Time-course of human motoneuron recovery
after sustained low-level voluntary activity

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ABSTRACT

Motoneurons often fire repetitively and for long periods. In sustained voluntary contractions the excitability of motoneurons declines. We provide the first detailed description of the time-course of human motoneuron recovery after sustained activity at a constant discharge rate. We recorded the discharge of single motor units (MUs, n=30) with intramuscular wire electrodes inserted into triceps brachii during weak isometric contractions. Subjects (n=15) discharged single motor units at a constant frequency (~10 Hz) with visual feedback for prolonged durations (3-7 minutes) until rectified surface electromyogram (sEMG) of triceps brachii increased by ~100%. After a rest of 1-2, 15, 30, 60, 120 or 240 s, subjects briefly resumed the contraction with the target MU at the same discharge rate. Each MU was tested with 3-4 rest periods. The magnitude of sEMG was increased when contractions were resumed and the target motoneuron discharged at the test frequency following rest intervals of 2-60 s (p = 0.001-0.038). The increased sEMG indicates that greater excitatory drive was needed to discharge the motoneuron at the test rate. The increase in EMG recovered exponentially with a time-constant of 28 s, but did not return to baseline even after a rest period of ~240 s. Thus, the decline in motoneuron excitability from a weak contraction takes several minutes to recover fully.

Abbreviations: sEMG, surface electromyography; MU, motor unit; MVC, maximal voluntary contraction; CI, confidence interval; ISI, interspike interval
INTRODUCTION

Motoneurons are the final common pathway of the motor system and are involved in every motor action, whether voluntary, involuntary, or reflex. Each motoneuron discharges at a frequency proportional to the synaptic current reaching the axon initial segment (Brownstone 2006). The size of this current depends on both intrinsic and extrinsic motoneuronal properties (for review Powers and Binder 2001; Nordstrom et al. 2007). In humans, studies involving maximal or submaximal voluntary contractions have provided insight into the nature and time-course of spinal and supraspinal factors that contribute to muscle fatigue (Gandevia 2001; Taylor and Gandevia 2008), including an important role of changes in intrinsic motoneuron properties (McNeil et al. 2009, 2011a, b).

Although human skeletal muscles are active only part of the day and usually contract at low intensities that do not induce fatigue (Kern et al. 2001), there is evidence that motoneurons involved in such contractions may nevertheless require increasing neural drive to maintain a constant level of output (Johnson et al. 2004; Riley et al. 2008). If synaptic input does not increase, motoneuron discharge rates will fall and their activity may eventually cease. Several lines of evidence indicate that this need for additional synaptic input caused by sustained activity reflects a progressive decline of intrinsic motoneuron excitability (Granit et al. 1963; Kernell 1972; Kernell and Monster 1982; Sawczuk et al. 1995; Powers et al. 1999). In reduced animal preparations, injection of constant current into the motoneuron soma or adjacent extracellular space generates an initially high discharge rate, which declines over a period of seconds to minutes (Granit et al. 1963; Kernell 1972; Kernell and Monster 1982; Speilmann et al. 1993). This phenomenon, termed spike-frequency adaptation, renders the motoneuron unable to sustain discharge activity without additional excitatory synaptic input (Powers et al. 1999; Nordstrom et al. 2007).

In humans, one cannot directly inject motoneurons with constant current to study spike-frequency adaptation. However, an inverse approach has been used to investigate human
motoneuron excitability in low-intensity contractions. Specifically, the discharge rate of a
motoneuron is maintained voluntarily and the amount of synaptic input or 'current' to the
motoneuron is estimated from the global excitatory drive to the motoneuron pool, derived from the
surface electromyogram (sEMG) (Johnson et al. 2004). To maintain a discharge rate, excitatory
neural drive has to increase and provide additional excitatory synaptic input if the excitability of the
motoneuron decreases during a contraction. Increased excitatory neural drive is also expected to
increase sEMG amplitude due to the recruitment of higher threshold motor units (MUs) and the
increase in MU discharge rate of motoneurons that do not have reduced excitability. With evidence
of MUs with higher and higher thresholds being recruited, sEMG amplitude becomes an indirect
measure of neural drive to the motoneuron pool and, therefore, the excitability of the motoneuron
whose discharge rate is clamped. Using this approach to study the first dorsal interosseous muscle,
Johnson and colleagues (2004) found sEMG increased ~25% during sustained low-intensity
contractions when the discharge rate of a MU was maintained. They concluded that human
motoneurons require progressively greater excitatory synaptic input to sustain a discharge rate and
argued this reflects intrinsic motoneuron fatigue.

When low-intensity contractions are maintained at a constant force for several minutes, the
decline in motoneuron excitability may cause MUs to stop their activity for seconds to minutes and
they are then re-recruited (Bawa et al. 2006; Bawa and Murnaghan 2009). There is preliminary
evidence that this break in MU activity allows intrinsic motoneuron processes to recover. Manning
and colleagues (2010) used transcranial magnetic stimulation and H-reflex stimulation and found
the decline in motoneuron excitability to be greater in the first half of these recovery periods, which
lasted on average 385 s. While advances have been made in understanding the development and
recovery of neuro-muscular fatigue (Gandevia 2001; Taylor and Gandevia 2008), much less is
known about how motoneuron excitability recovers from low-intensity contractions despite these
being much more common than strong fatiguing contractions.
The purpose of the present study was to derive the precise time-course of human motoneuron recovery after sustained low-intensity voluntary activity at a constant discharge rate. We used sEMG to estimate the amount excitatory neural drive required by single triceps brachii MUs to resume the same discharge rate at various times after sustained contractions. We hypothesized that motoneuron excitability would be reduced immediately after a sustained contraction and would recover over time. The aim of our studies was to determine the time course of the recovery of motoneurons after sustained activation.

