 0 1
lag (s)
G
Detrended force
2
0
-2
25 30 35 40 45 50 55
time (s)


First contraction
50
25
0
Force (% MVC)
0
10
20
30
40
50
60
time (s)
Middle contraction
50
25
0
Force (% MVC)
0
10
20
30
40
50
60
time (s)
Last contraction
50
25
0
Force (% MVC)
0
10
20
30
40
50
60
time (s)

SUBJECT #3



* CV of force
Percentage
3.5
0
CV of firing rates
18
* Cross-correlation between firing rates
Correlation value
1
Synchronization between firings
Synchronization Index
4
* Cross-correlation between firing rates and force
% synchronized motor unit pairs
100
* # recruited MUs
Number
8
0
20
40
60
80
100
% Endurance time

ALL 4 SUBJECTS