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Fatigue modulates synchronous but not asynchronous soleus activation during stimulation of paralyzed muscle

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Abstract

Objective—Electrical stimulation over a motor nerve yields muscle force via a combination of direct and reflex-mediated activation. We determined the influence of fatigue on reflex-mediated responses induced during supra-maximal electrical stimulation in humans with complete paralysis.

Methods—We analyzed soleus electromyographic (EMG) activity during repetitive stimulation (15 Hz, 125 contractions) in 22 individuals with complete paralysis. The bout of stimulation caused significant soleus muscle fatigue (53.1% torque decline).

Results—Before fatigue, EMG at all latencies after the M-wave was less than 1% of the maximal M-wave amplitude (% MaxM). After fatigue there was a fourfold ($p < 0.05$) increase in EMG at the H-reflex latency; however, the overall magnitude remained low ($< 2\%$ change in %MaxM). There was no increase in “asynchronous” EMG ~ 1 s after the stimulus train.

Conclusions—Fatigue enhanced the activation to the paralyzed soleus muscle, but primarily at the H-reflex latency. The overall influence of this reflex modulation was small. Soleus EMG was not elevated during fatigue at latencies consistent with asynchronous activation.

Significance—These findings support synchronous reflex responses increase while random asynchronous reflex activation does not change during repetitive supra-maximal stimulation, offering a clinical strategy to consistently dose stress to paralyzed tissues.

Keywords

Spinal cord injury; soleus; asynchronous EMG; H-reflex

INTRODUCTION

After spinal cord injury (SCI), electrical stimulation of paralyzed muscle is frequently used as a therapeutic intervention to precisely dose physiological stress to paralyzed tissues. Previous reports support that supra-maximal activation of muscle regulates muscle molecular transcription factors (Adams et al., 2011, Kunkel et al., 2011), muscle physiological properties (Shields and Dudley-Javoroski, 2006, Shields et al., 2006), and bone density (Dudley-Javoroski et al., 2012, Shields and Dudley-Javoroski, 2006) in humans with complete SCI. In this study, we examine if repetitive supra-maximal electrical

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stimulation and the associated muscle fatigue modulates synchronous and asynchronous reflex responses in human paralyzed muscle.

Electrical stimulation of skeletal muscle may yield a muscular force via a combination of peripheral and central mechanisms (Bergquist et al., 2011b, Collins, 2007). An electrophysiological analysis of skeletal muscle allows experimenters to delineate the various contributions to muscle activity. The muscle M-wave results from a direct activation of the motor nerve, yielding a wave of depolarization over the muscle sarcolemmal membrane (Lieber, 2002). Antidromic propagation of the stimulus can also elicit sporadic “backfiring” in the motor axon, yielding an F-wave (Kimura, 2001, Zappia et al., 1993). At low electrical stimulation intensities, activation of large peripheral sensory afferent fibers elicits H-reflexes (Pierrot-Deseilligny and Mazevet, 2000, Schieppati, 1987). Both axon mediated muscle activation (M-wave, F-wave) and reflex-mediated activation (H-reflex) contribute to skeletal muscle activity and may impact force generation during electrical stimulation of paralyzed muscle (Collins, 2007, Klakowicz et al., 2006). The H-reflex contribution to muscle force can be diminished via increased stimulation frequency (post-activation depression (Crone and Nielsen, 1989, Kohn et al., 1997, Shields et al., 2011)) or via increased stimulus intensity (antidromic collisions (Gottlieb and Agarwal, 1976). Single supra-maximal stimulation of paralyzed muscle should eliminate the synchronous reflex responses (H-reflex), but the effect of repetitive stimulation (fatigue) is uncertain.

Recent studies suggest that other “asynchronous” EMG events may contribute to muscle force (Bergquist et al., 2011a, Lagerquist et al., 2009, Merletti et al., 2011, Nickolls et al., 2004). These asynchronous EMG events do not occur at the typical latencies of the F-wave or H-reflex. Nickolls and colleagues reported that a burst of 100 Hz stimulation augmented subsequent force during 25 Hz stimulation, which they called “extra contractions” that persisted >1 second after the last stimulus pulse of a train (Nickolls et al., 2004). Bergquist and colleagues described the “asynchronous” EMG activity occurring between the M-wave and H-reflex latencies during sub-maximal stimulation (Bergquist et al., 2011a). In the present study, the EMG activity following a 1s rest between stimulation trains was analyzed to understand potential contributions of asynchronous EMG activity before and after fatigue.