METHODS

Fifteen subjects (5 female, 10 male, age 34.9 [22-60] yrs (mean [range])) with no known history of neurological disease or musculoskeletal injury to the right upper extremity participated in the study. Thirteen subjects participated in experiment 1 and 10 subjects participated in experiment 2, 8 of whom also participated in experiment 1. The experimental protocol was explained and written informed consent was obtained prior to the study. All study procedures conformed to the Declaration of Helsinki (2008) and were approved by the Human Research Ethics Committee at the University of New South Wales.

Experimental set-up.

Single MU activity was recorded with intramuscular electrodes custom-made from two 0.05 mm insulated stainless steel wire strands (California Fine Wire, Grover Beach, USA) wound together and threaded into a 1.5 inch 25-gauge hypodermic needle. The distal tips of the two wires were cut to expose their non-insulated cross-section (i.e. 0.002 mm²) and folded back to create two 1-mm barbs. After the skin was cleaned, the intramuscular electrode was inserted to a depth of ~1 cm in the mid-line of the lateral head of the right triceps brachii, 12-15 cm proximal to the olecranon. A pair of Ag-AgCl electrodes (Conmed ClearTrace ECG Sensor Electrodes, Utica, NY, USA) were placed laterally on either side of the intramuscular electrode insertion site (5 cm inter-electrode distance) to record sEMG activity from a larger sample of triceps brachii MUs (see...
A ground electrode was placed over the upper arm. Single MU activity was filtered (band-pass 30-5,000 Hz) and amplified (×3,000-10,000) with an isolated pre-amplifier and head stage (1902-10, 1902, Cambridge Electronics Design, Cambridge, UK) and digitised at 10,000 Hz using a 12-bit CED DAQ-card and Spike2 software (1401-Power, Cambridge Electronics Design, Cambridge, UK). The output of the amplifier was also connected to an audio amplifier and a window discriminator (DIS-1, BAK Electronics, Inc., Sandford, FL). The audio amplifier provided auditory feedback about the discharge rate of the target MU to the experimenters and subject. The window discriminator was used with a storage oscilloscope to discriminate the target MU on-line and send a TTL pulse for each identified MU action potential. Pulses were recorded with the DAQ-card and processed to give the subject visual feedback about the instantaneous discharge rate of the target MU (Fig. 1A) on a monitor located ~60 cm in front of the subject at eye level. The sEMG signal was filtered (16-1000 Hz), amplified (×2,000-10,000) and digitized (5,000 Hz) with the same system. Subjects sat at a table with their right arm supported in supination, 90° of shoulder flexion, and 100° of elbow flexion. The arm was fixed at the wrist to a stationary bar and the hand was relaxed and supported in a neutral wrist position. Isometric elbow extension force was measured in experiment 1. However, the low intensity of the contractions (18/20 subjects contracted at ≤ 4% maximum) meant that the force measures were susceptible to distortions from slight postural adjustments or breathing. Therefore, we did not analyse force data as it was not reliable and force was not recorded in experiment 2.

**Experimental protocol.**

In each experiment, subjects initially performed a slow elbow extension ramp contraction as experimenters monitored the intramuscular EMG signal for activity from the earliest recruited MU with action potentials sufficiently large and distinct in shape to be easily sorted with the window discriminator. Once this MU was recruited and identified, subjects adjusted the intensity of the contraction so that the target MU had a stable instantaneous discharge rate that could be maintained
for a prolonged duration. This target discharge rate was 1-3 Hz above the discharge rate at recruitment. Next, a 5-min rest was provided.

Experiment 1.

Subjects performed four trials. In the first trial, subjects produced an isometric elbow extension contraction with the target MU at the previously identified target rate. On the monitor, 10 s of instantaneous discharge rate data was continuously visible. The vertical scale of the display was 0-20 Hz, and there were two horizontal markers that indicated a band ± 0.5 Hz above and below the target discharge rate (Fig. 1A, B). Subjects were asked to maintain the instantaneous discharge rate of the target MU as precisely as possible within this target band. As subjects held the discharge rate for a few minutes, the overall amount of triceps brachii muscle sEMG activity increased and additional MUs appeared in the intramuscular recording. Subjects maintained the discharge rate of the target MU until instructed to stop. This instruction was given when the amplitude of rectified sEMG reached 2 times that at baseline or when the target MU could no longer be tracked reliably with the window discriminator, which sometimes occurred before sEMG amplitude doubled. The baseline, or start period, was defined as the initial 15 s of the trial when the target discharge rate was first reached. After subjects stopped their contraction, they were instructed to remain completely relaxed for a preset duration (30, 60, 120 or 240 s). Then, subjects resumed their contraction for an additional 30 s with the same target discharge rate of the target MU. Subjects then stopped the recovery contraction and had a 10 min rest before the start of the next trial. Each subsequent trial was performed in the same manner except that the duration of sustained contractions was set to the duration of the first trial. In most subjects the increase in sEMG amplitude in these subsequent trials was comparable to the increase in the first trial. Across the four trials, the duration of rest period between the sustained contraction and the subsequent recovery contraction (i.e. 30, 60, 120 and 240 s) were randomized across subjects. Once all trials were completed, subjects performed two brief maximal voluntary contractions (MVCs; 4-5 s, separated
by 2 minutes of rest) of the elbow extensor muscles to assess triceps maximal sEMG.

