Motor Unit Synchronization Is Increased in Biceps Brachii After Exercise-Induced Damage to Elbow Flexor Muscles

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Dartnall TJ, Nordstrom MA, Semmler JG. Motor unit synchronization is increased in biceps brachii after exercise-induced damage to elbow flexor muscles. J Neurophysiol 99: 1008–1019, 2008. First published January 2, 2008; doi:10.1152/jn.00686.2007. The purpose of this study was to determine the effect of eccentric exercise on correlated motor unit discharge (motor unit synchronization and coherence) during low-force contractions of the human biceps brachii muscle. Eight subjects (age, 25 ± 7 yr) performed three tasks involving isometric contraction of elbow flexors while EMG (surface and intramuscular) records were obtained from biceps brachii. Tasks were 1) maximum voluntary contraction (MVC); 2) constant-force contraction at various submaximal targets; and 3) sustained discharge of pairs of concurrently active motor units for 2–5 min. These tasks were performed before, immediately after, and 24 h after fatiguing eccentric exercise. MVC force declined 46% immediately after eccentric exercise and remained depressed (31%) 24 h later, which is indicative of muscle damage. For the constant-force task, biceps brachii EMG (~100% greater) and force fluctuations (~75% greater) increased immediately after exercise, and both recovered by ~50% 24 h later. Motor unit synchronization, quantified by cross-correlation of motor unit pairs during low-force (1–26% MVC) contractions, was 30% greater immediately after (n = 105 pairs) and 24 h after exercise (n = 92 pairs) compared with before exercise (n = 99 pairs). Similarly, motor unit coherence at low (0–10 Hz) frequencies was 20% greater immediately after exercise and 34% greater 24 h later. These results indicate that the series of events leading to muscle damage from eccentric exercise alters the correlated behavior of human motor units in biceps brachii muscle for ≥24 h after the exercise.

INTRODUCTION

Along with motor unit recruitment and rate modulation, the correlated activity of motor units is now regarded as an important physiological principle that regulates the mechanical output of a muscle, and has been included in many recent computational models of motor unit activity (Lowery and Erim 2005; Yao et al. 2000; Zhou and Rymer 2004). The correlated discharge of action potentials by motor neurons is caused by common synaptic input that is delivered through branched presynaptic inputs or by oscillatory drive from supraspinal sources (Farmer et al. 1993a,b; Sears and Stagg 1976). The correlated motor unit activity can be quantified in the time domain as motor unit synchronization or in the frequency domain as motor unit coherence, and an association between motor unit synchronization and high-frequency (~10–30 Hz) coherence suggests that these two phenomena may share similar mechanisms (Farmer et al. 1993a; Semmler et al. 2002, 2004). During voluntary contractions in humans, motor unit synchronization and coherence are often quantified simultaneously to provide complementary information about the adjustments made by the CNS during various tasks.

It has been known since the seminal study by Adams et al. (1989) that the correlated discharge of motor units in humans is not a fixed property of the CNS, but can vary depending on the details of the task that is performed. In human limb muscles, for example, changes in motor unit synchronization during isometric contractions have been observed when forces are exerted in different directions (Bremner et al. 1991), during performance of an action that requires significant attention (Schmied et al. 1999), and when object compliance has been altered (Kilner et al. 2002). Furthermore, correlated motor unit activity can be altered during changes in muscle length, because motor unit synchronization is enhanced during slow-lengthening contractions and low-frequency (2–12 Hz) coherence is reduced during shortening contractions (Semmler et al. 2002). These studies suggest that different isometric contractions and movements are controlled by altering the common synaptic input that is delivered to the involved motor units.

Along with the task-related adjustments, it is a commonly accepted view that certain types of exercise, such as fatigue or strength training, can enhance the correlated activity of human motor units (Gandevia 2001; Semmler 2002; but cf. Kidgell et al. 2006). One form of exercise that occurs during normal activities is termed eccentric exercise, which involves the active lengthening of muscle during tasks such as downhill walking, running, and lowering of an object held in the hand. Unlike concentric or isometric exercise, eccentric exercise is known to produce significant muscle damage and delayed onset muscle soreness (DOMS). Although the initial event that causes the muscle damage remains debatable (Proske and Morgan 2001; Warren et al. 2001), the effects of muscle damage result in a shift in the length-tension relation to longer optimal muscle lengths for peak force generation, a rise in whole-muscle passive tension and a reduction in maximal force production (Proske and Allen 2005). It is possible that the series of physiological and mechanical events leading to muscle damage (such as fatigue or pain) from eccentric exercise may act to influence the neural inputs responsible for correlated motor unit discharge. At present, there are no reports describing the discharge characteristics of motor neurons at the single motor unit level after eccentric exercise, and so the effect of

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exercise-induced muscle damage on correlated motor unit activity is unknown.

The aim of this study was to determine the effect of exercise-induced muscle damage on time- (motor unit synchronization) and frequency-domain (motor unit coherence) measures of correlated motor unit discharge during isometric contractions of biceps brachii. We tested a large sample of biceps brachii motor units before eccentric exercise, and at two time-points after eccentric exercise, in an attempt to distinguish between effects associated with fatigue and muscle damage (immediately after) and muscle damage alone (24 h after). Recent computational models of motor unit activity have indicated that motor unit synchronization can increase EMG and force fluctuations (Lowery and Erim 2005; Yao et al. 2000; Zhou and Semmler 2007). We therefore expected to see enhanced motor unit synchronization and coherence immediately after eccentric exercise that returned to baseline levels 24 h later. Such a finding would suggest that the factors associated with muscle damage from eccentric exercise contribute to increased correlated motor unit activity in human biceps brachii muscle.