The underlying mechanism and relative importance of asynchronous activation in the production and modulation of muscle force in human and animal models remains unclear (Bergquist et al., 2011a, Bergquist et al., 2011b, Frigon et al., 2011). These mechanisms are methodologically challenging to explore in human subjects with intact nervous systems: volitional descending co-contraction may interfere with the precise interpretation of reflex or innate motor neuron activation (persistent inward currents) (Dean and Collins, 2009).

In this study, we examine individuals with complete paralysis from SCI. Sensory loss and the absence of descending voluntary drive in this population eliminate the influence of voluntary contractions during electrical stimulation of paralyzed muscle. However, stimulation intensity and the sensory signaling from muscle fatigue may both modulate circuitry and subsequent muscle activation levels. Several studies support that muscle fatigue activates group III and IV free nerve endings in skeletal muscle (Kaufman et al., 1983, Rotto and Kaufman, 1988, Sinoway et al., 1993) and that activation of these afferent fibers modulates complex spinal reflex responses (Schmit et al., 2002, Wu et al., 2006). We designed these experiments to address whether two sources of sensory information, supra-maximal stimulation and metabolic signaling from muscle fatigue, modulate muscle activation (synchronous: H-reflex/F-wave; asynchronous: after cessation of stimulation).

The purpose of this study is to investigate whether muscle activity from synchronous or asynchronous sources is modulated during supra-maximal electrical stimulation and muscle

fatigue in humans with complete paralysis. We expect that synchronous reflex-mediated contributions to muscle force will systematically increase while asynchronous activation is minimally modulated. These findings will assist clinicians when dosing physiological stress to paralyzed tissues through electrical stimulation.

METHODS

Twenty-two individuals with SCI participated in the study (Table 1). The protocol was approved by the University of Iowa Human Subjects Institutional Review Board. All subjects provided written informed consent before participating. Inclusion criteria were motor-and sensory-complete SCI ((American Spinal Injury Association Impairment Score (AIS) --A) (Association, 2002) above T12, passive ankle dorsiflexion to neutral, and passive knee flexion to at least 90 degrees in a seated position. Exclusion criteria were lower motor neuron injury below T12, lower extremity trauma, pressure ulcers, or peripheral/systemic infection. We have previously demonstrated that repetitive supra- maximal soleus contractions can be safely performed in this population. This protocol induces an increase in several peripheral changes in muscle properties including increased relaxation time, decreased rate of relaxation, and long duration low frequency fatigue (Shields and Dudley-Javoroski, 2006).

Mechanical Recordings

Subjects remained in their wheelchairs during the test procedure. One randomly- selected ankle was stabilized in a system which measured isometric plantar flexion torque, as described previously (Shields, 1995, Shields and Dudley-Javoroski, 2006). The knee was positioned at ninety degrees of flexion and the ankle was in neutral joint position. With this configuration, the soleus muscle produces most of the plantar flexion torque (Sale et al., 1982). The foot was positioned on a rigid footplate instrumented with a force transducing load cell (Genisco AWU-250) placed directly underneath the metatarsal heads. The single-axis force transducer had a range of ± 5 V with linearity, hysteresis, repeatability and accuracy of 1.10, 1.22, 0.43 and 1.31% full scale, respectively (Shields, 1995). The foot was secured to the footplate via an ankle cuff with turnbuckles that directed force through the heel and into the footplate. A strap was secured tightly over the distal femur. This fastening system prevented upward movement of the heel during activation of the ankle plantar flexor muscles, helping to ensure that muscle contractions were isometric. We employed isometric contraction conditions because muscle length change introduces nonlinearity to the relationship between soleus stimulus input and torque output (Frigon et al., 2011). In addition, the influence of muscle length on M-waves is minimized during isometric contractions (Chang and Shields, 2002). Plantar flexor torque was calculated from the system in the manner previously described (Shields and Dudley-Javoroski, 2006). All force data were sampled at 1 kHz.

