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Effect of Electrical Stimulation on Muscle Recruitment as it Relates to Maximal Voluntary Contraction

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Effect of Electrical Stimulation on Muscle Recruitment as it Relates to Maximal Voluntary Contraction

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ABSTRACT

Regeneration of complex muscle tissue, particularly at the interface between distinct tissue types such as muscle and tendon, remains an intricate and challenging issue. One approach recommended for regeneration is electrical stimulation (ES), which involves delivering an electrical pulse into the body to induce muscle contraction. Despite its well-documented effectiveness with muscle and tissue hypertrophy in both clinical rehabilitation and tissue engineering, little is known in terms of monitoring direct muscle response to stimulation. The recruitment mechanism for muscular contraction with stimulation is unclear, and to understand the muscle capabilities, it is necessary to filter out the electrical stimulation pulses from electromyographic (EMG) muscle activity, as the frequency spectra often overlap, distorting the muscular signal.

The aim of this thesis is to investigate voluntary and ES-induced activation patterns in small (abductor pollicis brevis, APB) and large (vastus lateralis, VL) muscles, and to make recommendations for measuring optimal muscle recruitment with ES. In the first part, voluntary recruitment patterns for muscles of different sizes and fiber types are compared. Next, the efficacy of a stimulus-removal technique called empirical mode decomposition (EMD) is evaluated for use with multiple frequencies and contraction levels. This filtering method is then applied to EMG data to determine the optimal stimulation parameters in terms of greatest electrical activity emitted from the muscles. Finally, voluntary and ES-induced activation patterns are compared to evaluate differences in recruitment mechanisms.

The results of the investigation show differences between muscle types in terms of electrical activity measured with EMG. For both voluntary and electrically-induced contractions, smaller muscles with smaller fiber types output more electrical signal. EMD was successful in

removing stimulus artifact from the signal, and it was possible to compare muscle responses for varying levels of stimulation. Stimulation delivered at higher frequencies appeared to induce greater muscle response measured with EMG.

The author recommends further investigation of voluntary and ES-elicited muscle activations, particularly using protocols with greater participants and more varied muscle types.

I. INTRODUCTION

Electrical stimulation (ES) involves the application of electrical impulses delivered through the skin to elicit skeletal muscle contractions. Electrical stimulation has shown promising results in enhancing cell multiplication and tissue regeneration in connective tissues and in forming new collagen and muscles in injured tissues[1-3]. The technique has also been used extensively during rehabilitation and following neuromuscular injuries. Further advantages with using ES for tissue regeneration include the ability to provide treatment in a controlled exercise routine[4] and to localize and steer an electric field to activate specific locations of skeletal muscle cells.

Overall, there is considerable evidence supporting ES as an effective treatment modality on multiple levels, including increased muscle fiber vascularization and collagen synthesis [1-3] as well as enhanced functionality in patients with neuromuscular dysfunctions[5-8]. However, monitoring muscle activity during stimulation remains a challenge. As a result, the mechanism of action for ES in activating skeletal muscle is still disputed. The relationship between voluntarily activated muscle and ES-induced activation of healthy muscle should be established prior to full implementation of ES as a mechanism for muscle regeneration or recovery from injury.

Anatomy of a skeletal muscle

Skeletal muscle function relies on the coordination of the nervous and musculoskeletal systems. To complete a muscular contraction, the process begins at the spinal cord. The spinal cord is considered the first level of motor hierarchy. A motor neuron pool, located in the spine, contains the cell bodies of motor neurons capable of innervating skeletal muscle. Axons of motor neurons are contained in nerves that connect the spine to individual muscle fibers. To prompt a

muscle contraction, motor neurons release acetylcholine, a type of neurotransmitter, at the neuromuscular junction between the neuron and the muscle fiber. When acetylcholine binds to receptors on the muscle fiber, an action potential propagates along the muscle fiber triggering contraction (Figure 1).

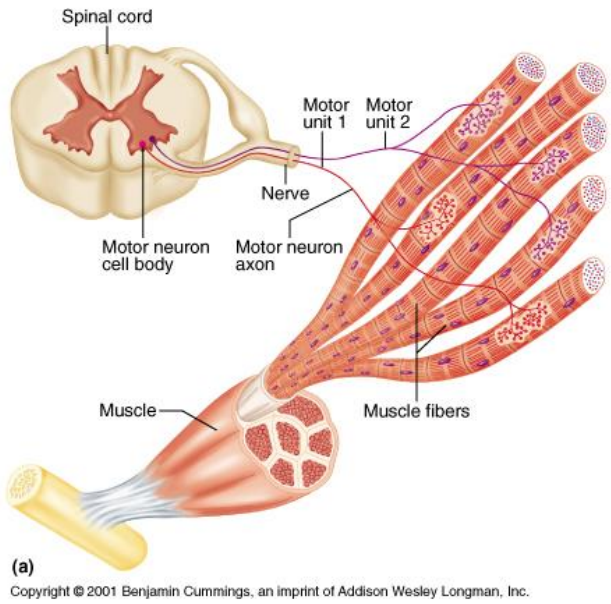

Figure 1: Anatomy of a Skeletal Muscle
Motor neurons make up motor units, which innervate muscle fibers[9].

A normal muscle is made up of hundreds to thousands of individual fibers. These are each then grouped into units called motor units, which differ according to function. A motor unit is composed of a motor neuron and the group of muscle fibers, which it innervates. There are two major types of motor units found in skeletal muscle: Type I and Type II. Typically, small motor units have smaller axon diameters, less fibers that make up the complete unit, and more fatigue-resistance. These motor units innervate slower fibers and are often referred to as Type I motor units. Larger motor units (Type II) innervate larger, faster fibers which are more fatigable.

The number of muscle fibers in a motor unit is directly related to the size of the motor neuron for that unit[10]. A small cell body has a lower excitation threshold to cause an action potential. Correspondingly, less input stimulation is required for the unit to fire. For example, a motor unit that has a smaller cell body also has a small nerve fiber that innervates fewer fibers upon activation than a larger motor unit, shown in Figure 2. For this reason, smaller, Type I motor units are more easily discharged and must be resistant to fatigue. Alternatively, Type II motor units can activate up to hundreds of muscle fibers synchronously with a single action potential from a motor neuron. Due to their direct relationship, fibers are generally referred to by their motor unit type, and 'Type I' or 'Type II' fiber compositions are often found in literature.

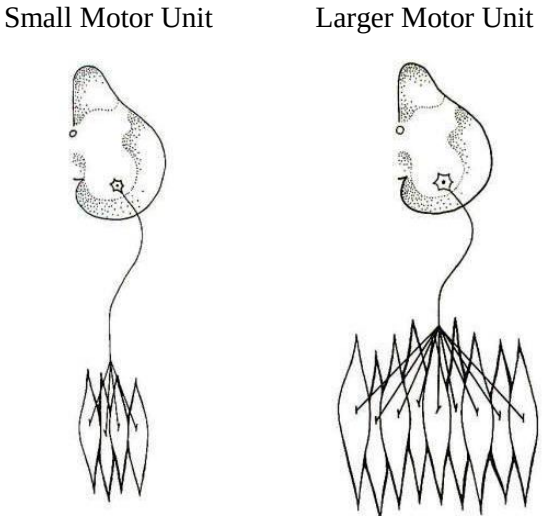

Figure 2: Small and Large Motor Units
Small and large motor units activate proportional numbers of fibers[11]

Muscles composed of smaller motor units are capable of finer, more precise control. This would be advantageous for muscles required for refined movements of the hand, such as the abductor pollicis brevis (APB) muscle in the thumb (Figure 3A). Alternatively, the quadriceps muscle group is responsible for powering movement of the entire leg, so fast activation of large amounts of muscle tissue would be more valuable. The vastus lateralis (VL), one of the muscles in the quadriceps (Figure 3B), is composed of 67% fast-twitch, Type II fibers[12,13], allowing fast

activation of many muscle fibers to coordinate functions such as walking and running. Respectively, the APB muscle is composed of 63% slow-twitch, Type I muscle fibers[12], permitting precise movements needed for turning dials and grabbing objects.

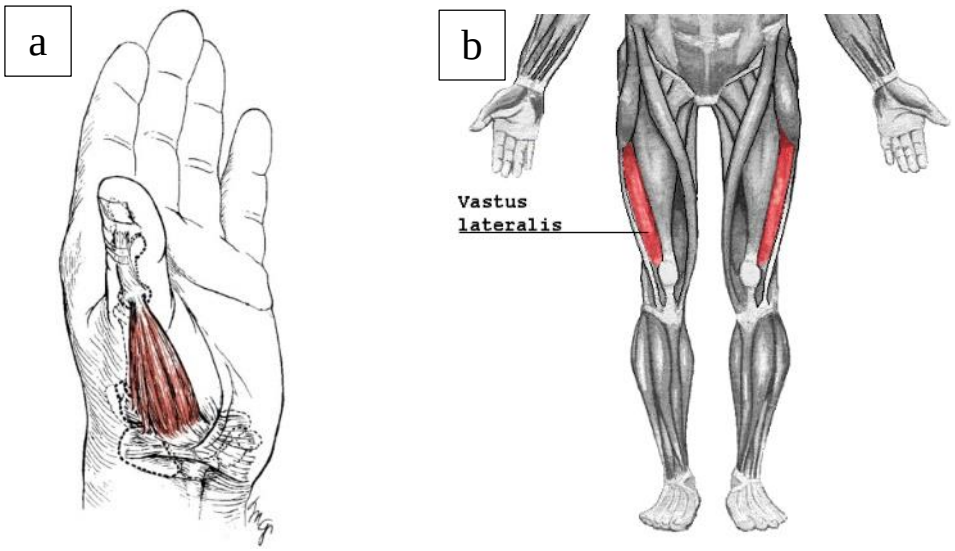

Figure 3: Abductor Pollicis Brevis and Vastus Lateralis Muscles
Illustrations of the (a) Abductor Pollicis Brevis[14] and (b) Vastus Lateralis Muscles [15].

Monitoring muscle response

Electromyography

With the release of an action potential from a single motor neuron, many muscle fibers can be activated synchronously. The action potential current from a motor neuron generates an electrical signal that can be recorded with an electrode. This process is called 'electromyography' (EMG). EMG recording can be done with a surface electrode on top of the skin, which records electrical activity from fibers directly underneath the electrode surface, or can be done with intra-muscular needles, for more precise recording of a single muscle fiber.

Due to its non-invasive nature, surface electrodes are more commonly used in clinical settings. The timing and amplitude of the electrical activity patterns recorded with surface EMG

(sEMG) electrodes reflect the total activity of motor neurons innervating the muscle at a given moment. The sEMG signal output from the muscles represents the sum of the individual motor unit action potential. For continuous fiber activation, a sequence of repeated action potentials is required. These are referred to as individual motor unit action potential trains (MUAPTs). An example of a sEMG muscle recording is displayed in Figure 4.

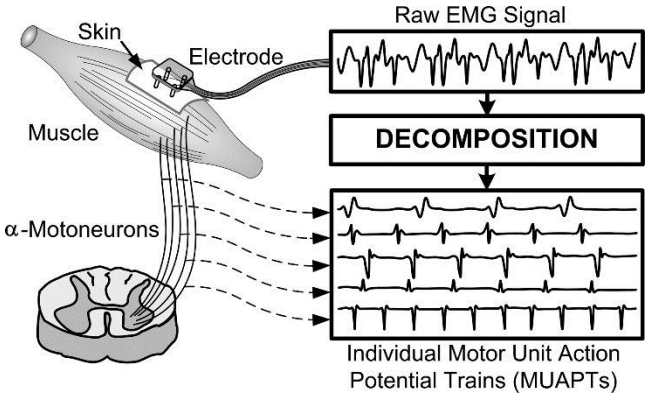

Figure 4: sEMG Recording of Compiled Motor Unit Signals[16]

By examining electrical activity measured with EMG, it is possible to indirectly measure muscle activation and fiber recruitment.

Voluntary muscle recruitment

A normal voluntary contraction follows a precise activation pattern, which involves the progressive recruitment of small, slower motor units followed by more larger sized, faster motor units to become engaged in the task[10,17-19]. This is called the 'size progression principle' and was first introduced by Henneman[17] in 1965. Typically, with voluntary progressive recruitment, each new motor unit activated adds their contractile potential onto the contractile force of the previous collection. This concept is known as spatial summation. If more fibers are needed to achieve the elicited response, neighboring muscle fibers will be recruited. Figure 5 illustrates the relationship between maximal voluntary contraction (MVC) force production and types of muscle fibers recruited using data from Henneman[10].