**Experiment 2.**

Subjects performed three trials identical to those in experiment 1 with the exception that the relaxation periods were 1-2, 15 and 30 s. Once the target MU was identified, but before the 5-min rest period that preceded the first trial, subjects were asked to practice the 1-2 s relaxation period several times. It was important that subjects completely relax their triceps brachii muscle after the sustained contraction and then quickly resume the isometric contraction with the same discharge rate of the target MU. Across the three trials, the relaxation periods between the prolonged sustained contraction and the subsequent recovery contraction (i.e. after rest periods of 1-2, 15 and 30 s) were randomized across subjects. Similar to experiment 1, subjects performed two brief isometric MVCs of the elbow extensor muscles after all trials were completed.

**Data analysis.**

For each trial, action potentials from the target MU were extracted with Spike2 software (Cambridge Electronic Design, Cambridge, UK). The algorithm identified MUs on the basis of size, shape, and timing. The discharge times of the target MU were manually reviewed to include unmatched action potentials and solve for instances in which two MU discharges were superimposed. Spike times were compared to recorded digital triggers from the window discriminator to ensure subjects were provided with accurate visual and audio feedback during the experiment. Once sorted, MU action potentials and discharge times from the target MU, as well as sEMG signals from the triceps brachii muscle were exported to Matlab (Mathworks, Natick, MA) for further analysis.

For each trial, mean sEMG signal was removed and then the signal was filtered (4th order, 40-450 Hz band-pass, zero-lag Butterworth) and rectified. The 400 ms window with the largest amplitude of rectified sEMG was determined across MVC trials and this value was used to normalize the rectified sEMG of sustained contraction trials. Next, for each trial, three 15 s periods
were identified and used to compute outcomes. The start period was the initial 15 s of the sustained contraction, the end period was the final 15 s of the sustained contraction, and the recovery period was the initial 15 s when the isometric contraction was resumed after the relaxation period. We calculated the mean rectified sEMG amplitude in each of the start, end and recovery periods. Additional measures were also calculated to determine whether the time-course of MU recovery was associated with characteristics of the sustained contractions or MU discharge activity. First, contraction duration and total MU discharge count were determined for each sustained contraction. Second, the mean discharge rate and the spike-to-spike variability (i.e. interspike interval coefficient of variation) of the target MU was determined for the start period of each trial. Spike-to-spike variability was used because it tends to be greater when MUs are active closer to their recruitment threshold, and MUs active closer to this recruitment threshold may undergo less intrinsic motoneuron fatigue with sustained activity (Riley et al. 2008). To confirm that the increase in sEMG amplitude during sustained contractions was associated with an increase in excitatory neural drive to the motoneuron pool of the elbow extensors, we measured the number of active MUs (action potential peak-to-peak amplitude > 20 μV) in the start and end periods of each trial. The number of recorded MUs was also determined for the recovery period of each trial to determine whether this measure of neural drive followed the same general pattern as sEMG amplitude. Because a change in the electrical properties of active muscle fibres can also lead to an increase in sEMG amplitude (Farina et al. 2004), we measured the MU action potential characteristic that best reflects these changes, namely, MU action potential duration (Fuglevand et al. 1989; Keenan et al. 2005). The average width of the action potential associated with the target MU was determined for the start and end periods of each trial.

Statistical analysis.

Descriptive parametric statistics (mean, 95% CI) and non-parametric statistics (median, interquartile range) were calculated to summarize group results and generate figures. One-way repeated-
measures ANOVA were performed to verify there were no differences in mean MU discharge rate, spike-to-spike variability, and sEMG amplitude across the start periods of the trials, and to verify there were no differences in the total number of MU discharges, the duration of sustained contractions and end : start sEMG ratio values across the sustained contractions for each trial. For these analyses, data from experiment 1 and experiment 2 were analyzed separately. One-sample t-tests were also used to determine whether differences in mean discharge rate between the start and recovery periods of each trial from experiment 1 and experiment 2 were significantly different from zero. This analysis was done to verify that the mean discharge rate of the target MU was similar in the start and recovery periods.

Ratios were calculated between mean rectified sEMG in the recovery and start periods (recovery : start). A ratio of 1.0 indicates that the mean rectified EMG amplitude was identical in the recovery and start periods. A ratio > 1.0 indicates that mean rectified EMG amplitude was greater in the recovery period compared to the start period (i.e., greater excitatory neural drive required to maintain the same discharge rate), whereas a ratio < 1.0 indicates that mean rectified EMG amplitude was greater in the start period. For each trial, a one-sample t-test was used to determine whether the ratio value was significantly different from 1.0. The same statistical analyses were performed for experiment 1 and experiment 2.