Electrical Stimulation and EMG Recordings

A probe over the tibial nerve in the popliteal fossa delivered electrical impulses to the plantar flexor muscles. The constant current electrical stimulator had a range of 0 to 200 milliamps at 400 volts. It was triggered by digital pulses from a data-acquisition board (Metrabyte DAS 16F, Keithley Instruments Inc., Cleveland, OH, USA) housed in a microcomputer under custom software control.

Surface EMG signals were recorded with a bipolar silver-silver chloride electrode with 0.8 cm diameter and 2 cm fixed inter electrode distance (Therapeutics Unlimited, Iowa City, IA). The recording electrode was positioned over the soleus muscle parallel to its fibers, approximately two cm medial to the midline of the calf and distal to the medial head of the

gastrocnemius. A ground electrode was placed over the anterior surface of the distal tibia. EMG signals were on-site pre-amplified by a factor of thirty-five, followed by mainframe differential amplification. The EMG amplifiers (Therapeutics Unlimited, Iowa City, IA) had an input impedance of 15 M Ω at 100 Hz, a frequency response of 15–1000 Hz, a common mode rejection ratio of 87 dB at 60 Hz, and a gain range of 1000–20,000 times. EMG signals were monitored on an oscilloscope. Data were stored for later offline analysis with DataPac 2k2 software (RUN Technologies, Mission Viejo, CA). The EMG sampling rate was 5 kHz.

Single-pulse stimulation (pulse width 500 μ s) was given to elicit soleus M-waves. The stimulation intensity was increased until maximal M-waves were obtained. This EMG value was calculated from the peak analysis and designated as MaxM. The stimulus intensity was set to 1.5 times the intensity associated with MaxM for the remainder of the protocol (supra-maximal stimulation). Because EMG may change as a result of the underlying muscle architecture (Chang and Shields, 2002, Frigon et al., 2007), we normalized all EMG signals to each preceding maximal M-wave within the train as well as to the single maximal M-wave generated at the start. Both methods yielded identical statistical results. Therefore, we only report the EMG as normalized to the initial MaxM in the results.

Experimental Protocol

A series of 10 stimulation trains (15 Hz, 7 pulses, 1333 ms between trains) was delivered to the tibial nerve to potentiate the soleus muscle (Shields and Chang, 1997). The fatigue protocol began 30 s after this warm up. During the fatigue protocol, the stimulator delivered a 10-pulse train (15 Hz; train duration 667ms) every two seconds (Figure 1A). A total of 125 fatiguing trains were delivered in succession. We have previously demonstrated that this fatigue protocol instigates significant changes in a range of paralyzed muscle physiologic properties: torque, torque-time integral, half-relaxation time and rate of relaxation, M-wave amplitude, and excitation-contraction coupling (low frequency fatigue)(Shields, 1995, Shields and Chang, 1997, Shields and Dudley-Javoroski, 2006, Shields et al., 2006). Peak plantar flexor torque was obtained for each train, and fatigue index (FI) was calculated as the percentage of the initial force (train #1) each subject was able to produce at the final contraction (train #125).

Partitioning EMG Events—Rectified soleus EMG was examined for each stimulus pulse in the 10-pulse trains. We identified M-waves beginning at 4 ms after the offset of the stimulus pulse (to exclude the stimulus artifact), ending at 35 ms (Figure 1B). We examined the EMG signal between 35 and 60 ms for electrical activity occurring after the M-wave (Post-M, Figure 1B). (Figure 1C) shows a representative example of the Post-M EMG broken into 5 ms latencies with a label indicating the approximate latency of the H-reflex (“H”). The peak amplitude and the integrated EMG were obtained for each post M-wave latency bin. Post-M-wave peak EMG and integral values were plotted: 1) according to stimulus train (to examine the effects of fatigue) and; 2) according to the within-train stimulus pulse number.

We sampled a 35 ms window of EMG immediately prior to the first pulse of each stimulus train (“Baseline” EMG, Figure 1B) in order to capture motor unit firing that was independent of a fixed latency. For the purposes of this assessment, the second contraction (Train 2) and the final contraction after fatigue (Train 125) were analyzed. The baseline of Train 2 captures any asynchronous EMG occurring after cessation of stimulation for Train 1. Likewise, the baseline of Train 125 captures any asynchronous events associated with Train 124. The peak amplitude and integral of the baseline EMG values were normalized to MaxM and compared before and after muscle fatigue.