METHODS

Eight healthy subjects [6 males, 2 females; age, 25 ± 7 (SD) yr] were recruited for this study. All subjects had no history of musculoskeletal pain or injury in the left arm or shoulder and had not participated in regular strength training for ≥6 mo. Written informed consent was obtained from all subjects before the beginning of the experiment. All experimental procedures conformed to the Declaration of Helsinki and were approved by the Human Research Ethics Committee at the University of Adelaide.

Subjects were seated in an experimental chair with the left forearm positioned vertically and the elbow joint at 90° flexion. The forearm was constrained in this position in a device designed to measure isometric elbow flexion force in the sagittal plane. Two wide nylon straps were used to secure the forearm in a supinated position throughout the experiment.

Surface EMG signals were recorded from the belly of the biceps brachii with bipolar electrodes (silver-silver chloride, 4 mm diam) placed ~2 cm apart (center to center). A grounding strap was positioned around the wrist to act as a reference. The surface EMG signals were amplified (100-1,000 times; V75-04, Coulbourn Instruments, Allentown, PA) connected to a speaker to provide audio-feedback of motor unit discharge during the low-force contractions. Single motor unit recordings were amplified (V75-02, Coulbourn Instruments) (1,000 times), band-pass filtered (90 Hz to 10 kHz; V75-48, Coulbourn Instruments), displayed on an oscilloscope, and recorded on digital tape.

As an indicator of a change in passive muscle tension after eccentric exercise, relaxed elbow angle was measured from the mid point of the acromion and coracoid process to the lateral epicondyle and to the mid point of the ulnar and radius styloid processes. The angle was measured with a goniometer while the subjects stood upright with their left arm relaxed by their side and their palm facing medially. A decrease in elbow joint angle after exercise represented a more flexed elbow, indicative of an exercise-induced rise in passive tension of the elbow flexor muscles. As an indicator of muscle soreness induced by the exercise, subjects rated the degree of pain exhibited in the muscle on a visual analog scale during forced extension of their elbow joint, where 0 represented no muscle soreness and 10 corresponded to the most intense muscle soreness imaginable. Relaxed elbow angle and muscle soreness were measured before exercise, at the end of the session on the first day after exercise (~1 h after), and 24 h later.

Each subject performed three tasks requiring isometric contraction of the biceps brachii muscle: 1) maximum voluntary contraction (MVC), 2) a constant-force task, consisting of a steady submaximal contraction at six different force levels, and 3) a motor unit task, consisting of sustained tonic discharge of biceps motor units at a constant, low mean discharge frequency. Surface EMG was recorded during all three tasks, and single motor unit recordings were obtained in the motor unit task. These tasks were performed on three separate occasions in the same subjects, which consisted of a baseline measure before exercise, a second session involving eccentric exercise (~1–4 wk after the first session), and a third session 24 h later. The data obtained in the same experimental session immediately after eccentric exercise were expected to include effects of fatigue and putative muscle damage on motor unit activity, whereas the data obtained 24 h after exercise examined the effects of muscle damage without fatigue, because it is known that maximum force following a fatiguing task recovers within 2 h (Walsh et al. 2004).

MVC force

The MVC task consisted of a ramp increase in elbow flexion force over a 3-s period from zero to maximum force and sustained for a further 3 s. Timing of the task was provided verbally by the experimenter and the subject was able to monitor the force displayed on an oscilloscope placed in front of them at eye level. To facilitate maximum force production, the experimenter provided verbal encouragement during the sustained maximum contraction. The subject performed three MVCs with ≥1 min rest between contractions, and the force was recorded on tape. The trial with the greatest MVC force was selected for analysis and used as the reference for the constant force task in each respective testing session.

Constant-force task

A force transducer (MLP-150, Transducer Techniques, Temecula, CA) located perpendicular to the forearm at the level of the wrist and mounted on a customized manipulandum was used to detect forces exerted during the tasks. The output from the force transducer was displayed on an oscilloscope and recorded on tape. A horizontal line on the oscilloscope screen showed the required target for each force level. Isometric contractions were performed at target forces of 2.5, 5, 10, 20, 35, and 50% MVC (% MVC refers to MVC recorded at the beginning of the respective testing session). These target forces were selected as it was expected that they would encompass the forces used during the motor unit task, with a larger number of contractions biased toward low force levels. Subjects were instructed to exert a steady
elbow flexion force for 15 s at each target force. One trial was performed at each target force level and the order of contractions was randomized. Each trial was scrutinized for erroneous force fluctuations because of concentration errors from the subject, and the trial was repeated at the appropriate force level if obvious errors occurred.