Double exponential decay functions have previously been used to model the time-course of spike-frequency adaptation (Sawczuck et al. 1995; Speilmann et al. 1993). It was not possible to capture the earliest motoneuron recovery (< 1 s), thus the time-course of motoneuron recovery was likely to be best described by a single exponential decay function. The ratio of recovery : start sEMG amplitude from experiment 1 and experiment 2 were combined and the best fit single exponential function was determined:

\[ \text{EMG amplitude ratio} = \text{ratio}_{240} + K \times \text{exp}(-t / \tau) \]

where \( \text{ratio}_{240} \) is the ratio of recovery : start sEMG amplitude for the 240 s rest trial, \( K \) is the
Correlation analysis was used to investigate the possible relationship between the ratio of recovery : start sEMG amplitude and characteristics of the sustained contractions or discharge activity of the target MU, including the length of the sustained contraction, the number of MU discharges during the sustained contraction, mean MU discharge rate, MU spike-to-spike variability, and the ratio of end : start sEMG amplitude. Pearson product-moment correlation coefficients were calculated for each trial (i.e. 1-2 s to 240 s rest periods). Data from the 30 s rest trials from experiment 1 and experiment 2 were pooled for this analysis. A Bonferroni correction was applied to the level of significance of these correlation analyses to account for the multiple use of the recovery : start sEMG ratio. The R statistical language (version 3.2.1) was used to perform statistical analyses. All statistical tests were two-tailed with the level of significance set at $\alpha = 0.05$.

RESULTS

Activity from single MUs was recorded as subjects performed 3-4 sustained low-intensity contraction trials. Across subjects, discharge activity of the target MU was recorded over a period of 40-60 min. Rectified sEMG amplitude increased in all sustained contractions and was accompanied by the recruitment of additional MUs in intramuscular recordings. Activity in 20 MUs was recorded in experiment 1 and 10 MUs in experiment 2. There was no difference in mean MU discharge rate or mean sEMG amplitude between the start periods of experiment 1 or 2 (Table 1). There was also no difference in contraction duration, overall number of MU discharges or magnitude of the increase in sEMG amplitude during sustained contractions of experiment 1 or 2 (Table 1). There was an ~ 2-fold increase in sEMG amplitude over the course of the sustained contractions, which corresponded to a mean increase from 2.6 to 5.1 % MVC sEMG amplitude. The main result of the present study is visible in the time-series data of individual subjects from experiment 1 (Fig. 2) and experiment 2 (Fig. 3). The instantaneous discharge rate traces confirm
that both subjects maintained relatively stable discharge rates of the target MU throughout each trial. Nevertheless, there was a gradual increase in sEMG activity during the course of the sustained contractions. This increase corresponded to an ~2-fold increase in sEMG amplitude by the end period of each trial. For the illustrated subject from experiment 1, rest periods of 240 and 120 s led to a return of sEMG amplitudes to levels comparable to those observed in the start periods (Fig. 2C, D). In comparison, for the same discharge rate of the target MU, sEMG amplitude was increased when the isometric contraction was resumed after rest periods of 60 and 30 s (Fig. 2A, B). The three trials from a single subject from experiment 2 are presented in Fig. 3. As in the previous example, sEMG amplitude was increased compared to start levels when the isometric contraction was resumed after a rest period of 30 s (Fig. 3C). The increase in sEMG amplitude in the recovery period was more pronounced as the duration of the rest period decreased to 15 and 1-2 s.

Although there was no difference in the mean discharge rate of the target MU between the start and recovery periods of all trials (Table 1), the resumption of the isometric contraction was accompanied by greater sEMG when rest periods were 60 s or less. In experiment 1, recovery : start sEMG ratios were not significantly different from 1.0 when rest periods were 240 s ($t_{19} = -0.38$, $p = 0.712$) and 120 s ($t_{19} = -0.968$, $p = 0.345$; Fig. 4A). However, ratio values were significantly increased when rest periods were 60 s ($t_{19} = 2.24$, $p = 0.038$) and 30 s ($t_{19} = -3.6$, $p = 0.002$). In experiment 2, the 30 s rest period was also associated with recovery : start sEMG ratios that were significantly greater than 1.0 ($t_9 = -9.09$, $p < 0.001$). Similarly, ratio values were significantly increased when rest periods were 15s ($t_9 = -4.17$, $p = 0.002$) and 1-2 s ($t_9 = -11.5$, $p < 0.001$; Fig. 4A).

Across subjects and experiments, there was an exponential decrease in the ratio of sEMG amplitude between the recovery : start periods as the duration of the rest periods increased ($r^2 = 0.29$; Fig. 4B). The exponential decrease had a time-constant ($\tau$) of 27.8 s. The modelled
recovery : start ratio was 1.40 after a rest period of 1-2 s, declining to 1.20 after a rest of 27.8 s. This time-constant indicates that the large initial increase in sEMG would be 63% smaller if the isometric contraction was resumed after a rest of 27.8 s.