To ascertain the average relative contribution of synchronous (H-reflex) and asynchronous (baseline EMG) activity during the contractions, we also calculated the peak EMG values and the ensemble averaged EMG values in the baseline and Post-M time intervals (Figure 1B). This allowed us to determine the latency at which increased EMG activity occurred consistently (as random latencies would equal zero during ensemble averaging). We next visually inspected the data and verified that over 90% of the defined peaks occurred within the latency of the H-reflex (40–44 ms). Finally, we calculated the peak EMG amplitude, for each latency, to determine whether the ensemble average underestimated the magnitude of the individual EMG peaks. The response to fatigue was consistent when analyzing the peak and ensemble averaged data; thus we only present the ensemble average in the figures.

Statistical Analysis

To examine EMG amplitude at several latencies before and after fatigue, the Peak H-reflex, integral, and ensemble-averaged EMG data for train #2 and train #125 were analyzed by a two-way (latency x train number) repeated measures analysis of variance (ANOVA) with post-hoc tests (Tukey) as indicated. Significance was set at $\alpha p < 0.05$.

RESULTS

Figure 2A depicts a representative example of soleus torque recorded at the beginning of stimulation and after repetitive stimulation (Train 125). Note the contractile slowing evident in Train 125. Figure 2B depicts group mean/SE soleus torque for the 125-contraction fatigue bout. From a maximum of 36.9 (2.5) Nm, soleus torque declined to 17.4 (2.0) Nm by contraction #125 (FI: 46.9).

During supra-maximal stimulation, peak EMG activity after the Post-M interval showed a linear increase from contraction 1 to contraction 125 (Figure 3A). Peak EMG activity for the total time during the Post-M interval ranged from a low of 1% MaxM early during the stimulation protocol to a high of 3% MaxM by the end of the stimulation protocol. The EMG integral showed a similar trend (Figure 3A), confirmed by the lack of a significant interaction between peak and integral % MaxM values ($p=0.085$, $F=1.735$) (Figure 3B). Only stimulus pulse #2 differed significantly from other stimulus pulses (1,3,5–9) (Figure 3B) ($p<0.001$, $F=5.555$). Overall, the integrated EMG activity behaved similarly to the peak EMG activity for the entire period (60 ms) after the M-wave.

Pre-Fatigue Contributions to Muscle Activation

We next analyzed the ensemble averaged EMG at all times after the M-wave for contraction 2 (Figure 4A). The ensemble averaged EMG activity peaked at a latency that is generally consistent with that of an H-reflex, suggesting that with supra-maximal activation there remains a small component (~2 %MaxM) of synchronous monosynaptic H-reflex activity. An individual analysis of the peak EMG for each subject confirmed that 90% of all the peak EMG signals occurred at the 40–44 ms latency.

We partitioned the EMG data into several 5-ms time bins (as shown in Figure 1C) to determine which Post-M latencies contained the greatest EMG activity. Figure 4B depicts mean (SE) ensemble averaged EMG activity for the pre-stimulus baseline and all Post-M latency bins from Train #2. No significant differences emerged among the baseline and post-M latencies for Train #2. Between the M-wave and the H-reflex (latency 35–39 ms), EMG activity was 0.88(0.26) %MaxM. EMG in the H-reflex latency bin (40–44 ms) then increased to 1.03(0.48) %MaxM, just a 0.15 %MaxM difference from the prior latency (Figure 4B). Analyzing peak EMG instead of ensemble averaged EMG revealed a similarly

small differential in the M-wave to H-reflex latency (1.60% MaxM) and the H-reflex latency (1.67% MaxM) (data not shown).

Post Fatigue Contributions to Muscle Activation

Repetitive activation caused a linear increase in peak EMG from train #1 to train #125 (as shown globally in Figure 3A). To determine the time after the M-wave that, on average, the EMG became most prominent, we again calculated the ensemble average across all subjects (Figure 5A). Post fatigue, the magnitude of EMG at the 40–44 ms latency was greater than the baseline and the 55–59 ms latency bins ($p < 0.05$, Figure 5B). Between the M-wave and the H-reflex (latency 35–39 ms), EMG activity was an average of 1.57(0.66) %MaxM (Figure 5B). EMG in the H-reflex latency bin (40–44 ms) reached an overall value of 2.49(1.35) %MaxM, a 0.92% MaxM difference from the prior latency. We next calculated the peak EMG for each subject, which demonstrated that the relative change across latency bins was statistically identical to the ensemble averaged EMG. Specifically, the peak EMG was 3.11% and 4.16% of MaxM for the 35–39 ms and 40–44 ms bins, respectively.