**Motor unit task**

The discharge of pairs of single motor units was recorded from the biceps brachii while subjects exerted a steady elbow flexion force. Subjects slowly increased the elbow flexion force until at least one motor unit that discharged action potentials repetitively was detected with each electrode. The subject was given audio feedback of the discharge from one motor unit, and a target force was provided on the oscilloscope screen which represented the force required to sustain the discharge for 2–5 min. The target force was occasionally adjusted during these trials so that at least one motor unit from each electrode could be accurately discriminated. The mean force used to activate the motor units throughout the trial was noted and the strength of the contraction relative to maximum force in that session (%MVC) was determined. Sampling from motor units that were active at relatively low forces minimized the effects of fatigue, and subjects rested for several minutes at the end of each trial. After the rest period, a different motor unit from one of the electrodes was selected, either by moving the electrode ≥0.5 cm to obtain a completely different motor unit waveform or by recording from a new electrode. No more than three different motor units were recorded from each electrode to reduce the likelihood of recording from the same motor unit on separate occasions.

**Eccentric exercise**

Controlled eccentric exercise with the biceps brachii muscle of the left arm was used to induce a minimum 40% reduction of isometric MVC force. This protocol was used to obtain similar muscle damage in all subjects, compared with the large variation in strength loss between subjects following a set number of eccentric contractions (Hubal et al. 2007). Furthermore, this eccentric exercise protocol has been shown to produce consistent changes in elbow flexors indicative of muscle damage, which include a shift in optimal muscle length, a cross-correlation histogram. Statistical significance of the peak in the cross-correlogram was determined using the methods described by Wiegner and Wierzbicka (1987). When no significant peak was observed, a standard peak width of 11 ms centered at 0 ms was used to quantify the strength of synchronization in that motor unit pair.

Cross-correlation histograms were not analyzed if the mean bin count was ≤4. The magnitude of the central synchronous peak in the cross-correlogram was quantified using two commonly used indices: 1) the index common input strength (CIS), which represents the frequency of synchronous discharges in excess of those expected because of chance for a period in which both motor units were tonically active (expressed as pulses/s,pps), and is mathematically independent of motor unit discharge rate (Nordstrom et al. 1992); and 2) the index E (Datta and Stephens 1990; Datta et al. 1991), which corresponds to the total number of extra counts within the peak above those expected due to chance, relative to the total number of reference unit discharges (expressed as pulses/trigger event, ppt) and is not influenced by discharge rate in rat hypoglossal motor neurons (Turker and Powers 2002).

Coherence analysis implemented in Matlab was performed on the coherence spectrum of the discharge times of the same pairs of motor unit units (Farmer et al. 1993a; Rosenberg et al. 1989; Semmler et al. 2004). The discriminated motor unit data were divided into contiguous, nonoverlapping epochs of 1.28 s that were made up of 256 bins. Each 5-ms bin was assigned a value of 1 when it contained a discriminated action potential and a value of 0 when no action potential was present, and these data were transformed into the frequency domain with a resolution of 0.8 Hz. Auto- and cross-spectra were averaged over the disjoint sections to obtain the coherence data for each motor unit pair, which resulted in a measure of linear association with values between 0 (completely independent) and 1 (completely dependent). Pooled coherence between the same pair of motor units was calculated (Amjad et al. 1997), allowing several independent recordings or segments to be combined. Values of coherence exceeding the 95% CI (Rosenberg et al. 1989) for the frequencies of interest (0–30 Hz) were regarded as significant. The computer. Signals were sampled at 200 Hz (force), 2,000 Hz (EMG), or 20 kHz (single motor unit recordings). Off-line analysis was performed with Spike 2 data analysis software (Cambridge, UK) and custom written scripts. The EMG from the MVC trial was full-wave rectified and averaged for a 1-s epoch centered around the point at which the maximum force was achieved. For the constant-force task, a 10-s sample of force from the middle of each trial was used to determine the mean and SD of force. The CV of force (SD/mean force × 100) was obtained from these values. The EMG from the biceps brachii was rectified and averaged over a user selectable window of 1 s corresponding to a stable portion of the EMG and force record. The EMG was normalized to the maximum EMG obtained during the MVC performed on that day to facilitate comparisons between subjects and across days.

A computerized spike-sorting algorithm (Spike 2, version 5, Cambridge Electronic Design) was used to discriminate single motor unit discharges. Action potentials belonging to a particular motor unit were identified based on waveform shape. For every trial, the interspike intervals (ISIs) of identified motor units were examined to ensure discrimination accuracy. In trials with a high number of errors (>5%), the motor unit recordings were reanalyzed on a spike-by-spike basis. The mean, SD, and CV of ISIs were obtained, and the mean frequency of the discharge times (1,000/mean ISI) was determined using custom-designed software written in Matlab (Mathworks, Natick, MA). Cross-correlation analysis was used to assess the amount of motor unit synchronization in pairs of concurrently active motor units detected with separate electrodes. The cross-correlation histogram had 201 bins that characterized the discharge behavior of one motor unit for 100 ms before and after each discharge of the reference motor unit. The cumulative sum (CUSUM; Ellaway 1978) technique was used to estimate the location and width of the central synchronous peak in the cross-correlogram. Statistical significance of the peak in the cross-correlation histogram was determined using the methods described by Wiegner and Wierzbicka (1987). When no significant peak was observed, a standard peak width of 11 ms centered at 0 ms was used to quantify the strength of synchronization in that motor unit pair. Cross-correlation histograms were not analyzed if the mean bin count was ≤4. The magnitude of the central synchronous peak in the cross-correlogram was quantified using two commonly used indices: 1) the index common input strength (CIS), which represents the frequency of synchronous discharges in excess of those expected because of chance for a period in which both motor units were tonically active (expressed as pulses/s,pps), and is mathematically independent of motor unit discharge rate (Nordstrom et al. 1992); and 2) the index E (Datta and Stephens 1990; Datta et al. 1991), which corresponds to the total number of extra counts within the peak above those expected due to chance, relative to the total number of reference unit discharges (expressed as pulses/trigger event, ppt) and is not influenced by discharge rate in rat hypoglossal motor neurons (Turker and Powers 2002).