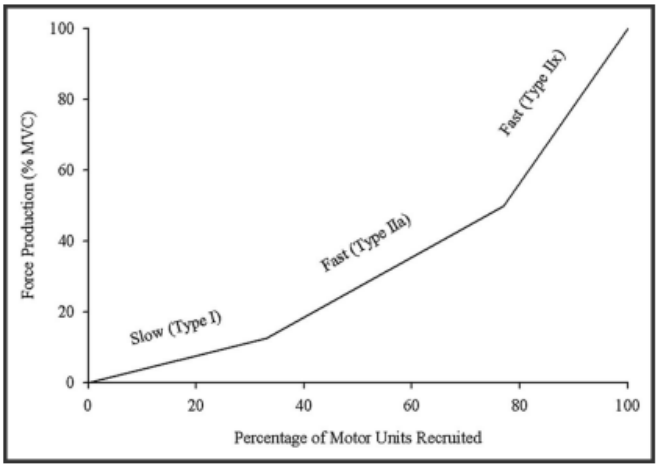

Figure 5: Representation of Progressive Recruitment During Voluntary Activation
Representation of progressive recruitment of motor units during voluntary activation of skeletal muscle. Displays slow (type I) and fast (type IIa and IIb) motor units. Data taken from Hennenman[10] and graphically represented by Gregory and Bickel[20].

MVC values are commonly used to standardize measurements among participants. By using MVC as a standard, it is possible to measure the degree of participation of the muscle fibers engaged in the contraction based on an EMG response[21], quantifying muscle recruitment. In fact, in most studies identifying the prevalence of fatigue with and without stimulation, participants are either electrically stimulated to get the maximal contraction value using a supramaximal stimulation twitch technique[22-24] or asked to voluntarily produce a force equal to a specific percentage of their maximal voluntary contraction (MVC). A dynamometer, or other force measurement mechanism, can be used as an indication tool to determine when a participant has reached a desired force level. By using a percentage of MVC instead of a standard force measurement, total muscle strength variability between participants and muscles are minimized, and recruitment can be compared. For example, Figure 6 shows results from participants engaged in voluntary contractions[21], comparing EMG electrical activity output (muscle activity) and percentage of muscular recruitment (%MVC). Because muscle electrical activity is related to activated motor units, according to this relationship, increased muscle recruitment is correlated with increased muscle action potential, and subsequently overall greater force output.

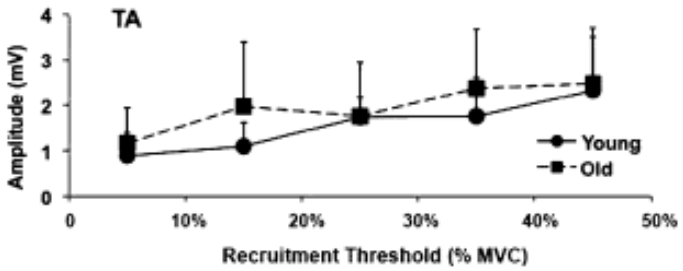

Figure 6: Relationship between Muscle Recruitment and Electrical Activity
Relationship between muscle recruitment (%MVC) and electrical activity (EMG amplitude in mV) from muscle fibers in the tibialis anterior muscle (TA) in two age groups[21]

Muscle response with Electrical Stimulation

Neuromuscular electrical stimulation (ES) is a therapeutic technique that uses electrical impulses delivered through the skin to elicit skeletal muscle contractions. Impulses are delivered via electrodes, similar to an action potential from the nervous system, causing muscle fibers exposed to the electrical field to contract. ES has been used clinically for rehabilitation to target skeletal muscle dysfunctions. Specifically, ES has been used successfully after central nervous system injuries, such as stroke or paralysis, to regain muscle function, improve motor control, and decrease muscle atrophy caused from disuse[5-8]. Electrical stimulation treatments are successful in enhancing cell multiplication in connective tissue and forming new collagen and muscles in injured tissues[1-3]. However, the mechanism of recruitment for ES in activating skeletal muscle is still disputed.

Parameters of ES and effects on muscle force production

ES parameters can have a significant effect on muscle force production and motor unit recruitment. Stimulation parameters typically manipulated include the following: electrode type and placement, waveform type, intensity, pulse frequency and duration. These are generally the settings that can be manipulated with a clinical stimulation machine, and each can change muscle force generation and performance.

To minimize variability with electrode placement, placement guidelines have been released by Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles (SENIAM). Surface electrodes are able to depolarize many fibers at once, and result in contractions, while needle electrodes can target one specific cell to elicit a twitch.

Electrical current can be sent into the body in two forms: alternative current (AC), where current changes direction repeatedly, or direct current (DC), where it flows in one direction. Typically, ES equipment delivers direct current in a pulsed format broken into phases. Pulsed current could be delivered as monophasic currents, with a one-directional flow of electrons, or biphasic currents, composed of two phases and a bidirectional flow of electrons, with a positive or negative phase over the baseline. A biphasic current is most commonly used with ES due to safety reasons. Often with biphasic currents, the first phase of the pulse (cathodic current) depolarizes cell membranes and neural activity is generated, while the second phase (anodic current) balances this depolarization so there is not charge accumulation, which could damage the tissue. Monophasic and biphasic current signals are shown below in Figure 7.

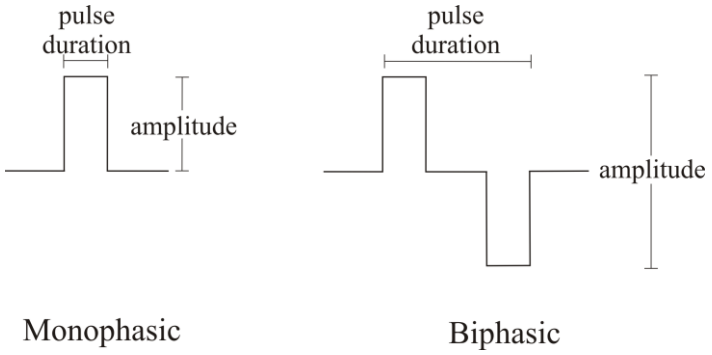

Figure 7: Monophasic and Biphasic Current Signal

Stimulations are then further classified by the parameters that make up the waveform: pulse intensity, frequency, and duration. Intensity is often referred to in milliamps (mA) of current delivered from the machine, and it often refers to the size, or amplitude of the electrical impulse delivered. Increasing the intensity causes greater electric charge to be delivered,

increasing the number of activated axons and motor units[25]. By increasing the intensity, force output increases linearly in both healthy and paralyzed muscle[26,27]. Pulse frequency refers to the rate at which stimulation is delivered into the body, expressed in terms of pulses per second (pps or Hz).

Generally, force-frequency relationships have a sigmoidal shape[28,29], shown in Figure 8, demonstrates an increase in frequency also results in an increase in force produced.

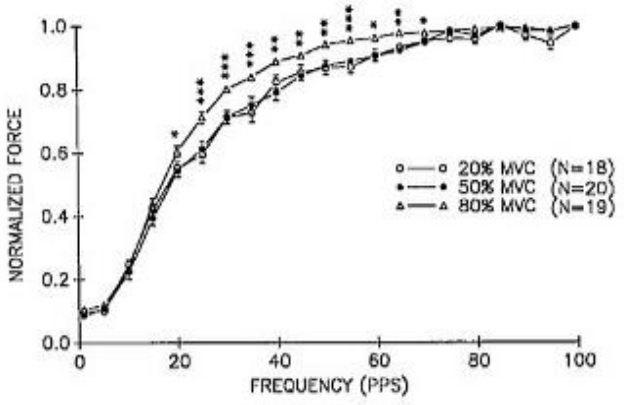

Figure 8: Force-Frequency Relationship
Force-frequency relationship for three intensities tested in terms of %MVC[28]

Frequency ranges vary depending on the target function for the stimulation treatment. Typically, 20Hz frequencies are adapted for preventing muscle atrophy or muscle wasting, while to improve muscle force, specifically in athletes, or engage in strength training, a much greater frequency must be used, possibly as high as 100Hz[30,31]. Most clinical treatment protocols use 20-50Hz[5,32], but in this study we would like to specifically target different types of muscle fibers and there has been evidence showing that specific types of muscles are targeted at separate frequencies. Slow-twitch and fast-twitch skeletal muscles have firing frequencies of 10Hz and 30Hz, respectively[33,34]. However, at higher frequencies above a certain threshold, stimulations can be delivered consecutively in a pulsed sequence, where there is not enough time between pulses to allow for full depolarization. This creates a cumulative effect involving multiple

stimuli, known as temporal summation, or tetanic contractions. Tetanic contractions are generally induced by 50 Hz stimulation[20]. Higher frequencies produce stronger muscle contractions, as seen in Figure 8, and are helpful to increase muscular strength, but also increase the likelihood for muscle fatigue to occur. To target separate types of muscles and compare the differences in muscle recruitment between slow-twitch (activated at 10Hz), fast-twitch (activated at 30Hz), and then finally the tetanic contractions (typically generated at 50Hz), an intervention that compares the three frequencies is needed.

Figure 9 shows representations of force outputs from a model of the feline caudofemoralis, a predominately fast-twitch muscle, while the muscle nerve is stimulated electronically at several frequencies[35]. Separate and discrete force twitches are seen at the lowest frequency tested, 15 Hz. When exposed to 37.5 Hz, the muscle releases successive twitch contractions causing summation. Contractions increase at first, but a stable level is achieved. Finally, higher forces and more summation is seen when the muscle is stimulated at 50 Hz.

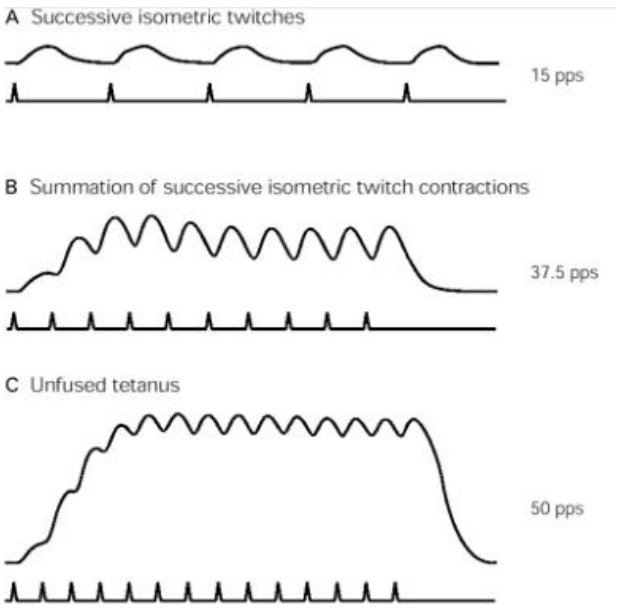

Figure 9: Force Output at Different Stimuli Frequencies
Force output from successive isometric stimuli delivered to the motor nerve at (a) 15 Hz, (b) 37.5 Hz and (c) 50 Hz [35]

Finally, pulse duration can be manipulated to result in additional motor unit recruitment as well. Pulse duration is the length of time of the pulse, measured in milliseconds (ms) or microseconds (μs). A hyperbolic relationship between pulse duration and intensity has been established. Using this relationship, by extending the duration of the pulse, it could be possible to get the same force production with less intensity required. In fact, increasing the duration has shown increased muscle force outputs without increasing fatigue[36,37].

By manipulating these stimulation parameters, it is possible to use ES to invoke stimulation and alter motor unit recruitment to achieve a task.

Recruitment with Electrical Stimulation

With the addition of electrical current to elicit a contraction, a change occurs in the distribution of motor unit recruitment. The exact mechanism of action for skeletal muscle contraction activated with ES is currently debated and can be summarized with two different theories: reverse selection recruitment, in which faster, more fatigable motor units are recruited first followed successively by slower units, and non-selective recruitment theory, which does not involve a size preference for recruitment.

Reverse selection recruitment theory

According to the reverse selection theory, larger fibers are recruited first, reversing the size-progression principle. Several studies have shown that the axons from larger motor units, which innervate faster, more fatigable fibers, are more easily depolarized[38,39]. Therefore, there is an advantage to activate these types of fibers first, followed by less easily depolarized, smaller, slower motor units, essentially reversing the voluntary progressive recruitment pattern. In fact, one study demonstrated that ES-evoked contractions showed a reversal of the size principal in 30% of tibialis anterior (TA) motor units[40]. Based on depolarization capacities, ES activates

larger, more fatigable units, progressively followed by smaller ones, which explains why often ES-stimulated muscles fatigue more quickly.

Non-selective recruitment theory

Contenders of the reversal theory believe that, in fact, there is no size selectivity involved in the recruitment of muscle fibers during ES. Instead, selection occurs based on random recruitment in the area near the stimulating electrode. The non-selective recruitment theory claims that the instances where reverse size progression was found is most likely due to more random occurrences rather than a designated pattern[20,41,42]. Twitch and force-frequency relationships were explored with the quadriceps muscle group at different ES intensities, and when forces were tested at 20,50, and 80% MVC, twitch times at 20% were not slower than 80%, as would have been predicted with a reversed recruitment order, and in fact there was no differences amongst the three levels[28]. The force-frequency relationship between 20 and 50% was also no different in the same study, favoring random recruitment of fibers in the area to perform the desired force response.

Muscle response studies with ES have commonly examined fatigue, as fatigue in a stimulated muscle occurs more quickly than a voluntarily-contracted muscle. In a normal voluntary contraction, fatigue-resistant, slow muscle fibers should be recruited first, followed by faster, more fatigable fibers. However, when fibers were exposed to different stimulation intensities (25 and 50% MVC), there was no difference in fatigue, lending more support to the non-selective recruitment theory for recruitment[43].