A 2-way repeated measures ANOVA indicated a significant interaction between fatigue state (contraction number) and latency ($p=0.025$, $F=2.700$). EMG activity in the 40–44 ms latency bin was approximately 2.4-fold higher after fatigue than before fatigue. For both the ensemble-averaged data and the peak EMG data, EMG increased as a function of contraction number (fatigue) during a time frame indicative of synchronous activity at the H-reflex latency. However, there was no modulation as a result of fatigue at a time 1 s after stimulation (baseline latency) that would be indicative of asynchronous firing. The poor association between fatigue and the change in asynchronous firing was confirmed by correlation ($r^2=0.005$).

DISCUSSION

The purpose of this study is to investigate whether muscle activity from synchronous or asynchronous sources is modulated during supra-maximal electrical stimulation and muscle fatigue in humans with complete paralysis. Various times after each M-wave were analyzed to assess both synchronous and asynchronous responses. The latency after each M-wave that showed the greatest EMG activity was 40–44 ms, consistent with that of the H-reflex. The time that showed the least EMG activity was the “baseline” interval. Thus while muscle fatigue modulated the synchronous responses after each M-wave it did not change the background activation level after 1 s. These findings support that during supra-maximal stimulation, fatigue reduces the effectiveness of the refractory period and antidromic collisions in human paralyzed muscle. Importantly, trains of supra-maximal stimulation do not enhance background asynchronous motor unit activity 1 s after each train.

Studies of subjects without SCI indicate that both central and peripheral mechanisms contribute to muscle fatigue during active maximal volitional contractions (Nordlund et al., 2004), however, the influence on synchronous firing is complicated by variations in central supra-spinal drive to the muscle. In this SCI cohort, we studied individuals with no supra-spinal drive and activated the muscle supra-maximally to insure that the drive to the muscle was constant. We expected that H-reflex amplitude would be suppressed during repetitive supra-maximal stimulation, as is usually observed, (Schindler-Ivens and Shields, 2000) via a combination of post-activation depression and antidromic collisions. As expected, we observed that the H-reflex was small (~1% MaxM) during supra-maximal stimulation in the pre-fatigue state and therefore was not a chief contributor to the overall soleus activation level.

In addition to H-reflexes, asynchronous activation may have contributed to the overall soleus torque. A recent study noted asynchronous EMG activity at a latency between the M-wave and the H-reflex (Bergquist et al., 2011a). That investigation used sub-maximal stimulus intensities in subjects without neurologic compromise; as such, comparisons with supra-maximal stimulus conditions are somewhat limited. The underlying mechanism of the asynchronous EMG activity was suggested to be persistent inward currents (PICs) within the spinal circuitry (Collins et al., 2001, 2002), but there was no validation to verify this mechanism. In the present study, pre-fatigue EMG activity at this latency (35–39 ms) was no different from the two following latencies (Figure 4B). After fatigue, EMG activity at this latency was significantly lower than EMG at the H-reflex latency. Together these findings support that minimal EMG activation occurred between the M-wave and H-reflex in these subjects with SCI.

In other studies, spontaneous motor unit firing could be observed after cessation of sub-maximal stimulation in humans without SCI (Collins et al., 2001, 2002). As this spontaneous discharge was observed in sleeping subjects and was absent after a nerve block, one group concluded that it was not due to voluntary activation and may instead be due to persistent inward currents (Collins et al., 2001). Non-sleeping subjects could halt the spontaneous discharge by attempting to “relax completely”; even after the subject asserted that they were already relaxed. In the present study, we observed no such spontaneous EMG activity 1 s after the cessation of supra-maximal stimulation in subjects with SCI.

Modulation of EMG after Fatigue

With fatigue, EMG at the H-reflex latency increased to approximately 2.5% MaxM. While the overall size of the H-reflex response remained small, the consistent growth of the H-reflex with fatigue is of interest. H-reflex amplitude is usually reduced during fatigue in electrically stimulated contractions (Duchateau and Hainaut, 1993, Garland and McComas, 1990). This is believed to reflect reflex inhibition of the alpha motor neuron pool (Garland and McComas, 1990). Motor neurons are believed to have a presynaptic inhibitory input from small diameter group III and IV afferents, activated by muscle metabolites that accumulate during fatigue (Duchateau and Hainaut, 1993, Garland and McComas, 1990, Kukulka et al., 1986, Woods et al., 1987). The growth of the H-reflex response when the muscle was fatigued suggests that other excitatory influences counteracted the inhibitory effect of group III and IV afferents.