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coherence estimates for each pair of motor units were normalized into z scores (Rosenberg et al. 1989) to allow for comparison of coherence between subjects and to facilitate statistical comparison for recordings on different days.

Statistical analysis

Elbow flexor force and EMG during maximal contractions were analyzed with a one-way repeated-measures ANOVA for the different exercise states (before, after, 24 h after). For the constant-force tasks, two-way repeated-measures ANOVA was used for exercise state (before, after, 24 h after) and target force (2.5, 5, 10, 20, 35, 50% MVC). Pairwise comparisons were performed with Fisher’s post hoc test. Statistical significance was set at 0.05 for all comparisons, and all values are reported as mean ± SE.

RESULTS

Indirect indicators of muscle damage

To ensure that the eccentric exercise resulted in muscle damage, several indirect indicators of muscle damage were measured before, immediately after, and 24 h after eccentric exercise. MVC force was significantly reduced immediately after the repeated eccentric contractions in all subjects (mean of 46 ± 3%, P < 0.001; Fig. 1A). This required an average of 125 ± 25 contractions with a mean exercise load of 9.4 ± 0.9 kg. Twenty-four hours after eccentric exercise, MVC force had only partially recovered and was still 31% lower than the MVC before eccentric exercise (Fig. 1A). No significant change in biceps brachii EMG was present during the MVCs recorded before (0.96 ± 0.20 mV), immediately after (0.74 ± 0.10 mV), and 24 h after (0.95 ± 0.20 mV) eccentric exercise (P = 0.19). The relaxed elbow joint angle was 14° more flexed immediately after eccentric exercise and 24 h later (P = 0.003; Fig. 1B). Subjects reported that muscle soreness during forced elbow extension was not different immediately after eccentric exercise compared with before eccentric exercise, although this increased ~10-fold 24 h after eccentric exercise (Fig. 1C).

EMG activity after eccentric exercise

To confirm the findings of previous studies, we measured biceps brachii EMG at a range of submaximal target forces before, immediately after, and 24 h after eccentric exercise. There was a significant main effect in the ANOVA for target force (F_{5,138} = 49.22, P < 0.001) and exercise state (F_{2,141} = 4.23, P < 0.01). Across all force targets, eccentric exercise increased EMG activity (expressed as a percentage of the maximum EMG recorded during the most recent MVC) from 10.6 ± 1.8% before to 22.4 ± 3.1% after eccentric exercise (P < 0.001). The EMG activity was still elevated 24 h after eccentric exercise (16.4 ± 3.0%, P < 0.01) compared with before eccentric exercise, but was less than that recorded immediately after eccentric exercise (P = 0.001). A significant exercise state × target force interaction was also present in the ANOVA (F_{10,84} = 2.93, P < 0.01). Average biceps brachii EMG measured immediately after eccentric exercise was 3 times greater at 20% target force, 2.3 times greater at 35% target force, and 1.7 times greater at the 50% target force compared with the EMG at the same target force before eccentric exercise (P < 0.001; Fig. 1D).
eccentric exercise (Fig. 2A). Biceps brachii EMG was also significantly larger immediately after eccentric exercise compared with 24 h after eccentric exercise at the 35% target force and significantly larger 24 h after eccentric exercise compared with before eccentric exercise at the 50% target force (Fig. 2A).

**Force fluctuations after eccentric exercise**

Force fluctuations were quantified as the CV of force at each of the submaximal target forces before, immediately after, and 24 h after eccentric exercise (Fig. 2B). There was a significant main effect in the ANOVA for target force ($F_{5,138} = 34.3, P < 0.001$) and exercise state ($F_{2,141} = 14.0, P < 0.0001$). For all target forces combined, post hoc analysis indicated that the CV of force was significantly greater immediately after eccentric exercise (3.7 ± 0.2%) compared with before eccentric exercise (2.1 ± 0.1%, $P < 0.001$) and 24 h after eccentric exercise (2.9 ± 0.3%, $P < 0.001$). The CV of force was also significantly greater 24 h after eccentric exercise compared with before eccentric exercise ($P < 0.001$). A significant exercise state × target force interaction was also present in the ANOVA ($F_{10,84} = 6.28, P < 0.001$). CV of force was significantly greater immediately after eccentric exercise compared with before eccentric exercise at all target force levels, with the largest differences occurring at the low forces (2 times greater at 2.5% MVC, 1.5 times greater at 50% MVC). Elbow flexor force fluctuations also remained significantly elevated 24 h after eccentric exercise compared with before eccentric exercise, but only at the low force levels (2.5 and 5% MVC).