The mechanism for motor unit recruitment will lead to differences in summation and fatigue progression. A variety of approaches have been used to support different theories for

recruitment with stimulation, but a determination has yet to be reached. Therefore, more research should be done to investigate recruitment patterns that occur voluntarily and elicited by ES.

Stimulation Artifact

One of the predominant challenges in studying muscle behavior when exposed to ES is the removal of the stimulation artifact. The stimulation artifact is due to three superimposed components[44]. First, current from the ES traveling through the muscle tissue crosses the recording electrodes, causing a voltage gradient. Next, a second current occurs due to the stray capacitance between the stimulating electrodes and ground, creating another voltage gradient. Finally, there is some electromagnetic coupling between the stimulating and recording leads, which further contributes to the stimulus artifact.

When exposed to muscle, the EMG output is generally spike-shaped followed by an exponential decay, similar to the one modeled by O'Keefe[45] in Figure 10. Following the stimulation, the electrically evoked myoelectric signal (m-wave) can be seen, representative of the muscle response[46]. Conduction latency is affected by the distance between the stimulation and recording sites. If the recording site is sufficiently far away from the stimulus location and the rate of stimulation is low, the artifact and muscle response will not overlap[47].

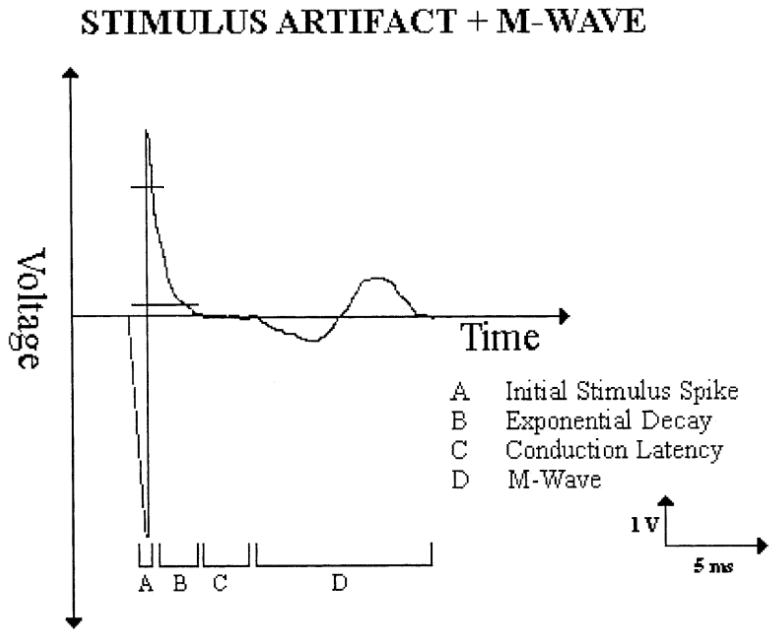

Figure 10: M-Wave with Stimulus Artifact Contaminated data (stimulus artifact + m-wave)[45]

To understand the muscle capabilities, it is necessary to filter out the electrical stimulation pulses from sEMG muscle activity, as the frequency spectras often overlap, distorting the muscular signal[48]. If electrical stimulation artifacts remain in the signal, subsequent analysis of the underlying muscular response is difficult. However, accurately blocking only the stimulation and not the muscle response remains a challenge[49] and has been attempted with varying techniques.

Stimulation Artifact Removal Options

Removal of the corrupting electrophysiological recordings has been attempted previously with sEMG using either hardware or software blocking techniques. Table 1 contains an overview of different methods and their limitations from a review by Heffer and Fallon[49].

Table 1: Commonly Used Artifact Rejection Techniques Regarding Four Criteria[49]

	Method	Signal and artifact can overlap in time	Signal and artifact can overlap in frequency	Artifact waveform can vary	Computationally efficient
Hardware-based	Filter	√	×	√	–
	Sample-and-hold	×	√	√	–
Software-based	Filter	√	×	√	?
	Template subtraction	√	√	?	?
	Sample-and-interpolate	√	√	√	√

Previous methods for eliminating the stimulus artifact include hardware blanking, software blanking with a comb filter or thresholds, and using a sample-and-interpolation method, but none have reported computationally effective results, specifically when using higher frequencies.

One of the most popular hardware options with EMG involves a blanking technique using a sample-and-hold design, where the stimulus pulse from the circuit triggers a response to stop the electrophysiological recording during the duration of the pulse[46,50,51]. However, if the stimulation waveform changes or if the interval is set for too long or too short, m-wave response may either be unnecessarily blanked out along with the artifact or some of the artifact may survive the editing. Alternatively, manipulating amplifier gains has been used as well, where stimuli gains of x 1000 are used, but the same inability to adapt to artifact changes applies[52,53]. Hardware filtering with a Chebyshev low pass filter at 550Hz was able to successfully remove high frequency elements of the stimulus, but not low frequencies[38].

Other artifact rejection techniques include using software-based blanking using mathematically derived templates[54-56], sample-and-interpolate techniques that average

neighboring pulses[49], or threshold-based blanking methods[45]. These methods all assume that the stimulation parameters are set and the distance between the stimulation and recorded electrodes are far enough apart so the m-wave and the artifact are not overlapping in time. Therefore, these techniques, although more flexible and adaptable to varying waveforms, cannot be used under all stimulation conditions. Overall, it is essential to remove as much of the artifact as possible, without eliminating much of the muscle response, in order to accurately examine the muscle response when exposed to stimulation. None of the filtering techniques commonly used are able to adjust to non-stationary and non-linear data to isolate the muscle response. Isolation of muscle activity from the artifact is crucial to determine the mechanism of muscle fiber recruitment with stimulation and presents the major challenge for researchers in the field.

In order to successfully isolate the muscle response from the ES signal, optimal muscle recruitment must be achieved. Therefore, the purpose of this study was to investigate muscle recruitment potential using two different muscles (APB and VL) and various frequencies and intensities of stimulation and compare the results with voluntary activations.

II. OBJECTIVES

The following are objectives of this study:

1 - Compare voluntary activation patterns in terms of force production and muscle recruitment for muscles of different fiber types and sizes.

2 - Effectively eliminate the stimulus artifact from a surface electromyography (sEMG) signal using a technique called Empirical Mode Decomposition (EMD). Evaluate the effectiveness of the removal procedure in eliminating the artifact for different frequencies.

3 - Compare electrically-elicited muscle responses at different frequencies in terms of maximal muscle recruitment for muscles of different fiber types and sizes.

4 - Compare voluntary and electrically-elicited contractions in terms of force production and muscle recruitment.

II. LITERATURE REVIEW

Voluntary and ES-activated motor units

Differences between voluntary and ES-activated muscle present additional challenges for interventions that combine both. Superimposing stimulation on voluntarily activated muscle has been explored for neuroprosthetic and therapeutic purposes[7,8,57,58]. This is known as combination, or 'hybrid' activation. Hybrid activation is more tolerable for human subjects[59], is capable of greater force output[60], and causes less fatigue[22,60,61] than ES-alone. However, voluntary and ES-induced muscle behavior can be unpredictable due to possible differences in activation

mechanisms. To the best of our knowledge, only a few papers have looked at muscle recruitment with a combination of voluntary and electrically-elicited contraction.

Generally, to get to the specific contraction level, standardized by individual participant, the intensity of the contraction is steadily increased until reaching a predetermined %MVC level. Ramping intensity until reaching a desired MVC[21] or to maximum tolerable intensity[62] has been used successfully in the past. Fling et al. looked at outcome measures in terms of 20% and 50% MVC such as recruitment threshold, EMG amplitude output, and intensity[21]. Binder-Macleod et al. electrically stimulated muscles until reaching thresholds of 20%, 50%, and 80% MVC with the same outcomes[28], although EMG data was not recorded and comparing muscle recruitment was based purely on force responses in terms of MVC. By standardizing the contraction for each individual in terms of their MVC, comparing differences in outcome measures is more generalizable.

Binder-Macleod et al. reported a force-frequency relationship sigmoidal in shape[28,29], shown in Figure 8, indicating an increase in frequency also results in an increase in force produced. Because 20%, 50%, and 80% MVC electrically-elicited contractions all displayed the same S-shape and no difference in twitch times, this data lends support to the non-selective theory of recruitment. According to the reversal recruitment principle, it would be likely that 20% MVC would produce faster twitch times and a difference in the force-frequency relationship when compared with 80% MVC. However, the data displayed characteristics that would be more likely prone from non-selective recruitment of muscle fibers more proximal to the area stimulated.

Another group, Frigo et al., collected EMG data with voluntary knee extension contractions of 100% and 40% MVC[63]. ES was then delivered to produce a force equal to 40%

MVC. At this point, voluntary contraction (40% MVC) was superimposed over the stimulation-elicited contraction (40% MVC) for a total force output of 80% MVC, and then the stimulation element was eliminated, dropping back to voluntarily-contracted 40% MVC and then to 0, shown in Figure 11.

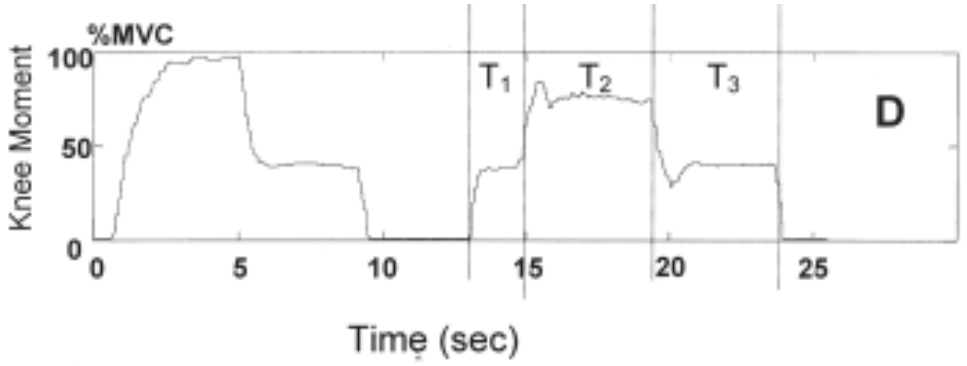

Figure 11: Force Pattern in Two Phases
Phase 1 included voluntary contraction at 100% and 40% MVC. Phase II included 2s stimulation causing 40% MVC force, then 4s of voluntary 40% MVC superimposed on the stimulated contraction, then 4s of only voluntary 40% MVC[63]

Although the protocol examined voluntary and stimulated contraction conditions, Frigo's outcomes did not focus on muscle recruitment but instead on stimulation filtering efficiency by comparing signal-to-noise ratios for electrode placement, frequencies, and different filtering techniques. Comb filtering plus blanking stimulation pulses was found to be more effective in eliminating the stimulation artifact but preserving the muscle response than comb filtering by itself.

Stimulation Artifact Removal

One of the main reasons why voluntary contraction with stimulation is so relatively unexplored is due to the difficulty in isolating muscle response data from stimulations that are contained in the same spectra, regardless if the contraction is elicited voluntarily or electrically. Generally, hardware and software filtering techniques are template-based with fixed features and

many limitations, specifically when using higher frequencies. These methods also assume that the stimulation parameters are set and the distance between the stimulation and recorded electrodes are far enough apart so the m-wave and the artifact are not overlapping in time. Therefore, these techniques cannot be used under all stimulation conditions.

One recent development has addressed the limitation in adaptability of typical filters by introducing an 'adaptive matched filter'[64]. More robust than traditional filters, this approach simulates contaminated EMG signal to determine ideal parameters to use with an adaptive filter based on a genetic algorithm, which is then applied to the real contaminated EMG signal. Although more flexible and adaptable, this process relies on the local features of the signal to develop a template for filtering, increasing computational difficulty.

To address many inconsistencies in signal processing, a method was needed to analyze non-stationary and non-linear data while preserving signal information in the time and frequency domains. Empirical mode decomposition (EMD) is a signal processing method proposed by Huang et al., and has been applied to removing stimulus artifacts previously[65,66]. Using this technique, the EMG signal is decomposed into physically meaningful intrinsic modes using an EMD algorithm. One of the most difficult complications of stimulus removal is the overlap of stimulus and m-wave in the time domain, and overlap of the power-spectrum in the frequency domain. EMD signal processing appears to be the better option for difficult stimulus removals, as it is dynamic, computationally efficient, and not based on a template.

The use of EMD to remove the stimulation artifact has only been validated with one subject at one stimulation frequency[66]. Further research must be done to determine if this technique can be applied to additional frequencies and muscles. Therefore, this investigation tested the efficacy of the EMD removal technique when used with multiple muscles and different frequencies.

III. SIGNIFICANCE

There is an increasing need for novel treatment options for difficult-to-treat areas such as the rotator cuff. ES has shown positive results in research and clinical settings for these types of areas. However, knowledge on the optimal parameters of ES to be used in rehabilitation with muscles of different sizes and muscle types is lacking. Additionally, the recruitment pattern of muscle fibers activated with stimulation is crucial to fully understand muscle response using this modality, and this is currently unknown. This protocol aims to address both these areas by comparing the abductor pollicis brevis (APB) and vastus lateralis (VL) muscle when engaged in stimulation at various frequencies and intensities. Furthermore, it aims to evaluate the effectiveness of a stimulation removal procedure, EMD.