Alternately, the apparent increase in H-reflex amplitude could perhaps reflect enhanced F-wave contributions due to increased excitability of motor axons. Although no previous studies have examined the effects of fatigue on the tibial nerve F-wave in humans with SCI, one study of paralyzed thenar muscle showed that F-waves appeared less frequently after fatigue induced by repetitive stimulation (Butler and Thomas, 2003). Moreover, the onset latency of the tibial nerve F-wave (46–53 ms) (Mesrati and Vecchierini, 2004, Zappia et al., 1993) is generally longer than the latency of the elevated EMG observed in the present cohort (~40 ms). For these reasons, we believe that changes in F-wave persistence (that is, enhanced motor neuronal excitability/backfiring) were unlikely to explain the growth of the post-M EMG we observed after fatigue.

Interestingly, at no time after the stimulus train was there evidence of asynchronous firing of motor units, which would have suggested that fatigue enhanced intrinsic motor neuron activity (e.g. persistent inward currents). Thus, while fatigue did enhance reflex contributions to soleus activation in a synchronous response, the asynchronous response was not increased.

Relative Contributions of Synchronous and Asynchronous Mechanisms

Given recent observations of synchronous, reflex-mediated EMG activity during repetitive stimulation (Bergquist et al., 2011a), we wished to explore whether reflex pathways could contribute to soleus torque during stimulation conditions that were unfavorable to the appearance of H-reflexes (supra-maximal stimulation). There is limited information on the relative contributions of synchronous and asynchronous mechanisms in the spinal cord injured model. A popular experimental protocol uses a 100 Hz stimulus burst to yield torque augmentation during subsequent 20 Hz stimulation (Bergquist et al., 2011a). In one study (Lagerquist et al., 2009), but not in another (Frigon et al., 2011), torque augmentation was abolished when a peripheral nerve block was applied, adding uncertainty to the issue of a central (spinal) mechanism for the torque augmentation. We did not employ this high-frequency torque augmentation protocol in our SCI population because stimulation at 100 Hz (in isometric loading conditions) carries a risk of bone fracture (Hartkopp et al., 1998). Supra-maximal stimulation at physiologic frequencies (e.g. 15 Hz) offers a balance between large (but not excessive) contractile forces (Shields and Dudley-Javoroski, 2006) and avoidance of frequency-related failure of neuromuscular transmission (Chang and Shields, 2002). In rehabilitation, supra-maximal stimulation has shown usefulness in protocols designed to ameliorate bone density decline via restoration of physiologic levels of mechanical loading (Dudley-Javoroski and Shields, 2008, Shields and Dudley-Javoroski, 2006). Our data supports that supra-maximal stimulation can modulate muscle force in people with complete paralysis without giving rise to unpredictable asynchronous activity.

In the present study, we conceptualized soleus torque as the summed outcome of M-wave activity, possible asynchronous motor unit discharge, and the synchronous H-reflex (and possible F-wave). The contributions of activity after the M-wave (0.88% MaxM) and the H-reflex (1.03 % MaxM) (Figures 4 and 5) were sufficiently small that peripheral mechanisms (M-wave) predominated (~98% of total electromyographic activity). The relative contribution of synchronous mechanisms intensified after fatigue (Figure 5B) but remained small (<2.5% MaxM). Although this model is an oversimplification, it illustrates that peripheral mechanisms were by far the largest contributor to the overall change in soleus torque after fatigue. However, it is important to point out that M-wave activity did not modulate torque in a strictly linear manner. Nonlinearities in torque development were likely introduced by mechanisms peripheral to sarcolemmal propagation (M-wave). For example, in skinned fibers, torque augmentation after high-frequency stimulation can occur because of enhanced sensitivity of myofibrillar elements to calcium (Abbate et al., 2002). Torque can likewise be modulated by myosin light chain phosphorylation (Tubman et al., 1997) and alterations in calcium handling (Chin et al., 1997, Dutka et al., 2005, Inashima et al., 2003). The inability to measure these sources of torque nonlinearity is an inherent limitation of any electromyographic investigation of factors that contribute to force. Other investigators have likewise highlighted the complicating influence of muscle intrinsic factors to measurement of torque modulation during repetitive stimulation (Frigon et al., 2011).