Across all trials for each rest period, there were no correlations between recovery : start sEMG ratio values and the number of MU discharges in the sustained contraction (r = -0.07 – 0.30 (range), p > 0.254), the duration of the sustained contractions (r = -0.55 – 0.31, p > 0.103), mean MU discharge rates (r = -0.38 – 0.30, p > 0.275) or start period spike-to-spike variability (r = -0.07 – 0.29, p > 0.221). However, end : start sEMG ratio values were positively correlated with recovery : start sEMG ratio values (r = 0.07 – 0.65, p = 0.002 – 0.773). Only the correlation for the 60 s rest trial reached the corrected level of significance (r = 0.64, p = 0.002); the three other trials were not statistically significant once the level of significance was corrected for multiple comparisons: 15 s (r = 0.65, p = 0.043), 120 s (r = 0.43, p = 0.050) and 240 s (r = 0.53, p = 0.015).

The pattern of positive correlations indicates that, irrespective of the duration of the rest period, MUs associated with larger increases in sEMG amplitude during sustained contractions tended to also be associated with larger amplitude sEMG signals when contractions were resumed.

We used sEMG to estimate the amount of synaptic input to the target MU. As shown in the sample data in Fig. 5, the increase in sEMG amplitude during a sustained contraction was accompanied by progressive recruitment of additional MUs. Across subjects, there was an increase in the number of MUs recorded from fine-wire electrodes over the course of the sustained contractions (see Fig. 6). Overall, the median number of recorded MUs was 1 [inter-quartile range: 1, 3] at the start of the sustained contractions; this value increased to 3.5 [3, 5] by the end of the contractions. Additionally, the number of active MUs recorded from fine-wire electrodes in the recovery period of each trial was in line with the recovery time-course found using recovery : start sEMG ratios (see Fig. 6). Specifically, the median number of recorded MUs was 4 following a brief 1-2 s rest period; this value decreased to 2 MUs for rest periods of 15-60 s and 1-1.5 MUs for rest
periods of 120-240 s.

Brownstone and colleagues The mean duration of MU action potentials was determined for the start and end periods in 20 of the target MUs. This measure was unreliable in 10 of the target MUs because of excessive superpositions of other recorded MUs in the end period of the trial. Overall, the duration of MU action potentials increased by 7.9% (95%CI: 2.0-13.8%) over the course of the sustained contraction.

DISCUSSION

In the present study, sustained voluntary contractions reduced the excitability of human single motoneurons. Our findings provide a systematic analysis of the time course of their recovery. To derive the precise time-course of recovery, the drive required by these motoneurons to resume their activity at the same discharge rate was determined at several times after the sustained contraction. Results indicate that motoneuron excitability recovers exponentially over time, with more than half the recovery occurring in the first minute and full recovery taking more than 240 s. Despite the minimal muscle fatigue associated with weak contractions such as these performed here (< 10% MVC), there is a gradual decline and slow recovery of motoneuron excitability. This is likely an ongoing process in all active motoneurons.

We have interpreted an increase in sEMG as an increase in excitatory drive to the motoneuron pool of the investigated muscle. As was recently concluded, sEMG can serve as a useful approximation of the amplitude of the neural or synaptic drive to muscle in certain controlled conditions (Enoka and Duchateau, 2015). The quasi-linear relationship, for fresh muscle, between sEMG amplitude and voluntary isometric force, which is in turn correlated with voluntary activation, suggests that sEMG reflects the level of recruitment and firing of motoneurons within a pool (e.g. Bélanger and McComas, 1981; Lawrence and De Luca 1983; Milner-Brown and Stein1975; Patla et al. 1982; Perry and Bekey, 1981). As MU activity depends on the level of net excitatory input to the motoneurons, sEMG acts as an indirect index of excitatory drive. The
amplitude of sEMG is also influenced by the electrical properties of the active muscle fibres (Farina et al. 2010, 2014; Hicks et al. 1989; Vandervoort et al. 1983). Here, we noted an ~8% increase in MU action potential duration over the course of the sustained contractions. While some simulation studies have shown that increased MU action potential duration in 50% MVCs can cause greater signal cancellation and thus result in lower amplitude sEMG (Keenan et al. 2005), others have found that increased MU action potential duration in 30-80% MVCs lead to an increase in sEMG amplitude (Fuglevand et al. 1989). However, from these studies we suggest that the effects of MU action potential duration are likely to be minimal at the very low contraction intensities used here.

Also, repetitive contractions can cause a progressive increase in the amplitude of electrically induced M-waves (Hicks et al. 1989; Vandervoort et al. 1983), but only during contractions above ~75% MVC (Vandervoort et al. 1983). On the other hand, the number of active MUs recorded from our indwelling fine-wire electrodes increased by the end of the sustained contractions. This recruitment of additional MUs is strong evidence that the increase in sEMG reflects an increase in neural drive that has occurred over the course of the sustained contractions and continues in some of the recovery contractions.

The increase in sEMG in our study with sustained activation of a MU at a constant rate is consistent with previous studies in a variety of muscles of both high and low intensity submaximal voluntary contractions, in which increases in sEMG were seen at the same time as MU firing rates decreased or were held steady (Garland et al. 1994; De Luca et al. 1996; Carpentier et al. 2001; Adam and De Luca, 2003, 2005; Johnson et al. 2004; Riley et al. 2008; Manning et al. 2010). However, the mechanism for the decline in excitability of active motoneurons relative to other motoneurons in the pool is uncertain. While possibilities include changes in afferent input, neuromodulatory input, or changes in intrinsic properties of the motoneuron, afferent and neuromodulatory inputs are unlikely to be targeted to one motoneuron over others in the same pool. Hence, an alteration in intrinsic properties that depends on the repetitive activation of the
motoneurons has been postulated.