Overall, no study has focused specifically on the percent muscle recruitment of two different muscles and three different frequencies engaged in electrical stimulation with a non-fatiguing protocol. Binder-Macleod et al. recorded muscular data while ramping up intensity to MVC thresholds of 20, 50, and 80%[28], although different muscle sizes were not tested and they did not focus on percent muscle recruitment required to contract. Fling et al. examined 0-50% MVC forces in terms of recruitment thresholds in small (first dorsal interosseous, FDI), and large (tibialis anterior, TA) muscles[21], but did not compare muscle responses elicited with electrical stimulation as well.

The APB and VL muscle were selected for a few reasons. First, the biomechanical properties of both have been well-studied and EMG data on both muscles are available. The APB is a small hand muscle and has predominately more slow-twitch fibers, while the VL muscle is a large leg muscle with more fast-twitch fibers[12]. Overall, these two muscles have opposite muscle fiber ratios and differ in size, so they are ideal to compare muscle recruitment. Muscle

recruitment between small hand and large leg muscles has been compared previously[21], but focused on the first dorsal interosseous (FDI) and tibialis anterior (TA), which also have different fiber ratios. In fact, muscle recruitment associated with abduction of the thumb and extension of the knee have been monitored with EMG[67], but no one has examined recruitment for both muscle groups with and without electrical stimulation. If a relationship is discovered between these muscles with varying fiber type concentrations, the results could be generalized to other muscles with varying fiber distributions.

Additionally, isolating muscle response data from stimulations that are contained in the same spectra is a continuous challenge for researchers in the ES field, and particularly challenging to create a filtering system that is able to adjust to varying artifact waveforms where a template method would not be applicable. Previous filtering methods have significant limitations, specifically when analyzing high frequency data. Some advantages in using EMD as a removal process are that it is (1) fast and data-adaptive and (2) it is not template-based and so additional inputs are not required[65]. EMD is more comprehensive than time, FFT, or wavelet filtering by itself, as decomposition preserves elements in each mode, instead of separating the artifact and EMG frequencies and then filtering them. The use of EMD to remove the stimulation artifact has only been validated with one subject at one stimulation frequency[66]. Further research must be done to determine if this technique can be applied to additional frequencies and muscles. Therefore, this investigation evaluates the efficacy of the EMD removal technique when used with multiple muscles and different frequencies.

Once the artifact is removed, it is possible to examine activity output from the muscle when engaged in contractions with ES. Muscle response to different levels of ES frequency has been examined in terms of variation in muscle thickness[68] and change in muscle performance during fatigue[30,69,70]. However, muscle activity generated from different frequency levels of ES

in a non-fatiguing protocol has never been explored. This is most likely due to the challenge of removing the stimulus artifact from an electromyography (EMG) signal with higher frequencies. Typically, removal of the artifact has been easiest when the muscle is activated by stimulation at lower frequencies, so lower rates have been most commonly used with surface EMG. Higher rates are relatively unexplored, and so a range of 10, 35, and 50Hz will be used to examine muscle response in this study. There is evidence that fiber firing rates differ according to muscle type, and it could be possible to target specific types at individual frequencies. An intervention that compares muscle activity when exposed to ES at three frequencies is needed.

Finally, there is not yet a determination on the accurate mechanism of motor unit recruitment with stimulation. By using Henneman's model for voluntary progressive recruitment[10] and examining the relationship between the force production (% MVC) and EMG response (% muscle recruitment), we can compare correlations for voluntary and stimulated contractions. The trend will help to reveal the method of recruitment that is occurring and add support to the associated mechanism theory: selective, reverse, or non-selective.

Overall, information generated with this procedure, outlined with objectives 1 through 4, will give valuable information regarding motor unit recruitment with stimulation with a combination of voluntary and electrically-elicited contractions.

IV. METHODOLOGY

Participants

Ten healthy participants were recruited for this study, including both males and females (mean age: 24.4 ± 2.5 years). A homogenous population of young, active participants was selected to control for any differences between activity level and its effect on muscle performance. All participants recruited had no history of musculoskeletal problems, cardiovascular problems, or orthopedic diagnoses such as arthritis, ligament injuries, meniscus

tears, joint instability, etc. Participants with a known allergy to Ag-AgCl surface electrodes or adhesive were excluded. The protocol was reviewed and approved by an IRB and written consent was obtained from all participants prior to participation.

Design

This study is a repeated measures within-participants design. Muscle response data was recorded during voluntary and electrically-elicited contractions at three different frequencies (10, 35, 50 Hz), measuring electrical activity from both small (APB) and large (VL) muscles.

Instruments

Electrical Stimulation device

The electrical stimulation was applied using Respond Select® neuromuscular electrical stimulation system (Empi, Inc., St. Paul, Minnesota). The Respond Select system is typically used in clinical settings and can deliver between 0 and 100 mA of stimulation at rates between 2 and 150 Hz. Pulse width is typically set to 300us. Bipolar surface electrodes, which are the most non-invasive option for delivering stimulation to the body, were connected to the stimulator and placed directly over the muscles to be activated.

Electromyography (EMG)

EMG data was collected continuously with a physiological modeling system (Nexus-10, MindMedia B.V., Netherlands). Surface electrodes were used to collect muscle response data with the EMG. Ag-AgCl surface electrodes were placed on the muscle belly parallel to the muscle fibers directly over the 'motor' points of the muscles to be activated. These locations were determined using Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles (SENIAM) electrode placement guidelines.

Dynamometer

Isometric force data was collected with a hand-held dynamometer (MicroFET2 HHD, Hoggan Health Industries, USA), Figure 12a. The device and dynamometer procedure has been validated previously[71]. MVC was collected with the dynamometer at maximal force and then used to calculate 25%, 50%, and 75% of MVC force.

Experimental Set-Up

Pre-gelled silver-silver chloride (Ag-AgCl) self-adhesive surface electrodes were placed on the skin over each muscle belly after skin preparation. Skin preparation procedure consisted of cleaning the skin area underneath the electrodes by using alcohol rub and shaving excess hair if needed. Stimulation electrodes were placed on the muscle's motor points according to SENIAM electrode placement guidelines over the muscle motor point. A reference electrode was placed on a bony site at the distal end of the ulna. EMG electrodes were placed directly between the active and indifferent ES electrodes to record muscle activity between the two. The direction of the EMG electrode placement was placed longitudinally, parallel to the muscle fibers, over the muscle belly. To avoid possible artifacts, all the electrodes and wires were fixed on the skin by using adhesive tapes.

To test the selected quadriceps muscle, VL, all participants were seated on an upright chair so that their feet were not touching the floor and the back of their knee joint at the edge of the chair seat[72], with their hand resting on a flat surface, as shown in Figure 12b. A belt was used to stabilize the dynamometer during recording, increasing consistency across repeated measurements[72]. The chair has straps around the waist and ankle to isolate only the quadriceps muscle group. Similarly for the thumb muscle, all other hand and forearm muscles were isolated

with the setup shown in Figure 12c. The participant was required to sit upright at a 90-degree angle with the shoulder and outside of arm pressed against the wall.

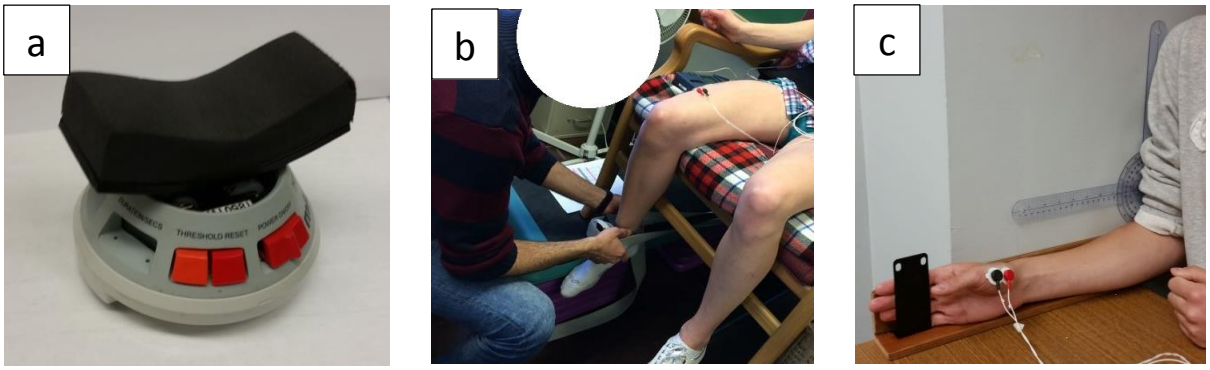

Figure 12: Experimental Set-up
(a) Hand-held dynamometer used to measure isometric extension force for use with the (b) chair with belt stabilization set-up validated by Bohannon[72] and (c) arm stabilization set-up with elbow bent at 90 degrees.

Procedure

Part One: MVC Contractions

Each participant underwent isometric knee and thumb extensions. EMG data was collected while undergoing a maximal voluntary contraction (MVC), held for four seconds and then released back to 0, accompanied by verbal encouragement from the research assistant. The value from the dynamometer at maximal force was recorded and then 25, 50, and 75% MVC thresholds were calculated. The participant then engaged in three more contractions for each of the three MVC threshold values (± 10%). The range of the measurements used is due to the sensitivity of the dynamometer; it is recommended to use a range. The dynamometer was used as an indication tool to determine when the participant reached 25%, 50%, and 75% MVC force values, and the contraction was held for 4s each time before dropping back to 0. Figure 13A describes the procedure for the voluntary contractions.

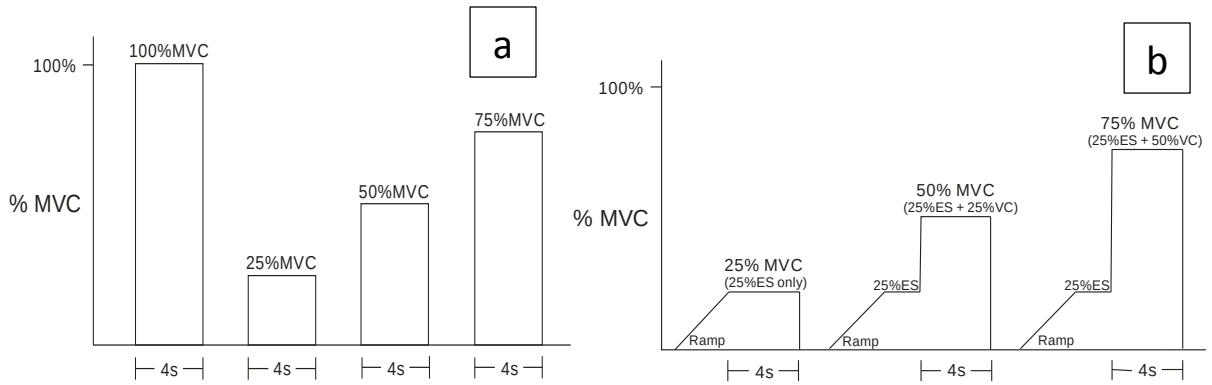

Figure 13: Muscle Activation in Terms of Force Levels
(a) Voluntary and (b) ES-induced muscle activation in terms of force levels (%MVC). Each contraction is held for 4s. Force is determined as indicated from the dynamometer.

Part Two: ES-Elicited Contractions

Randomizations between muscles (APB and VL) and frequencies (10, 35, and 50 Hz) were done between subjects to prevent learning and cross-over effects. Electrical stimulation was applied using Respond Select® neuromuscular electrical stimulation system (Empi, Inc., St. Paul, Minnesota). Stimulation was delivered via a biphasic wave with a pulse width of 300us in a range of intensities (increasing from 0 mA-80 mA (safe range) until 25% MVC is reached) and three frequencies (10 Hz, 35 Hz and 50 Hz). Timing information and signal characteristics were collected from the EMG recording device indicating the time when the stimulation pulse sequence began and ended. Participants first became familiar with the device and the feeling of the electrical stimulation. The researcher slowly increased the intensity up to 30mA for approximately 3 seconds for assimilation. sEMG data was collected during each of the following conditions: 25% (ES-elicited contraction only), 50% (25% ES-elicited contraction + 25% voluntary contraction), and finally 75% (25% ES-elicited contraction + 50% voluntary contraction), displayed in Figure 13B. Electrical stimulation was delivered starting at 0 and increasing intensity until reaching 25% MVC (+/- 10%). Once reaching the desired force threshold of 25% MVC, the stimulation was held at a constant intensity for 4s. After one minute rest, ES was again ramped in intensity until reaching 25% MVC. Participants were then asked to

voluntarily contract their related muscles (VL or APB) to reach 50% and then 75% of their MVC, as shown in Figure 13B. Data was collected continuously and 50% and 75% were labeled on EMG recordings.