Clinical Interpretation and Implications

The results of this study are specific to supra-maximal stimulation and show clear differences from protocols that use sub-maximal stimulus intensities. Sub-maximal stimulus conditions likely enhance the relative contribution of synchronous (H-reflex) and asynchronous activation of muscle (Collins, 2007). Sub-maximal stimulation in a subject with SCI may introduce M-wave variability and force instability due to 1) inconsistent motor axon recruitment (because the slightest movement of the electrode will change the number of axons stimulated) and 2) asynchronous firing patterns that may emerge when refractory mechanisms and antidromic collisions are no longer in force. The “safety factor” when activating the nerve at 1.5 times MaxM offers insurance that axonal recruitment will

remain uniform even if slight probe movement occurs when the muscle contracts. To achieve a similar level of uniform recruitment with sub-maximal stimulation would require the use of pad electrodes directly over the muscle belly, which would eliminate the possibility of selective examination of M-waves and H-reflexes.

Uniformity and predictability of evoked muscle contractions are important in the clinical use of electrical stimulation protocols. Such protocols often aim to administer a precisely-defined dose of mechanical load via muscle contraction as an anti-atrophy and anti-osteoporosis countermeasure (Dudley-Javoroski et al., 2011, Dudley-Javoroski et al., 2012, Dudley-Javoroski and Shields, 2012 (in press), Shields and Dudley-Javoroski, 2006). The work of previous groups indicates that sub-maximal stimulus intensities are permissive to asynchronous EMG events (Bergquist et al., 2011a). This introduces an element of unpredictability in the administration of a dose of mechanical load to human tissues. The present study supports that supra-maximal intensity stimulation is not accompanied by a significant contribution of random, asymmetric EMG events. Supra-maximal stimulation, administered at physiologic frequencies (e.g. 15 Hz), therefore, is a useful strategy for administering known levels of physiologic stress to paralyzed tissues.

Methodological Considerations

We defined EMG activity according to latency criteria, using previous work which observed activity between the M-wave and the H-reflex latency (Bergquist et al., 2011a, Lagerquist et al., 2009). We used the ensemble average to independently determine where the muscle activity intensified during repetitive stimulation. This study showed that most EMG occurred at a time that is generally consistent with the H-reflex latency. We verified (visually) and calculated individual peaks for each subject to test if ensemble averaging the data attenuated the effect. Under all methods of analysis, the 40–44 ms latency demonstrated the greatest change in EMG during repetitive supra-maximal stimulation. For subjects with shorter limbs, the H-reflex response could indeed begin earlier than the starting latency that emerged (40 ms, Figure 1C). However, our data support that on the whole, EMG before fatigue in the latency between the M-wave and H-reflexes is no higher than baseline and is comparable to the neighboring latencies. After fatigue, EMG peaks consistently appeared at the 40–44 ms latency, suggesting that increased motor unit excitability overrode the inhibition induced by antidromic collisions and refractory conditions. Importantly, no asynchronous firing occurred at a time between contractions (“baseline” latency). It is possible that motor neuron firings occurred at other latencies that were not measured during the 1.3 seconds between contractions. However, we did not visually observe these bursts at supra-maximal intensities.

CONCLUSIONS

During supra-maximal stimulation at 15 Hz, synchronous (H-reflex) activation of the soleus muscle is minimal, though significant, in humans with complete SCI. This study confirms that under these test conditions, asynchronous activation after the cessation of stimulation is likewise minimal. The additional sensory input associated with muscle fatigue enhanced the synchronous activation to the soleus muscle (H-reflex). At no time did asynchronous bursts of motor unit firing occur. Peripheral factors such as muscle intrinsic properties and direct activation from the M-wave appear to play a much greater role than synchronous or asynchronous activity when activating human paralyzed muscle with supra-maximal stimulation. The small, but consistent synchronous reflex modulation and the lack of random asynchronous reflex modulation supports that supra-maximal electrical stimulation offers a consistent strategy to stress muscle and bone tissue in humans with SCI.