Several studies have mapped the time-course of the spike-frequency adaptation that results from these intrinsic changes. In reduced animal preparations, injection of a constant-current into the motoneuron soma initially causes a high discharge rate, which rapidly declines and is followed by a more gradual decline that persists up to minutes (Granit et al. 1963; Kernell 1972; Kernell and Monster 1982; Sawczuk et al. 1995). A similar time-course occurs for extracellular stimulation of cat medial gastrocnemius motoneurons (Spielmann et al. 1993). These authors also noted that despite a 24% decrease in discharge rate over time, nearly all slow-type motoneurons discharged for the entire 240 s stimulation, which underscores the slow 'late' phase of spike-frequency adaptation.

Given the duration and intensity of sustained contractions in the present study, mechanisms involved in this late adaptation are particularly germane to our results. These include (i) increased conductance decay time related to Ca\(^{2+}\)-activated K\(^+\) channels, (ii) increased Na\(^+\)-K\(^+\) pump activity, (iii) increased Na\(^+\) channel inactivation, and (iv) decreased persistent inward currents (see Nordstrom et al. 2007, for a review). Little is known about the recovery time-course of each mechanism. Sawczuk and colleagues (1995) suggest that rat hypoglossal motoneurons show partial recovery from late adaptation after a break of ~10 s. As is evident in Figure 3 of Brownstone and colleagues (2011), recovery of a cat lumbar motoneuron takes 2-2.5 minutes following 4 mins of intermittent current injection. Our findings extend those observations to provide a systematic analysis of the time-course of the global recovery of motoneurons following a low-intensity contraction.

The current study shows that the time-course of motoneuron recovery was exponential, with a time constant of 28 s. Thus, after a low-intensity sustained contraction, 63% of the recovery occurs within ~30 s, after which recovery is considerably slower. While the amplitude of the sEMG recovery : start ratios were significantly increased for rest periods of 60 s or less, the modelled recovery revealed ratios > 1.0 across all time points, which indicates full recovery may take several
minutes. During low-intensity sustained contractions, some motoneurons temporarily stop their discharge activity (~200-400 s) and the excitability of these cells is reduced in the first half of the rest period compared to the second half (Bawa et al. 2006; Bawa and Murnaghan 2009; Manning et al. 2010). This timeline is compatible with the exponential time-course reported in the present study. Nevertheless, there is currently no evidence to indicate that when MUs are derecruited during sustained low-intensity contractions, they may be re-recruited only after the decline in excitability has recovered; this warrants further investigation.

Spike-frequency adaptation is greater in larger motoneurons and is related to the higher discharge rates and total spike counts (Kernell and Monster 1982; Spielmann et al. 1993). Thus, the total amount of motoneuron activity in the present study could have influenced the time-course of recovery. However, Spielmann and colleagues (1993) did not observe an effect of discharge rate or count on spike-frequency adaptation in slow-type motoneurons, which are the type likely recorded in the present study. In line with these results, we found no association between MU recovery and measures of activity (e.g. discharge rate, contraction duration or start period ISI variability). In contrast, sEMG recovery : start ratios were positively correlated with end : start sEMG ratios. Based on our sample of 30 MUs, the motoneurons that have undergone the greatest reduction in excitability over the course of a sustained contraction will also be those that have recovered the least at given times after the contraction has stopped.

It is likely that activity dependent changes in intrinsic motoneuron properties play an important role in muscle fatigue (McNeil et al. 2009, 2011a, b). During strong sustained contractions, reduction in excitability of individual active motoneurons may contribute to motor unit slowing and cessation of firing, and therefore decrease force production. In prolonged weak contractions, similar effects on the active motoneurons may lead to rotation of activity between MUs, and may require extra voluntary drive to maintain performance. The current findings indicate that once reduced excitability has developed, a rest of 30 s is not sufficient to allow full recovery of
the affected motoneurons.

In conclusion, the present study has confirmed that the motoneurons active in low-intensity sustained contractions experience a progressive decline in excitability. Importantly, we have discovered that the time-course of recovery from this type of decline in motoneuron excitability is best fitted by an exponential decay in which ~63% of the recovery of motoneuron excitability occurs within 30 s, with full recovery taking more than 240 s. This finding is consistent with the long recovery periods of previously active MUs to be re-recruited and may contribute to the disproportionate increase in effort that occurs in weak constant-force contractions that are sustained for tens of minutes (Smith et al. 2007; Søgaard et al. 2006) and to perceived fatigue that occurs in diseases such as stroke, multiple sclerosis and chronic fatigue syndrome.
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Figure 1. Experimental set-up. A) Activity from a triceps brachii motor unit (MU) was recorded with an intramuscular electrode. Electrodes on either side of the intramuscular electrode recorded the surface electromyogram (sEMG) from the same muscular head of the triceps brachii. In each of the 4 trials, subjects performed an isometric elbow extension contraction and maintained the stable discharge rate of a target MU. The discharge rate was selected in the 1st of 4 trials, and was 1-3 Hz above the discharge rate of the MU when it was first recruited. Subjects received visual and auditory feedback of the discharge rate of the target MU, and their hand was supported by a strap suspended from the ceiling. B) During the experiment, experimenters visualized target MU discharge rates (Inst fq) calculated from window discriminator digital pulses (spikes), intramuscular MU recordings (wire EMG), filtered and rectified sEMG signals (rect sEMG) and unprocessed EMG signals. Subjects were instructed to stop the sustained contraction when the amplitude of the rectified and filtered sEMG signal reached 2.0 times baseline (horizontal line). Baseline was the mean rectified sEMG amplitude during the start period, which was the initial 15 s at the target discharge rate.