**Motor unit recordings**

Figure 3 shows recordings obtained during the motor unit task before and after eccentric exercise in one subject. In this subject, 100 eccentric contractions with a 9.6-kg load induced a 40% reduction in MVC force that recovered to a 25% reduction in MVC 24 h after eccentric exercise. To activate the pair of motor units throughout the trial, the subject exerted a mean force of 7.2 N (3.4% MVC) before exercise and 3.9 N (2.9% MVC) after exercise. Despite the decline in force, the mean biceps brachii EMG increased from 3.0% (0.19 mV) of maximum EMG before eccentric exercise to 4.3% of maximum EMG (0.27 mV) after eccentric exercise. The mean motor unit firing rate before eccentric exercise was 9.0 (motor unit 1) and 8.0 Hz (motor unit 2), whereas it was 9.9 (motor unit 3) and 11.1 Hz (motor unit 4) after eccentric exercise. Mean motor unit discharge rate variability was not influenced by eccentric exercise in this subject and was 12.9 (motor unit 1) and 10.1% (motor unit 2) before eccentric exercise, whereas it was 13.0 (motor unit 3) and 10.3% (motor unit 4) after eccentric exercise. The synchronization index CIS was 0.45 for the motor unit pair studied before eccentric exercise and 0.70 for the pair studied after eccentric exercise (Fig. 3G).

For the motor unit task, a total of 296 motor unit pairs (376 individual motor units) were obtained from eight subjects in all recording sessions, with 105 motor unit pairs obtained before eccentric exercise, 92 motor unit pairs immediately after eccentric exercise, and 99 motor unit pairs obtained 24 h after eccentric exercise. A similar number of motor unit pairs were recorded in each subject in each of the three recording sessions: this was ~13 motor unit pairs per subject before eccentric exercise, 11 motor unit pairs immediately after eccentric exercise, and 12 motor unit pairs 24 h after eccentric exercise. The number of motor unit pairs exhibiting significant short-term synchronization was 58 (55%) before, 65 (71%) immediately after, and 69 (70%) 24 h after eccentric exercise.

Figure 4 shows the discharge characteristics from pairs of motor units obtained before, immediately after, and 24 h after eccentric exercise. The mean discharge rate was influenced by exercise state (Fig. 4A; $F_{2,201} = 10.75, P < 0.001$), but the mean discharge rate variability was not (Fig. 4B; $F_{2,201} = 1.29, P = 0.28$). Post hoc analysis indicated that the mean discharge rate was 11% (1.5 Hz) greater immediately after eccentric exercise compared with before eccentric exercise ($P < 0.0001$). The mean discharge rate was still significantly elevated by 5% (0.5 Hz) 24 h after eccentric exercise compared with before eccentric exercise ($P < 0.05$; Fig. 4A), but this was significantly lower than the rate immediately after eccentric exercise ($P < 0.01$). For motor units discharging at the lower rate in each pair, the discharge rate immediately after eccentric exer-
cise (12.0 ± 0.3 Hz) was significantly greater than the discharge rate before eccentric exercise (11.0 ± 0.2 Hz, \( P < 0.001 \)) or 24 h after (11.3 ± 0.2 Hz, \( P < 0.05 \)) eccentric exercise. Similarly, for the motor units firing at the higher rate in the pair, the discharge rate immediately after (13.4 ± 0.3 Hz, \( P < 0.001 \)) and 24 h after eccentric exercise (12.6 ± 0.2 Hz, \( P < 0.01 \)) was significantly greater than before eccentric exercise (11.8 ± 0.2 Hz).

Motor unit synchronization

All 296 motor unit pairs were included in the analysis of the strength of motor unit synchronization (Fig. 4, C and D). For those pairs lacking a significant peak, synchrony was quantified over 11 bins centered at time 0. The mean strength of motor unit synchronization was influenced by exercise state for the synchronization index CIS (Fig. 4C, \( F_{2,293} = 8.29, P < 0.001 \)) and the index E (Fig. 4D, \( F_{2,293} = 7.95, P < 0.001 \)). The synchrony index CIS increased by 30% (0.13 pps) at both time points after eccentric exercise (Fig. 4C), whereas the synchronization index E increased by 26% (0.009 ppt) immediately after and by 30% (0.010 ppt) 24 h after eccentric exercise (Fig. 4D). The duration of the significant synchronous peaks (data not shown) was not influenced by eccentric exercise (\( F_{2,293} = 0.31, P = 0.73 \)). Mean duration of significant synchronous peaks was 18.8 ± 1.0 ms before (\( n = 58 \)), 18.4 ± 0.9 ms immediately after (\( n = 65 \)), and 18.1 ± 1.0 ms 24 h after eccentric exercise (\( n = 69 \)). The mean force exerted during the motor unit trials was significantly lower immediately after (7.3 ± 1.4 N, 7% MVC, \( P < 0.001 \)) and 24 h after eccentric exercise (10.3 ± 2.3 N, 7% MVC, \( P < 0.001 \)) compared with before eccentric exercise (22.6 ± 3.4 N, 11% MVC).