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Highlights

1. The soleus muscle H-reflex is larger after fatigue induced by supra-maximal repetitive stimulation.
2. “Asynchronous” EMG bursts, consistent with fluctuations in muscle force, were not prevalent during supra-maximal stimulation.
3. Supra-maximal stimulation, administered at physiologic frequencies (e.g. 15 Hz), is a useful strategy to administer fixed levels of physiologic stress (force) to paralyzed tissues.

Figure 1.

(A) Representative example of soleus torque and rectified EMG during a 10-pulse stimulus train. (B) Representative example of soleus rectified EMG between the first two stimulus pulses in a train. We sampled EMG from the pre-stimulus baseline, the M-wave, and the “Post-M” interval. (C) Representative example of EMG from the “Post-M” interval depicted in (B). We divided this interval into 5-ms bins for analysis. The 40–44 ms bin (“H”) corresponds to the H-reflex.

Figure 2.

(A) Representative example of soleus torque at the first and the final contractions of a repetitive stimulation protocol. Note the decline of force and the contractile slowing apparent at Train 125. FI = fatigue index, the percent of initial torque remaining at contraction 125. (B) Mean (SE) soleus torque over the 125-contraction bout.

Figure 3.

(A) Soleus EMG peak and integrated amplitude for the “Post-M” interval of 125 contractions (See Figure 1 for description). Values are mean (SE) EMG for all 10 stimulus pulses expressed as a percent of the maximal M-wave. The “baseline” EMG amplitude or integral (See Figure 1B) has been subtracted from the Post-M EMG amplitude or integral for each contraction. (B) Soleus peak and integrated EMG for each of the ten stimulus pulses in the stimulation trains. Values are mean (SE) for 125 contractions; “Baseline” EMG has been subtracted. The integral for pulse 10 is omitted because data recording of the 10th event of each train concluded only at the onset of the next train. The long duration of each 10th event (~1400 ms) compared to the duration of events 1 thru 9 (67 ms) yielded an artificially elevated 10th pulse integral for each train. * = significantly different from pulses 1, 3, 5–9 ($p < 0.001$).

Figure 4.

(A) Ensemble averaged EMG for the “Post-M” interval of contraction #2 (see Figure 1 for description). The latency of the H-reflex is denoted. (B) Statistical comparison of EMG for contraction #2, arrayed in latency bins. “-35 – 0 ms” corresponds to EMG captured between the pre-test warm-up contraction and contraction #1 (“baseline EMG”, Figure 1B). Values are mean (SE) for all within-train stimulus pulses (1–10) and for all subjects.

Figure 5.

(A) Ensemble averaged EMG for the “Post-M” interval of contraction #125 (see Figure 1 for description). The latency of the H-reflex is denoted. (B) Statistical comparison of EMG for contraction #125, arrayed in latency bins. “-35 – 0 ms” corresponds to EMG captured between contraction #124 and contraction #125 (“baseline EMG”, Figure 1B). Values are mean (SE) for all within-train stimulus pulses (1–10) and for all subjects. * = significantly higher than baseline and the 55–59 ms latency bins ($p < 0.05$)

Table 1

Subject Demographics

Subject	Age	Gender	Years post SCI	SCI level	Height (cm)	Weight (kg)
1	56	M	11.00	T6	170	102.3
2	38	M	12.82	T1	177	52.3
3	46	M	2.39	C5	185	90.9
4	68	M	12.51	T5	185	84.1
5	31	M	2.13	C6	185	77.3
6	33	M	13.71	T4	180	68.2
7	52	M	5.27	T7	170	81.8
8	47	M	0.07	C7	185	109.1
9	32	F	0.05	T5	165	59.1
10	27	F	0.39	T5	165	80.5
11	26	M	0.04	C5	167	56.8
12	30	M	1.23	T9	180	111.4
13	25	M	3.52	T9	185	68.2
14	57	F	3.44	C6	160	52.3
15	46	M	0.06	T6	172	81.82
16	30	M	0.03	T6	187	104.6
17	38	M	0.02	C5	187	67.3
18	18	M	1.25	T4	172	58.2
19	39	M	2.02	T4	187	90.9
20	25	M	2.96	T4	187	68.2
21	25	M	4.42	T4	187	77.3
22	21	M	0.02	C6	180	77.3