Figure 2. Individual subject data from experiment 1. Rectified surface electromyography (sEMG) and motor unit (MU) instantaneous discharge rate data from all four trials from a typical subject. The gradual increase in sEMG amplitude over the course of the sustained contraction is visible between the start and end periods of each of trial: 30 s rest period (A), 60 s rest period (B), 120 s rest period (C), and 240 s rest period (D). The white rectangles in each prolonged sEMG trace indicate the start, end and recovery periods, each 15 s in duration, measured for each trial. Below each sEMG trace is the instantaneous discharge of the target MU during the sustained contraction and after the various rest periods when the subject resumed the contraction. Note the stable discharge rate within and between trials. When the subject resumed the isometric contraction, sEMG amplitude was similar to the start period after the 240 s and 120 s rest periods, whereas it was increased after the 60 s and 30 s rest periods. Start and recovery period rectified sEMG is shown on the right; white lines and black arrows indicate mean sEMG amplitude for these periods.

Figure 3. Individual subject data from experiment 2. Rectified surface electromyography (sEMG) and motor unit (MU) instantaneous discharge rate data from all three trials from a typical subject. The gradual increase in sEMG amplitude over the course of the sustained contraction is visible between the start and end periods of each of trial: 1-2 s rest period (A), 15 s rest period (B), and 30 s rest period (C). The white rectangles in each prolonged sEMG trace indicate the start, end and recovery periods, each 15 s in duration, measured for each trial. Below each sEMG trace is the
instantaneous discharge of the target MU during the sustained contraction and after the various rest periods when the subject resumed the contraction. Note the stable discharge rate within and between trials. When the subject resumed the isometric contraction, sEMG amplitude was greater in the recovery period compared to the start period for all three trials, especially for shorter rest periods. Start and recovery period rectified sEMG is shown on the right; white lines and black arrows indicate mean sEMG amplitude for these periods.

Figure 4. Ratio of rectified surface electromyography (sEMG) amplitude between the recovery and start periods. (A) The ratio of sEMG amplitude (recovery : start) calculated for each trial is presented for experiment 1 (grey circles) and experiment 2 (black circles). sEMG amplitude was similar between the start and recovery periods when the rest period after the sustained contraction was 120 and 240 s. This lead to ratio values that were not significantly different from 1.0 (p > 0.05). When rest periods were reduced to 60 s or less, sEMG amplitude in the recovery period was significantly increased (* p < 0.038). Mean [95% confidence interval] shown. See text for detailed statistical results. (B) sEMG recovery : start ratio values from experiment 1 and 2 were fitted with an exponential decay function. The best-fit function had a time constant (τ) of 27.8 s, which indicates that the modelled increase in sEMG recovery : start ratio at 1-2 s is reduced by 63% after 27.8 s. ratio240 is the ratio of recovery : start sEMG for the 240 s rest trial.

Figure 5. Individual subject data for a 30 s rest trial illustrating the increase in rectified surface electromyography (sEMG) amplitude and associated recruitment of additional motor units (MUs) over the course of a sustained contraction. Top panel shows sEMG signal (5 Hz low-pass, zero-lag, 4th order Butterworth Filter) increasing over the course of the ~5 min sustained contraction. The bottom panel shows the raw signal recorded via the indwelling fine-wire for the same trial. During the start period of the sustained contraction (i.e. initial 15 s), a single MU was active (○), the target MU. Over the course of the sustained contraction, the fine-wire electrode captured the progressive recruitment of 3 additional MUs (□, Δ, ◃), with the activity of a total of 4 MUs being recorded in the end period of the sustained contraction. After a rest period of 30 s, resumption the isometric contraction with the target MU active at the same discharge rate was associated with one additional MU (□) being recorded by the fine-wire electrode as well as a larger amplitude sEMG signal compared to the start period (white dashed line).

Figure 6. Number of motor units (MUs) (and interquartile range) recorded from indwelling fine-wire electrodes during the start (○), end (Δ) and recovery (■) period of each trial. There was a median of 1 MU recorded during the start period of all trials. By the end period of the sustained contractions, there was a median of 3-4 MUs recorded by each fine-wire electrodes. Thus,
additional MUs had been recruited during the sustained contraction. The median number of MUs recorded in the recovery period of each trial grossly mirrored the exponential decay observed for the recovery : start sEMG ratio. Following a 1-2 s rest period, the median number of active MUs remained at 4. However, this number decreased to 2 MUs after rest periods of 15-60 s, and it returned to baseline for rest periods of 120-240 s.
Table 1. Motor unit and surface EMG characteristics.