Regression analysis was used to examine the association between motor unit discharge rate and the strength of motor unit synchronization throughout the three recording sessions. No significant association was observed between motor unit discharge rate and the strength of motor unit synchronization with the index CIS or E when obtained before or after eccentric exercise. However, a weak but significant positive association was found between the synchrony index CIS and motor unit discharge rate 24 h after eccentric exercise (\( r^2 = 0.131, P < 0.001 \)).
Motor unit coherence

Coherence analysis was performed on the same 296 pairs of motor units used for cross-correlation analysis obtained before, immediately after, and 24 h after eccentric exercise (Fig. 5). For the coherence analysis, the number of 1.28-s data segments used was similar for all three sessions and was 85 ± 3 segments before eccentric exercise, 88 ± 3 segments after eccentric exercise, and 85 ± 2 segments 24 h after eccentric exercise. The typical pattern of coherence observed in biceps brachii motor units includes a single large-amplitude, low-frequency peak that occurs from 0 to 10 Hz, with minimal coherence above 10 Hz (Fig. 5A). Statistically significant coherence that occurred above the 95% CI was observed from 0 to 6 Hz when measured before, immediately after, and 24 h after eccentric exercise. When averaged for each individual motor unit pair, the peak frequency (exercise state, \( F_{2,293} = 3.2, P < 0.05 \)) was significantly higher 24 h after eccentric exercise (1.9 Hz) compared with before (1.5 Hz, \( P < 0.05 \)) eccentric exercise, but was not different from the peak frequency immediately after eccentric exercise (1.7 Hz). For statistical comparison, the mean coherence z scores are shown for the low (0–10 Hz) and high (10–30 Hz) frequency bands (Fig. 5B). A significant interaction in the ANOVA was present for exercise state × frequency band (\( F_{2,586} = 7.22, P < 0.001 \)). Post hoc analysis indicated that the mean coherence z scores from 0 to 10 Hz increased by 20% (\( P < 0.001 \)) immediately after eccentric exercise and by 34% (\( P < 0.001 \)) 24 h after eccentric exercise compared with before eccentric exercise. No significant difference was found for mean coherence z scores at 10–30 Hz between testing sessions.

For all motor unit pairs, linear regression analysis revealed a significant association between motor unit synchronization and high-frequency (10–30 Hz) coherence (\( r^2 = 0.20, P < 0.001 \)). The association was greatest 24 h after eccentric exercise (\( r^2 = 0.23 \)), intermediate after eccentric exercise (\( r^2 = 0.19 \)), and least before eccentric exercise (\( r^2 = 0.10, \) all \( P \leq 0.001 \)). No significant associations were found between motor unit synchrony and 0- to 10-Hz coherence at any time point.

DISCUSSION

Eccentric exercise was used in this study to examine the neural adjustments following exercise-induced muscle damage. In line with previous reports (Lavender and Nosaka 2006; Leger and Milner 2001b; Semmler et al. 2007), we showed that...
An assessment of motor unit synchronization was performed in this study by cross-correlation analysis of the individual discharge times of pairs of concurrently active motor units. Motor unit synchronization analysis is a powerful technique that reveals subtle functional connections within the human CNS and their role in the execution of voluntary movements. For example, motor unit synchronization is lower during index finger flexion compared with abduction (Bremner et al. 1991), but is greater during lengthening compared with shortening muscle contractions (Semmler et al. 2002). These findings are interpreted as a change in the number and/or excitability of branched corticospinal inputs used to perform these tasks (Farmer et al. 1990; Schmied et al. 1999). However, most measurements of the strength of motor unit synchronization in humans are performed in hand or wrist muscles, which have strong corticospinal projections to motor neurons (Clough et al. 1968; Phillips and Porter 1964). In contrast, corticospinal inputs to biceps brachii motor neurons are generally weaker than in hand muscles (Palmer and Ashby 1992), and this is reflected in reduced synchrony in proximal muscles (Kim et al. 2001). We also found that the strength of motor unit synchronization in biceps brachii was weak, with values similar to those obtained previously (Keen and Fuglevand 2004). After eccentric exercise, we observed a ~30% increase in the strength of motor unit synchronization assessed using two commonly used indices (CIS and E; Fig. 4). Although we also found a significant (~11%) increase in motor unit discharge rate immediately after exercise, this was not responsible for the increased synchrony, because there was no association between motor unit discharge rate and synchronization before or immediately after eccentric exercise using either index.

For voluntary isometric contractions of fresh muscle, the prevailing view is that motor unit synchronization arises from common synaptic input received by motor neurons from either branched last-order interneurons or cortical efferents that receive common input (Datta et al. 1991; Farmer et al. 1990, 1993b; Mantel and Lemon 1987; Moritz et al. 2005; Schmied et al. 1999), but does not seem to be influenced by peripheral afferents (Farmer et al. 1993a, 1997). However, the mechanisms responsible for an increase in motor unit synchronization after eccentric exercise are less clear, because the exercise-induced muscle damage is likely to cause physiological adjustments within the muscle that influence both the spinal and cortical control of movement. For example, the decline in force immediately after eccentric exercise is caused not only by muscle damage but also is likely to involve neuromuscular fatigue from the repetitive eccentric contractions. It is a widely accepted view that, along with changes in cortical and spinal excitability, fatigue is associated with an increase in motor unit synchronization (Gandevia 2001). However, this view is based on computer simulations (Kleine et al. 2001; Yao et al. 2000) or experimental studies using indirect assessments from the surface EMG (Farina et al. 2002), which are likely to be misleading (Farina et al. 2004). Nonetheless, a decline in maximum voluntary isometric force from fatigue recovers within 2 h (Walsh et al. 2004), and we observed increased motor unit synchronization 24 h after exercise, suggesting that fatigue is not responsible for the increased motor unit synchronization 24 h after eccentric exercise. Similarly, muscle pain...
can result in altered cortical and spinal excitability (LePera et al. 2001), and DOMS could conceivably alter motor unit synchronization by afferent inputs acting at both these levels. However, synchrony was enhanced immediately after eccentric exercise, as well as 24 h later, whereas DOMS was only present 24 h after eccentric exercise. This suggests that pain is not the primary mechanism driving the change in motor unit synchronization after eccentric exercise.