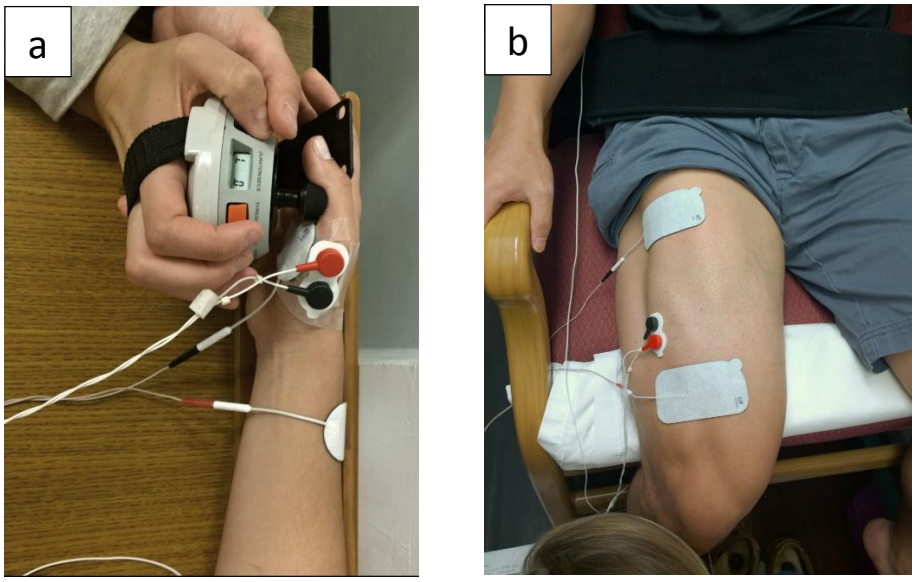

Figure 14: Force Measurement During Isometric Contractions
Force measurement during isometric contractions of the (a) APB and (b) VL.

Data Analysis

Signal Processing

The recorded EMG signal was first filtered using a Butterworth filter (20-500Hz cutoff, 5^{th}-order) using the native software of the device, BioTrace + (MindMedia B.V., Netherlands). Data was then transferred to Matlab® for all other processing.

Voluntary Signal Processing

The last two seconds of data was removed and used for further analysis. Voluntary signals were full-wave rectified. Surface EMG amplitudes and integrated EMG activity (iEMG) were computed for comparisons between the two muscles and their recruitment levels based on

preset force generation (% of MVC). Additionally, the Fast Fourier Transform (FFT) of each signal was used to obtain the power density spectrum by using the Discrete Fourier Transform Algorithm in Equation 1, where k=0,…,N-1. The conversion of data to the frequency domain allows us to see the frequencies at which fibers become activated.

Equation 1: Discrete Fourier Transform

$$X_k = \sum_{n=0}^{N-1} x_n e^{-i2\pi k \frac{n}{N}}$$

The median frequency (MDF) was then calculated using Equation 2 based on the power spectrum for each muscle at each %MVC activation level.

Equation 2: Median Frequency

$$\text{MDF} = \sum_{k=1}^{M} P_k$$

ES Signal Processing

For stimulated contractions, the entire four seconds of constant contraction was put through a process called empirical mode decomposition (EMD), outlined in Figure 15 and 16.

EMD involves the decomposition of a signal into intrinsic mode functions (IMFs) and a residual using sifting amplitudes[65]. Sifting has two functions: eliminate riding waves and smooth asymmetrical amplitudes. To perform the sifting process, first, the cubic spline envelope of local maxima and minima is determined, and the mean of the envelope (m_1) subtracted from the original signal to give h_1, the first component. This process is shown in Figure 15.

Equation 3: Intrinsic Mode Function Step 1

$$x(t) - m_1 = h_1$$

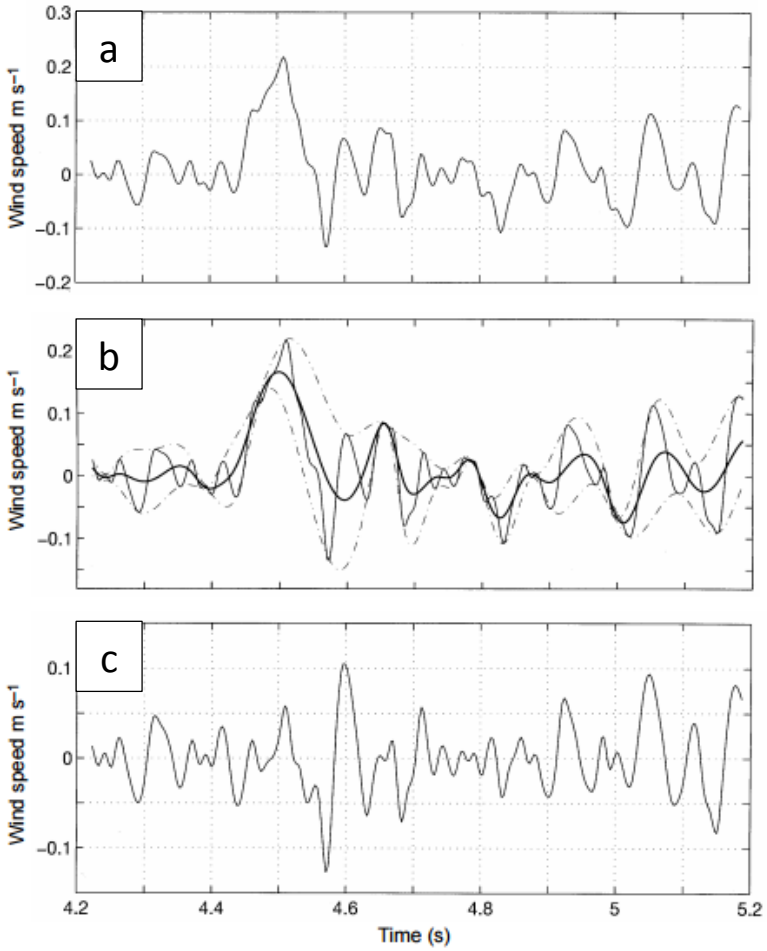

Figure 15: Illustration of the Sifting Process Using EMD
(a) the original signal (in terms of wind speed), (b) data in solid grey line, upper and lower envelopes in dot-dash lines and mean in thick black line, and (c) the difference between the data and m_1. This is now h_1[65].

The new mean (m_{11}) is subtracted from h_1 to get h_{11}, the next component. This is repeated k times until h_{1k} is an IMF. Each IMF represents an oscillation mode embedded into the data. The IMF conditions have been met when the following two conditions are satisfied:

(1) The number of local maxima/minima and number of zero crossings must be either equal or differ at most by 1.

(2) The mean value of the envelopes, one defined by the local maxima, the other by the local minima, is 0.

Equation 4: Intrinsic Mode Function Step 2

$$h_{1(k-1)} - m_{1k} = h_{1k}$$

At this point, $h_{1k}=c_1$, the first IMF component, which is subtracted from the original signal x(t), and the process continues until finally a residual r_n remains.

Equation 5: Intrinsic Mode Function Step 3

$$x(t) = \sum_{i=1}^{n} c_i + r_n$$

For this dataset, the first 4 IMFs were eliminated, as the stimulus artifact dominated the signal in those levels. The signal was then reconstructed by adding the remaining IMFs (Figure 16).

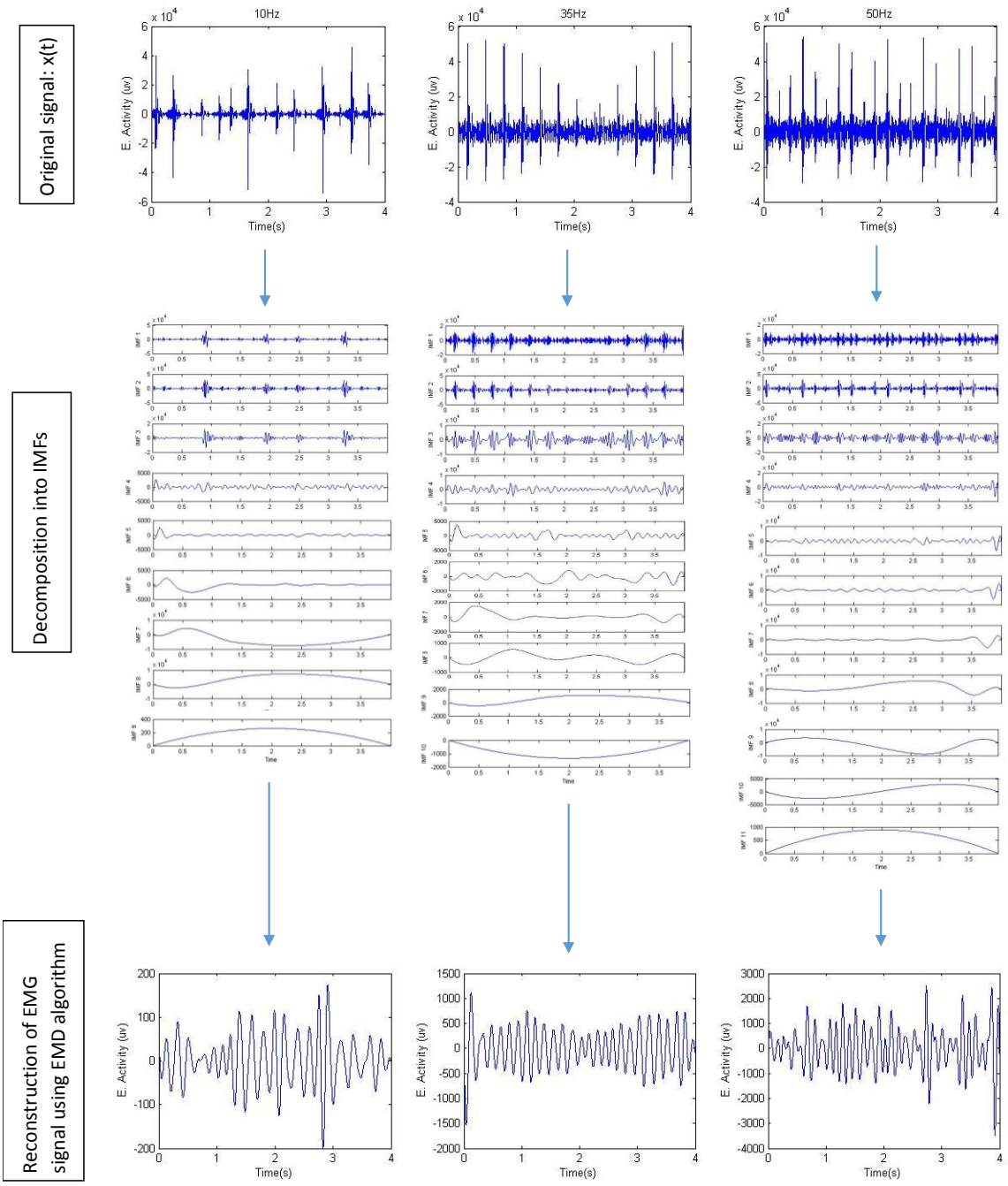

Figure 16: Steps of Empirical Mode Decomposition
Steps of Empirical Mode Decomposition with 10 Hz, 35 Hz, and 50 Hz stimulated muscle.

Post-EMD reconstruction, the data was cut to the center two seconds of the signal to minimize any effect from neighboring features. The signal was rectified prior to analysis.

Signal Parameters

Post-EMD, filter performance tests were conducted including a comparison of signal-to-noise (SNR) values and power reduction (PR) ratios, similar to the validation tests done by Qiu et al.[64].

SNR is the ratio of the mean energy of the control, or 'clean' EMG signal to the mean energy of the noise[73]. In this case, electrical activity (measured in uV) from the voluntary signal at 25% MVC, which has no stimulation artifacts, was used to compare against ES-only 25% MVC before and after EMD-processing. In equation 6, x(t) refers to the input signal measured at electrically-induced 25% MVC, and v(t) to the voluntary signal collected at the same level. An increase in SNR indicates an increase in artifact removal. Percent change was calculated between SNR generated from pre-EMD to post-EMD.

Equation 6: Signal-to-Noise Ratio

$$SNR = 10\ log_{10} \frac{\sum v(t)^2}{\sum (x(t) - v(t))^2}$$

Additionally, a power reduction calculation (PR) was used as a more accurate representation of SNR, as the voluntary signal is not a perfect, 'clean' signal to compare against. The PR is an indirect measurement but indicates the extent of the removal process. To address the limitations of the SNR, the ratio between power of the original signal z(t), and then power of the filtered post-EMD output signal y(t) was computed, using equation 7. In this manner, pre- and post-filtering can be compared directly.

Equation 7: Power Reduction Equation

$$PR = 10\ log_{10} \frac{\sum z(t)^2}{\sum y(t)^2}$$

Finally, a measurement of the efficiency of the removal technique proposed by Frigo et al. [63], was calculated using data from the 'hybrid' activation output, at 50% MVC, using equations 8-10 (pre- and post EMD processing)[63]. The efficiency index is a combination of ratios: operative signal to noise ratio, or OSNR, (voluntary when stimulation is present) and virtual signal to noise ratio, VSNR (voluntary when stimulation is not present). In these equations, Y_{STIM} refers to the mean electrical activity at 25% MVC when induced by ES-only, Y_{VOL} is the mean activity during voluntary contraction at 25% MVC, and $Y_{STIM+VOL}$ is the signal activity output at 50% MVC (which is composed of 25% MVC ES-only elicited force, and 25% voluntary force superimposed on top).