<table>
<thead>
<tr>
<th>Rest duration</th>
<th>Mean discharge rate (Hz)</th>
<th>ISI COV</th>
<th>EMG (%MVC)</th>
<th>duration (s)</th>
<th>MU discharges (count)</th>
<th>END:START EMG ratio</th>
<th>START – RECOVERY period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Exp 2</td>
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<tr>
<td>1-2s</td>
<td>10.9 [8.9 – 11.0]</td>
<td>15.8 [13.2–18.4]</td>
<td>3.0 [2.3 – 3.7]</td>
<td>262.3 [214.3 – 312.3]</td>
<td>2937 [2500 – 3374]</td>
<td>1.8 [1.6 – 2.0]</td>
<td>-0.11 [-0.45 – 0.37]</td>
</tr>
<tr>
<td>15s</td>
<td>10.5 [9.6 – 11.4]</td>
<td>16.7 [13.2–20.2]</td>
<td>3.1 [2.3 – 4.0]</td>
<td>256.2 [210.2 – 302.2]</td>
<td>2858 [2417 – 3298]</td>
<td>1.7 [1.5 – 1.9]</td>
<td>0.20 [-0.45 – 0.37]</td>
</tr>
<tr>
<td>30s</td>
<td>10.8 [9.9 – 11.7]</td>
<td>16.6 [14.9–18.3]</td>
<td>3.0 [2.2 – 3.8]</td>
<td>269.7 [206.1 – 333.3]</td>
<td>2874 [2421 – 3327]</td>
<td>1.8 [1.6 – 1.9]</td>
<td>-0.08 [-0.68 – 0.52]</td>
</tr>
<tr>
<td>Exp 1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>30s</td>
<td>10.9 [10.2 – 11.6]</td>
<td>16.2 [13.8–18.6]</td>
<td>2.7 [1.8 – 3.6]</td>
<td>263.7 [229.3 – 298.1]</td>
<td>3146 [2851 – 3443]</td>
<td>2.3 [1.9 – 2.7]</td>
<td>-0.06 [-0.34 – 0.24]</td>
</tr>
<tr>
<td>60s</td>
<td>11.1 [10.5 – 11.7]</td>
<td>15.6 [13.0–17.6]</td>
<td>2.3 [1.5 – 3.1]</td>
<td>251.2 [208.5 – 293.9]</td>
<td>2994 [2655 – 3333]</td>
<td>2.3 [2.0 – 2.6]</td>
<td>-0.05 [-0.26 – 0.37]</td>
</tr>
<tr>
<td>120s</td>
<td>11.0 [10.3 – 11.7]</td>
<td>16.6 [13.9–19.3]</td>
<td>2.1 [1.3 – 2.9]</td>
<td>270.0 [237.7 – 302.3]</td>
<td>3190 [2960 – 3419]</td>
<td>2.4 [2.1 – 2.7]</td>
<td>-0.23 [-0.44 – 0.23]</td>
</tr>
<tr>
<td>240s</td>
<td>10.9 [10.2 – 11.6]</td>
<td>14.7 [13.1–16.3]</td>
<td>2.5 [1.7 – 3.3]</td>
<td>257.1 [224.6 – 289.6]</td>
<td>3118 [2831 – 3405]</td>
<td>2.2 [1.8 – 2.6]</td>
<td>-0.03 [-0.38 – 0.44]</td>
</tr>
</tbody>
</table>

Data from experiment 1 (Exp 1) and experiment 2 (Exp 2) of calculated differences across trials for mean discharge rate, interspike interval coefficient of variation (ISI COV) surface EMG (sEMG) amplitude, sustained contraction duration, number of motor unit discharges, and end : start sEMG ratio. The mean discharge rate of the target motor unit did not differ between the start and recovery periods. All values are mean [95% CI].
A

subject feedback

pre-amplifier

time (s)

Inst fq (Hz)

spikes

wire EMG (mV)

rect sEMG (mV)

sEMG (mV)

start period

B

0 10 20

0 10 20 30

0 0.01 0.02

0 0.0 0.1

0.0 0.01 0.02

0.0 0.02 0.03

0.0 0.01 0.02

0.0 0.02 0.03

triceps brachii

surface EMG

fine-wire EMG

reference electrode

armbar

rect sEMG (mV)

sEMG (mV)

wire EMG (mV)

spikes

Inst fq (Hz)

time (s)
A

EMG (% MVC)

InstFq (Hz)

time (s)

START

END

RECOVERY

30 s

2%MVC

B

EMG (% MVC)

InstFq (Hz)

time (s)

START

RECOVERY

60 s

2%MVC

C

EMG (% MVC)

InstFq (Hz)

time (s)

START

RECOVERY

120 s

2%MVC

D

EMG (% MVC)

InstFq (Hz)

time (s)

START

RECOVERY

240 s

2%MVC
**A**

EMG amplitude ratio = ratio240 + K * exp(-t / τ)

EMG amplitude ratio = 1.07 + 0.35 * exp(-t / 27.8)

time constant (τ) = 27.8 s

**B**

EMG amplitude ratio = ratio240 + K * exp(-t / τ)

EMG amplitude ratio = 1.07 + 0.35 * exp(-t / 27.8)

time constant (τ) = 27.8 s
MUs recorded with fine-wire electrode (count) vs. rest period duration (s)

- **START**
- **END**
- **RECOVERY**

Time intervals: 1-2, 15, 30, 60, 120, 240