The increase in motor unit synchronization was observed immediately after eccentric exercise and remained elevated 24 h later, suggesting that the factors responsible for this change are likely related to the effects of muscle damage that are present at both of these time points. Muscle spindle afferents, which provide feedback to the CNS about mechanical changes in the muscle, are a potential source of increased motor unit synchronization because of their wide divergence of inputs to motor neurons and interneurons in the spinal cord (Mendell and Henneman 1971). There is increased reliance on sensory feedback during changes in muscle length (Burke et al. 1978), and there is increased motor unit synchronization during lengthening contractions (Semmler et al. 2002), so it is possible that changes in muscle spindle afferents as a result of eccentric exercise could contribute to the observed increase in motor unit synchronization. However, it has been shown previously in the anesthetized cat that there is no change in the spindle afferent response following a series of eccentric contractions that lead to substantial muscle damage, suggesting that muscle spindles are spared from damage during eccentric exercise (Gregory et al. 2004). An alteration in fusimotor drive that increases the sensitivity of the muscle spindle after eccentric exercise could enhance spindle afferent feedback onto motor neurons. This seems unlikely because the available evidence indicates a reduced stretch reflex sensitivity after stretch-shortening cycle exercise that induces muscle damage (Avela et al. 1999).

Finally, an increase in motor unit synchronization could be caused by common inhibitory inputs to the motor neurons (Turker and Powers 2001), although this is likely to broaden the width of the central synchronous peak, which was not observed in this study. Nonetheless, alterations in recurrent inhibition, which is particularly biased toward low threshold motor units (Katz and Pierrot-Deseilligny 1999), could induce changes in common inhibitory inputs to motor neurons at the spinal level after exercise. For example, the injection of l-acetylcarcarnitine induces a reversible increase in recurrent inhibition (Mazzocchio and Rossi 1997), which results in an increase in motor unit synchronization (Del Santo et al. 2006; Mattei et al. 2003), but it is not known if there is a change in recurrent inhibition after exercise-induced muscle damage. Furthermore, a similar effect could arise from reciprocal inhibition, because we have shown an increase in antagonist muscle co-activation after eccentric exercise using an experimental design similar to this study (Semmler et al. 2007). At present, the cause of the increased motor unit synchronization is unknown, but it is likely to include an interaction between several different mechanisms that could produce excitatory and/or inhibitory effects acting within the CNS.

Motor unit coherence

Motor unit coherence is a frequency domain measure of the strength of common oscillatory input to the motor neurons that is believed to originate in cortical and subcortical areas, including the motor cortex (Farmer et al. 1993a; McAuley et al. 1997; Rosenberg et al. 1989). Measurements of single motor units during low-force isometric contractions of intrinsic hand muscles have previously established that motor neurons receive common rhythmic inputs in the frequency bands 1–12 and 16–32 Hz (Farmer et al. 1993a). Several studies have shown an association between motor unit synchronization and high-frequency coherence (Farmer et al. 1993a; Halliday et al. 1999; Semmler et al. 2004), suggesting that both these features of correlated motor unit activity arise from similar mechanisms. These are likely to include corticospinal inputs (Datta et al. 1991; Farmer et al. 1993b). However, the coherence spectrum for biceps brachii motor units is not the same as it is in hand muscles. For example, in biceps brachii motor units, we found a large-amplitude, low-frequency oscillation that peaks at 1–2 Hz, with minimal high-frequency coherence, which is in agreement with previous studies (Farmer et al. 1993a; Kim et al. 2001). These findings suggest that the relative contribution of the oscillatory inputs at low and high frequencies is different in arm and hand muscles, and supports the observations of a reduced corticospinal projection to proximal as opposed to distal muscles (Clough et al. 1968; Phillips and Porter 1964). Nonetheless, we did observe a weak but significant association between motor unit synchronization and high-frequency (10–30 Hz) coherence that was stronger after eccentric exercise, suggesting that the weak inputs generating synchronization in biceps brachii also contribute to high-frequency coherence. Despite this association, no significant increase in 10- to 30-Hz coherence in biceps brachii motor units was observed after eccentric exercise, possibly because of a shift in high-frequency coherence that included values >30 Hz that were not quantified in the analysis.

Although the motor unit coherence values obtained in this study were relatively weak (Fig. 5), it has been shown previously that an average of ~20% (and ≤40%) of the tremor signal can be accounted for by oscillatory motor unit activity at 1–12 and 15–30 Hz in distal muscles, even when the low-frequency component of the pooled motor unit coherence was <0.1 (Halliday et al. 1999). Furthermore, theoretical analysis based on data from the first dorsal interosseous muscle (FDI) has shown that motor unit coherence makes a significant contribution to tremor when the motor unit–motor unit coherence is >0.03 (Christakos et al. 2006). These findings suggest that the pooled coherence values obtained in this study may still be functionally relevant, particularly if the oscillatory activity is occurring over a broad population of motor units. This is particularly relevant considering that the biceps brachii contains ~5 times more motor units than intrinsic hand muscles (Johnson et al. 1973), which increases the probability of correlated motor unit discharges in different motor unit pairs. In contrast, the high-frequency coherence between 10 and 30 Hz observed in biceps brachii in this study was minimal, and we suggest that it is not of sufficient magnitude to be functionally relevant.