Equation 8: Operative Signal-to-Noise Ratio

$$OSNR = (Y_{STIM+VOL} - Y_{STIM})/Y_{STIM}$$

Equation 9: Voluntary Signal-to-Noise Ratio

$$VSNR = Y_{VOL}/Y_{STIM}$$

Equation 10: Efficiency Index Equation

$$EI = OSNR/VSNR = (Y_{STIM+VOL} - Y_{STIM})/Y_{VOL}$$

EI values represent the efficiency in extracting the voluntary signal in the presence of electrical stimulation. Values should decrease post-EMD processing and become closer to 1, as the voluntary component becomes more pronounced and detectable. Ideally, the muscle activity change from 25% to 50% (voluntary response once isolated from the stimulation), represented in the numerator of the ratio, should be close to the voluntary change from 0 to 25%, displayed in

the denominator. Therefore, the comparison of both components should give a ratio closer to 1 after the artifact is eliminated.

Normality tests for the SNR, PR, and EI datasets revealed normally distributed data. A repeated measures ANOVA was run to compare the differences pre and post-filtering, and compare between muscles and stimulation frequencies.

Statistical Analysis

Voluntary Activation Pattern Comparison

Prior to statistical analysis, tests for normality for iEMG and MDF distributions were completed. Both data sets revealed normally distributed values and model residuals. Raw force data from the dynamometer were compared between muscles at maximal contraction using a Student's t-test. Linear regression analyses were performed to evaluate if the electrical signal outputs (iEMG and MDF) can be explained by the proportional increases in MVC and the recruitment levels. Muscle type was introduced as a potential moderator in the linear regressions to determine if separating groups based on the interaction of muscle type and contraction level showed a better fit with the signal output data. Finally, to evaluate specific differences between recruitment levels and muscles, ANOVAs and Tukey post-hoc tests were conducted.

Evaluation of effectiveness of artifact removal

Tests for normality for sEMG artifact removal measurements were conducted. The data was revealed to be normally distributed. An ANOVA test was run to detect differences in frequency levels per muscle, as well as a comparison between muscles. Tukey post-hoc tests were also conducted.

Comparison of muscle responses at different frequencies

First, an ANOVA was used to compare the effect of frequency levels (Hz) and muscle type on intensity required to reach 25% MVC force output. An ANOVA was also used to compare electrical activity data between muscles and contraction type (stimulated muscle at 10, 35, and 50Hz, and voluntarily contracted muscle) when muscles were engaged at 25% MVC. Again, Tukey post-hocs were used to determine specific differences.

For hybrid activation, linear regression analyses were performed to evaluate if the electrical signal outputs could be explained by the frequency of stimulation and contraction level (%MVC). To determine specific differences, moderators were included in linear regression analyses, including frequency and muscle type. ANOVA and Tukey post-hoc tests were conducted for further comparison for all tests.

Finally, the voluntary component was isolated from 50% and 75% MVC data by subtracting ES-only muscle response, using equation 11. Mean values from 50% and 75% MVC are considered outputs from hybrid activation, Y_{Hybrid}. $Y_{25\%ES}$ represents the mean electrical activity detected at 25% MVC (ES-only), and $Y_{IsolatedVR}$ the isolated voluntary response.

Equation 11: Isolated Voluntary Response

$$Y_{IsolatedVR} = Y_{Hybrid} - Y_{25\%ES}$$

An ANOVA was run on isolated voluntary response from 50% and 75% MVC, followed by Tukey post-hocs to determine if there were differences between stimulation frequencies and muscle types.

For all tests, p-values were set at $p<0.05$ to determine significance and values were reported in terms of mean ± SE. SAS V9.4 was used for all statistical analysis.

Calculation of effect sizes

Finally, for all outputs, effect sizes were calculated for each outcome by calculating the standardized mean difference for each sample. The standardized mean difference, *d*, is the difference between the mean of the APB muscle and the mean of the VL muscle for each contraction level or stimulation frequency, divided by pooled standard deviation[74]. The standardized mean change provides an indication of the strength of a relationship between variables, rather than a significance level which reflects the probability of the observed change due to chance. The effect size index, *d*, follows a normal distribution with a range from negative infinity to positive infinity with zero as the null value. The magnitude of the standardized *d* value can be interpreted as 0.25, 0.5, and 0.8 for small, medium, and large effects on the outcomes of interest, according to Cohen's classification[75]. Effect sizes were calculated using a custom coding calculator created by Heudo-Medina et al.[76].

V. RESULTS

Comparison of voluntary muscle responses for different muscle types

We calculated the iEMG for each participant under all four activation levels for both muscles (25, 50, 75, and 100% MVC) and compared the means (Figure 17A). The corresponding linear regression model explained 82% of the variance in data. Our analysis showed that increased voluntary activation is a strong predictor for increased electrical activity emitted from the muscles. Additionally, the results, displayed in Table 2, revealed a significant difference in electrical activity for the different contraction levels, as well as between the two different muscles. The electrical activity generated from APB (smaller size with more type I fibers) was

consistently larger than VL (larger size and more type II fibers) ($p<0.001$). Contraction level moderated significantly the differences between muscles, specifically when increasing from 25 to 50% MVC.

Table 2: Linear Regression Results for Voluntary Signal Outputs (*n*=10)

	iEMG (uV·s)				Norm. EA (uV)				MDF (Hz)			
Predictor	**Mean (SE)**	**β**	***d* (95%CI)**	**R^2**	**Mean (SE)**	**β**	***d* (95%CI)**	**R^2**	**Mean (SE)**	**β**	***d* (95%CI)**	**R^2**
Contraction level				0.82				0.81				0.64
25	291.89 (66.88) §,‖	-234.79	8.36 (5.62,11.10)		28.75(2.62) ‡,§,‖	-75.05¶	0.65 (-0.25,1.55)		92.25 (4.57)	-0.65	2.50 (1.33,3.67)	
50	613.74 (147.22) ‖	-170.16	12.07 (8.23,15.91)		55.23(3.94) †,§,‖	-53.46¶	1.42 (0.44,2.40)		90.62 (4.80)	0	1.82 (0.77,2.86)	
75	836.76 (180.73) †	-115.19	15.90 (10.90,20.91)		78.41(5.18) †,‡,‖	-33.44¶	1.66 (0.65,2.68)		85.62 (3.64)	1.45	1.02 (0.09,1.95)	
100	984.60 (196.59)		15.55 (10.65,20.45)		100†,‡,§				86.89 (4.52)		1.27 (0.30,2.26)	
Muscle												
APB	1187.57 (117.42)	1357.28¶			72.26(4.92)	0.00¶			100.45 (3.30)	19.71¶		
VL	175.93 (18.00)				58.93(4.65)				77.24 (1.22)			
Contraction level x Muscle												
APB												
25	512.61 (89.31) ‖	-915.84¶			32.68(4.58) ‡,§,‖	7.74			108.10 (5.03)	12.01		
50	1091.67(201.361) ‖	-401.42			65.05(6.02) †,§,‖	18.52¶			104.20 (7.07)	7.45		
75	1482.75(211.75) †,‖	-65.31			91.29(7.50) †,‡	24.73¶			92.75 (6.19)	-5.45		
100	1663.25(243.20) †,‡				100 †,‡				96.75 (7.69)			
VL												
25	71.17(9.10)				24.81(2.13) ‡,§,‖				76.39 (2.63)			
50	135.81(14.51) †				45.41(2.81) †,§,‖				77.04 (2.54)			
75	190.77(18.31) †				65.52(4.48) †,‡,‖				78.49 (2.53)			
100	305.96(41.00) †				100†,‡,§				77.04 (2.38)			

Note: iEMG – integrated EMG, Norm. EA – normalized electrical activity, MDF- median frequency. Symbols (significance at p<0.01): †-significant difference from 25% MVC, ‡-significant difference from 50% MVC, §-significant difference from 75% MVC, ‖-significant difference from 100% MVC, ¶-Linear regression model significance.

Assuming the maximum number of motor units are recruited at maximal force production (100% MVC), electrical activity at maximal contraction for each participant was converted to 100% motor units recruited in order to compare the recruitment patterns of the two muscles on a normalized scale (Figure 17B). Again, the linear regression model of the normalized electrical activity (NEA) revealed a statistical difference between contraction levels and muscles, with a significant moderator effect of the contraction levels on muscle type. The percentage of motor units recruited was significantly larger in the APB than VL at 25, 50 and 75% MVC ($p<0.05$), even when both muscle activity outputs were normalized to their largest value. There is a more gradual increase in motor units recruited for VL than APB until 75% MVC, where a large proportion of motor units are recruited in the VL in order to reach maximal contraction (100% MVC).

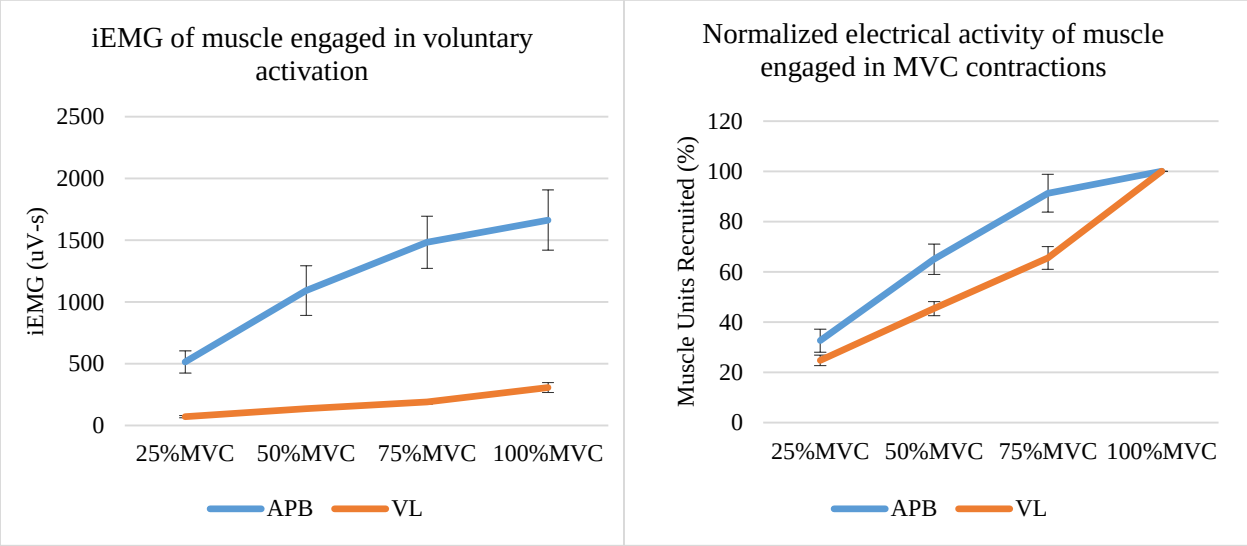

Figure 17: iEMG and Normalized Electrical Activity
(a) iEMG and (b) normalized electrical activity (Norm. EA) output of muscle engaged in voluntary activation for both APB and VL.

Expectedly, maximal force measured with the dynamometer during the contraction was significantly larger for VL than APB (mean force output: APB = 34.91 ± 1.91 lbs, VL = 311.55 ± 30.45 lbs, $p<0.001$).

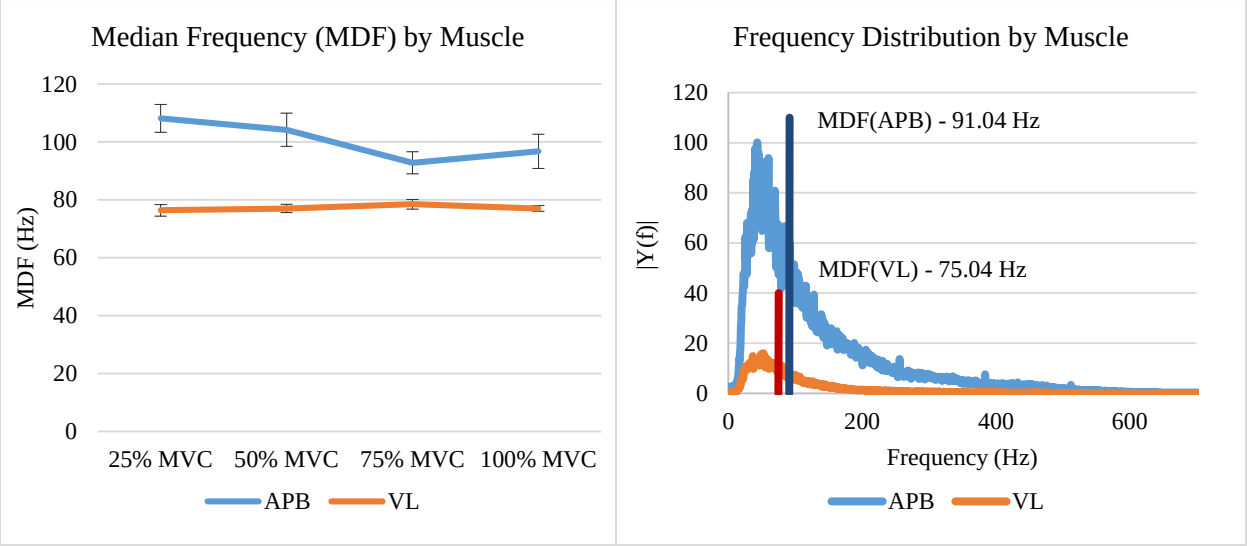

Figure 18: MDF and Frequency Distributions of APB and VL Muscles
(a) MDF and (b) mean frequency distributions for thumb (APB) and quadriceps (VL) muscles.

To undergo further analysis regarding firing type, the MDF values were calculated using the power spectrum (Figure 18). Overall, there was a difference in MDF between muscles of different sizes ($p<0.001$), but no difference between levels of muscle contraction ($p>0.05$). The linear model generated explained 64% of the variability in the MDF data set ($R^2 = 0.64$). There was no moderator effect, indicating no significant change when introducing the interaction between muscle and contraction level to the model. MDF values did not change when contraction was increased.

Mean power spectrums for both muscles were plotted on Figure 18B to compare muscles' frequency distribution. APB shows a greater total number of frequencies detected, consistent with increased electrical activity seen with the iEMG.

Evaluation of effectiveness of stimulation artifact removal

Table 3: Pre and Post EMD Processing SNR, PR, and EI for 10Hz, 35 Hz, and 50Hz

	APB*			VL*		
	10Hz	35Hz	50Hz	10Hz	35Hz	50Hz
SNR (dB)						
Pre-EMD	-21.09±1.33	-24.59±1.79	-27.31±1.82	-38.63±1.33	-44.33±1.50	-43.09±2.18
Post-EMD	0.20±0.62	-0.76±1.40	-0.84±1.30	-6.57±2.22	-18.75±1.34	-13.57±2.14
PR						
	26.38±1.82	26.21±1.61	28.58±1.25	31.24±1.38	25.02±1.08	28.58±1.62
Efficiency Index						
Pre-EMD	9.00±3.55	44.81±26.42	37.72±7.76	72.48±27.81	125.20±47.21	136.61±40.77
Post-EMD	0.30±0.08	0.46±0.18	0.48±0.23	1.57±0.26	4.94±1.10	5.17±1.26

*Note: * Significant differences ($p<0.05$) were found between pre- and post processing for all categories and all levels of stimulation.*

SNR increased post-EMD for APB and VL. Original pre-filtered signals had a SNR value of approximately -25 dB for APB and -40 dB from VL. Percent change was calculated using pre- and post-EMD values. Post-filtering, the signals experienced an average 98% and 69% reduction in 'noise', respectively. For our purposes, 'noise' was treated as the 'stimulation artifact'.

Power reduction (PR), the ratio between pre-filtered and post-filtered stimulation data (25% MVC data with ES) displayed higher values. The results in Table 3 indicate when comparing pre- vs. post-EMD, there was a reduction in amplitude 26-31 times. This means that by eliminating the first four IMF levels which were clearly dominated by the artifact, there was a reduction in energy in the signal of approximately 28 times. For power reduction, there was no difference between muscles or stimulation rates.

Finally, efficiency indices decreased with filtering closer to 1 with EMD for APB and VL. Efficiency indices, which compare the isolated voluntary activation from 25% to 50%MVC

post-stimulation muscle data (numerator) to the normal voluntary activation from 0 to 25%MVC (denominator), revealed higher levels for VL than for APB. Again, post-filtering, there was no significance between stimulation frequencies.

Comparison of muscle responses at different frequencies

Required intensity to reach 25% MVC

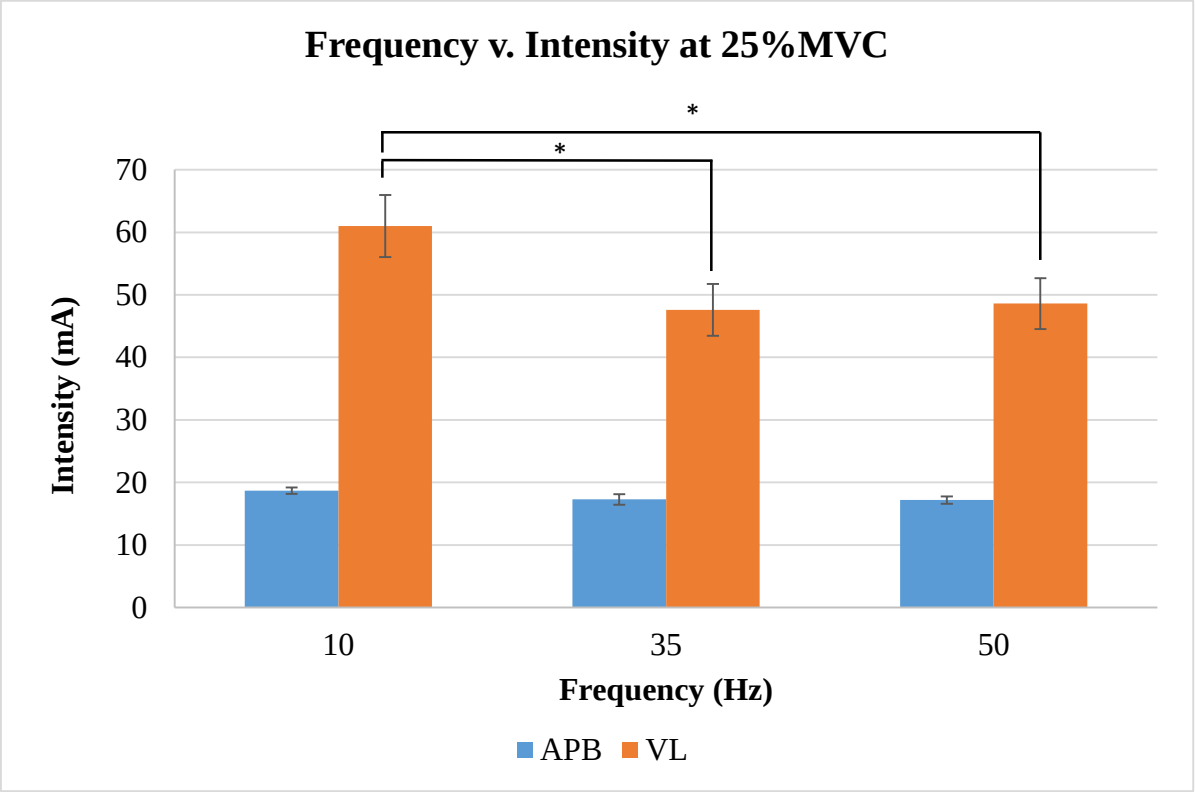

Figure 19: Frequency-Intensity Response of APB and VL Muscles at 25% MVC
Frequency-Intensity response detected through sEMG activity in two muscles (APB and VL) at 25% MVC.

For VL, stimulation with 10Hz required higher intensities ($p<0.05$) to reach 25% MVC than higher frequencies, although there was no difference between 35 and 50Hz (Figure 19). Additionally, the small muscle of the hand (APB) required significantly less intensity at all frequencies than the larger VL muscle (mean APB: 17.73±0.40mA, mean VL: 52.40±2.700mA, d = 8.39 (5.65, 11.14), $p<0.001$) to reach the same contraction level (25% MVC).

ES-induced muscle response at 25% MVC

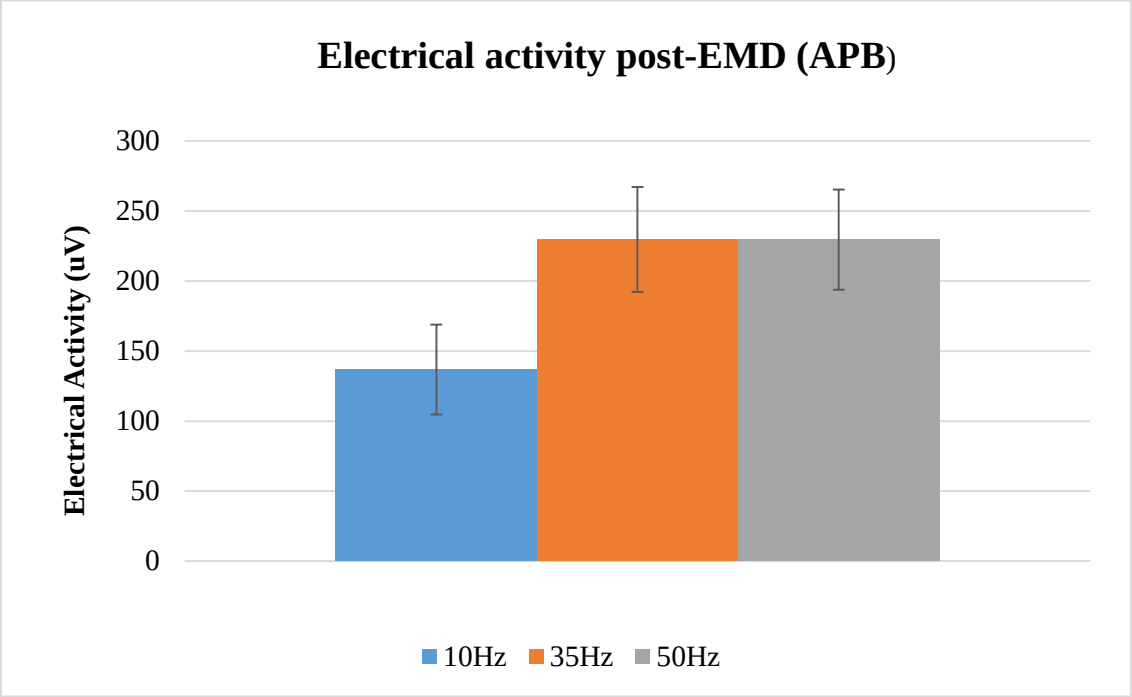

Figure 20: Electrical Activity (Post-Processing) Generated by Stimulating APB at 25% MVC

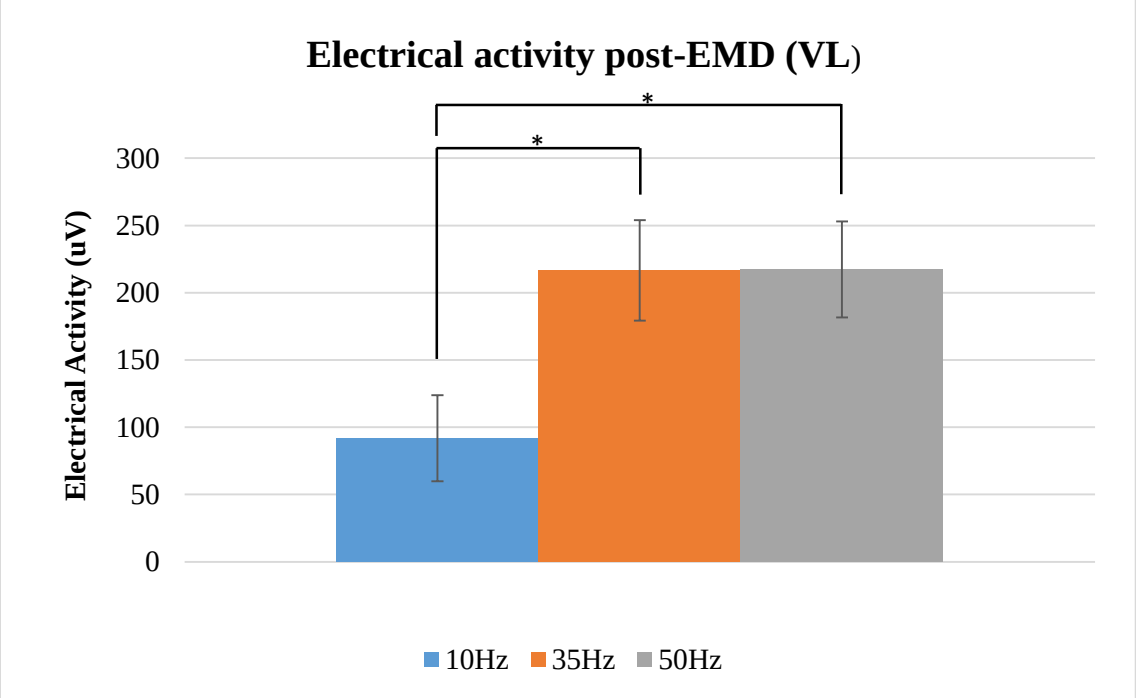

Figure 21: Electrical Activity (Post-Processing) Generated by Stimulating VL at 25% MVC

When both muscles were engaged in ES-induced contraction at 25%MVC, electrical activity outputs from the muscles were compared in terms of three frequency levels (10, 35, and 50 Hz). For APB, although there was less electrical activity output from the muscle when stimulated at 10Hz, this is not a significant difference. In fact, there was no significant difference between the activity outputs from the muscle from any of the three frequency levels. However, when VL was engaged in a 25%MVC contraction, there was a significant difference between 10Hz and the higher frequencies, 35 and 50Hz.