Along with motor unit synchronization, motor unit coherence can be modulated according to the requirements of the task. Reduced oscillatory input to motor neurons has been observed during concentric contractions (Kakuda et al. 1999; Semmler et al. 2002), and is enhanced during a precision grip task when the compliance of the manipulated object is in-
creased (Kilner et al. 2002). However, these studies involve alterations in motor unit coherence at frequencies >5 Hz, which is likely to include inputs from stretch reflex mechanisms (6–12 Hz; Christakos et al. 2006), corticospinal cells (10–30 Hz; Farmer et al. 1993a), and the sensorimotor cortex (20–40 Hz; Murthy and Fetz 1996). In contrast, we found increased low-frequency (0–10 Hz) coherence, with a peak frequency of 1–2 Hz, immediately after eccentric exercise and 24 h later. Motor unit coherence at these frequencies may reflect differences in a feature of the central command known as “common drive” (De Luca et al. 1982), which is considered a source of the low-frequency oscillations that are manifested in the smoothed firing rates of concurrently active motor units (De Luca and Erim 1994; Lowery et al. 2007; Myers et al. 2004). Although the origin of low-frequency coherence has not been identified, it is unlikely to arise from muscle spindle afferents, because common drive has been observed in the orbicularis oris muscle of the lower lip that is accepted as containing no muscle spindles (Kamen and De Luca 1992). Several studies have shown that low-frequency (0–15 Hz) coherence is not correlated with motor unit synchronization (Farmer et al. 1993a; Halliday et al. 1999; Kilner et al. 2002; Semmler et al. 2002, 2004), and it has been observed following cortical and capsular stroke, suggesting a possible subcortical origin (Farmer et al. 1993a). Therefore the increased low-frequency coherence that was observed immediately after and 24 h after exercise-induced muscle damage is likely to be caused by CNS adjustments above the level of the spinal cord but not including the corticospinal pathway.

Mechanisms of increased EMG and force fluctuations

It is now well established that eccentric exercise results in an increase in EMG and force fluctuations during submaximal contractions (Lavender and Nosaka 2006; Leger and Milner 2001a; Saxton et al. 1995; Semmler et al. 2007). While there is no doubt that eccentric exercise results in damage to muscle fibers, it is important to note that a reduced contribution from single motor units to the net force is not responsible for the increase in EMG, because the submaximal target forces are normalized to the new (lower) maximum force capability of the muscle. The increased EMG and force fluctuations are greatest at low forces (Semmler et al. 2007), which suggests that adjustments in motor unit activation play an important role in this phenomenon. Several computer simulation studies suggest that motor unit synchronization can alter the amplitude of the EMG signal because of summation of motor unit potentials at the surface of the muscle (Kleine et al. 2001; Yao et al. 2000; Zhou and Rymer 2004). We have shown that there is an increase in the correlated activity of motor units during submaximal contractions after exercise-induced muscle damage and that this persists for 24 h. However, the partial recovery of EMG and force fluctuations at 24 h suggests that correlated motor unit activity plays only a minor role in the EMG and force changes after eccentric exercise.

The physiological factors that can influence the amplitude of the EMG include the timing of the motor unit action potentials and the membrane properties of the muscle fibers (see Farina et al. 2004). During sustained contractions that induce muscle fatigue, a decrease in the muscle fiber conduction velocity will have a profound effect on the EMG (Bigland-Ritchie et al. 1981; Stalberg 1966), because there will be a greater overlap between action potentials and increased action potential summation (Keenan et al. 2005). Furthermore, the electrophysiological properties of the muscle fibers are likely to change because of the membrane damage resulting from eccentric exercise, which will contribute to this effect. The possibility exists that an increase in motor unit synchronization, combined with a prolonged action potential duration (caused by muscle fatigue and damage), results in a more than proportional increase in EMG that is experienced immediately after the eccentric exercise (Keenan et al. 2006; Yao et al. 2000). The rapid recovery of the muscle fiber membrane properties from fatigue would suggest that the small increase in EMG observed 24 h after eccentric exercise may be caused only by the effects of increased correlated motor unit activity. The role of changes in the muscle fiber membrane properties to the increased EMG after exercise-induced muscle damage remains to be explored.

In conclusion, we recorded from single motor units in the biceps brachii muscle to examine correlated motor unit activity after exercise-induced muscle damage. Substantial changes in motor unit activity were found after eccentric exercise, which included an increase in motor unit synchronization and low-frequency (0–10 Hz) coherence, suggesting that one or more factors associated with muscle damage contribute to the increased correlated behavior of biceps brachii motor units. However, the change in correlated motor unit activity did not match the recovery of EMG and force fluctuations 24 h after exercise, suggesting that it plays only a minor role in the impaired neuromuscular performance after exercise-induced muscle damage. The mechanisms responsible for the change in correlated motor unit activity with exercise-induced muscle damage remain to be determined.

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