Comparison of muscle responses voluntarily and electrically-elicited

Hybrid activation muscle response at 50% and 75% MVC

Hybrid activation was modeled in Figure 22 (APB) and Figure 23 (VL), and outputs from the linear regression analysis are contained in Table 4. Electrical activity was modeled in a linear regression by muscle type, frequency, and contraction level (R^2=0.23). There were significant differences between all three factors ($p<0.05$). All possible interactions between the different factors (muscle type, frequency, and contraction level) were not significant and were not included in the model. Overall, significantly less electrical output was seen when adding voluntary contraction to supplement the stimulation contractions for all stimulation frequencies ($p<0.05$).

According to the results from repeated measures ANOVA and post-hoc Tukey tests, there were significant differences in electrical activity at some stimulation levels. As seen in Figure 22, the trend between increasing contraction levels and electrical activity output from the muscles was positive for all stimulation frequencies (10, 35, and 50 Hz). However, significant differences between increasing contraction levels were seen only with the lower frequency levels: 10 and 35

Hz ($p<0.05$). For VL, the trend between contraction level and muscle activity was positive for all stimulation frequencies as well (Figure 23). However, electrical activity in terms of increasing contraction levels was significant only when stimulation was delivered at 10 Hz ($p<0.05$).

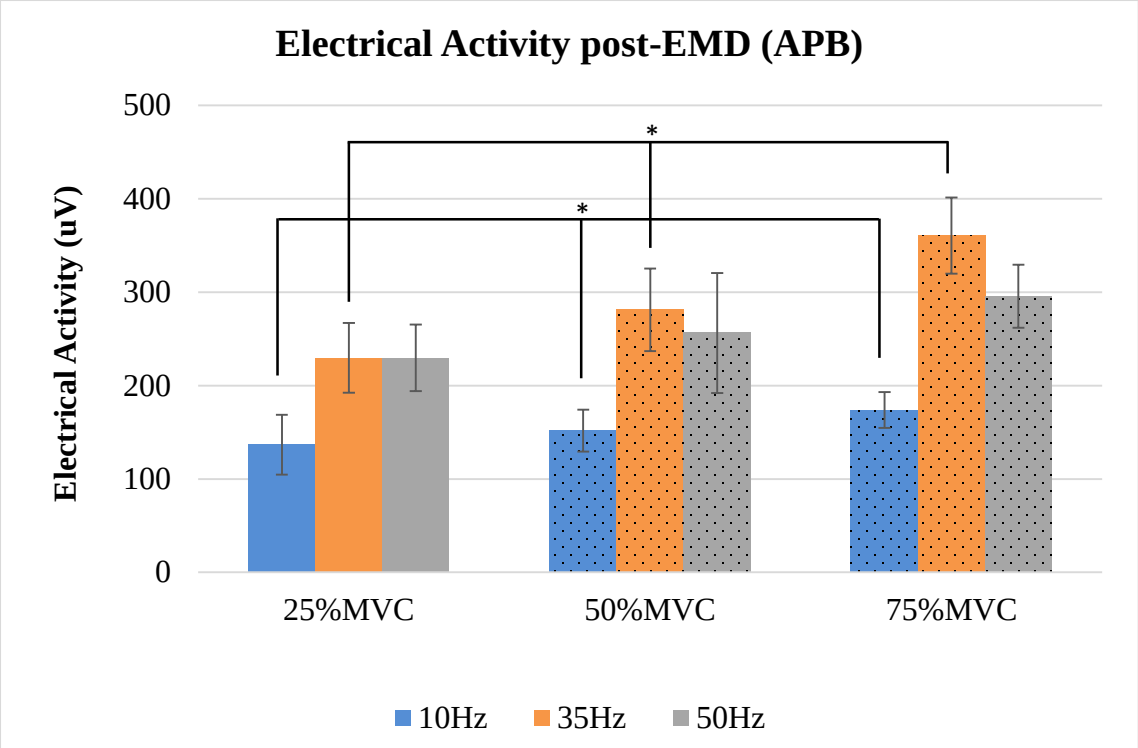

Figure 22: Electrical Activity Generated During ES-Elicited APB Tests
Electrical activity generated at 25% MVC (ES only), 50% MVC (25% ES +25% Vol), and 75% MVC (25% ES + 50% Vol) for APB post-EMD processing. *p<0.05, all contraction levels were significant to each other.

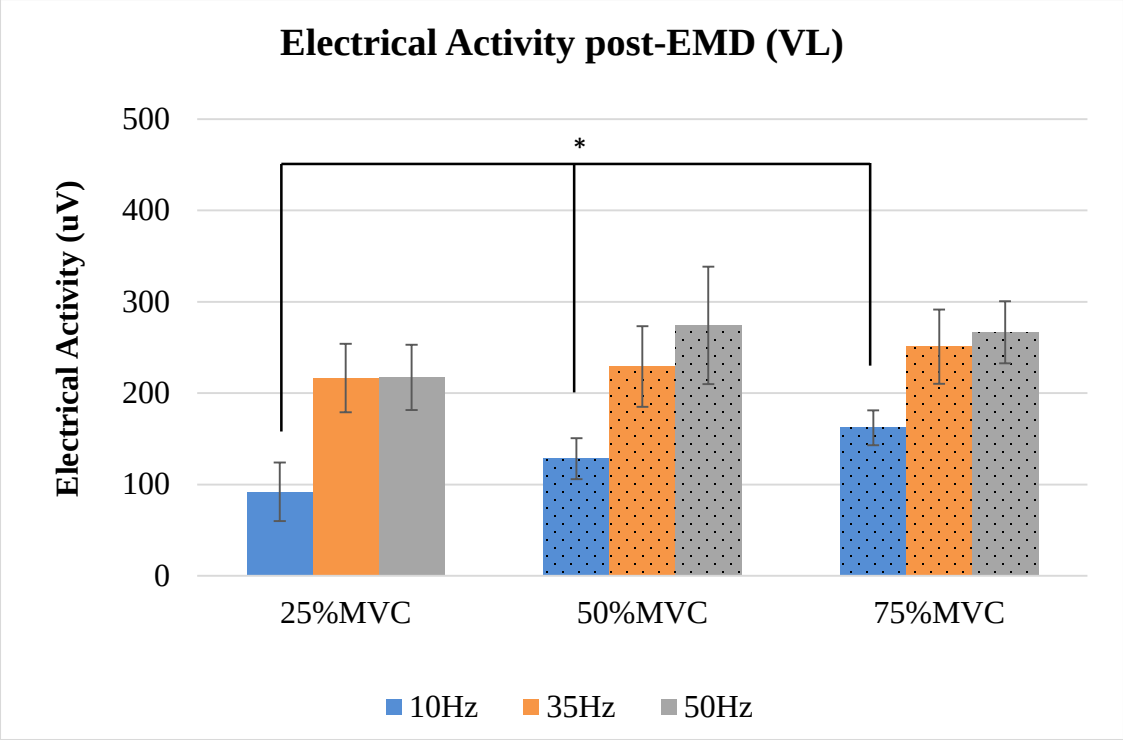

Figure 23: Electrical Activity Generated During ES-Elicited VL Tests
Electrical activity generated at 25% MVC (ES only), 50% MVC (25% ES +25% Vol), and 75% MVC (25% ES + 50% Vol) for VL post-EMD processing. *p<0.05, all contraction levels were significant to each other.

Table 4: Electrical Activity (uV) During Hybrid Activation.
Results from linear regression test (*n*=10)

	Electrical Activity (uV)			
Predictor	**Mean (SE)**	**β**	***d*(95%CI)**	**R^2**
Muscle				0.22
APB	235.1(14.3) §	30.9*		
VL	204.2(14.35)			
Frequency				
10 Hz	140.9(10.5) ||,#	-115.83*	1.24 (0.28,2.19)	
35 Hz	261.3(17.3) ¶	4.70	2.04 (0.96,3.12)	
50 Hz	256.7(18.5) ¶		0.26 (-0.62,1.14)	
Contraction level				
25	187(15.1) ‡	-64.68*	0.89 (-0.03,1.81)	
50	220.2(18.5)	-31.49	0.63 (-0.27,1.53)	
75	251.7(18.3) †		1.81 (0.77,2.85)	

Note: Significance was set at p<0.05. Differences between groups were determined through ANOVAs and Tukey post-hoc tests. Symbols (significance at p<0.01):-Linear regression model significance, †-significant difference from 25% MVC, ‡-significant difference from 75% MVC, §-significant difference from VL, ¶-significant difference from 10Hz, ||-significant difference from 35Hz, #-significant difference from 50 Hz.*

Isolated voluntary muscle response at 50% and 75% MVC

Hybrid activation contains ES-induced and voluntary components, and so the voluntary component was isolated by subtracting the output from 25% MVC (pure ES-elicited muscle response). Values were graphed in Figure 24. The results of the ANOVA showed no significance between the contraction levels, muscle types, or frequency. However, the effect sizes were calculated as d =1.34 (-2.32, -0.38) between the two contraction levels, 50% and 75% MVC. According to Cohen's classification, this is a large effect (d > 0.8) on the electrical activity when changing the contraction force. The effect size between muscles was calculated at d = 0.56 (-1.44, 0.35), indicating a medium effect (d > 0.5) on the EMG electrical activity caused by different muscles.

Overall, significantly less electrical output was seen when adding voluntary contraction to supplement the stimulation contractions than the initial activity generated from 0-25% MVC (pure ES-elicited response) for all frequencies ($p<0.05$).

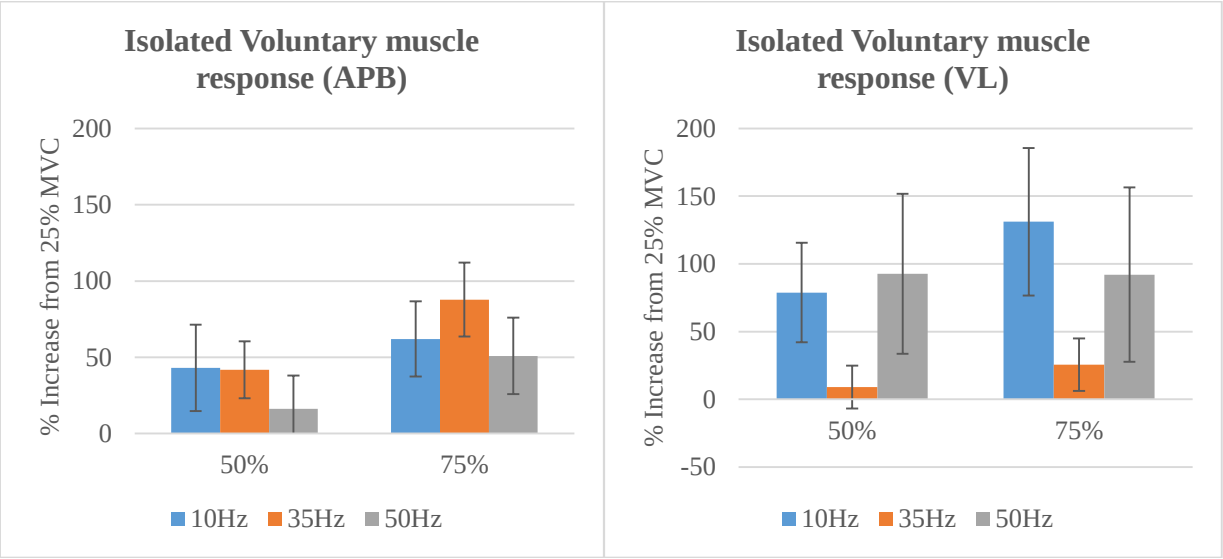

Figure 24: Isolated voluntary muscle response post-ES removal
Isolated voluntary muscle response percent increase post-ES removal (after removal of 25% MVC data) for (a) APB and (b) VL muscles.

VI. DISCUSSION

Comparison of voluntary muscle responses for different muscle types

For each muscle, EMG measurements portrayed the normal muscle recruitment progression. Higher contraction levels recruited more muscle fibers and generated higher EMG activities, a trend initially proposed by Henneman[10]. Our data showed that APB generated more electrical activity, but less force output, than VL. Fling[21] reported electrical output data for the small thumb and large leg muscle as recorded with needle electrodes. Although the needle electrodes used were able to measure more precise activity from the two muscles and therefore a much higher electrical amplitude was detected, Fling discovered the same behavior in electrical activity of small and large muscles as ours[21]. Fling proposed the electrical activity difference between the muscles to be due to a higher number of fibers clustering in smaller muscles, and the increased amplitude due to an increased number of action potentials recorded with the electrode.

One feature of surface EMG recordings is the electrical activity detected in the signal reflects the size and distance of the closest muscle fibers to the recording surface electrode. If fibers are more densely located closer to the electrode surface, more action potentials are detected and there will be greater electrical amplitude recorded with EMG. The APB is predominantly made up of slow Type I fibers that are generally smaller in width. In this manner, greater numbers of fibers can be clustered into a smaller area, crucial to control the fine motor skills that are required to operate the thumb. Knight and Kamen[77] reported that superficial muscle units are larger than deep motor units in the VL. Biologically, intramuscular regionalization could allow for more conservation of heat, higher access to blood flow and more efficient oxidative metabolism for fibers in deeper locations[77], features consistent with Type I, slow fibers. It might be possible that the EMG signal is detecting activity from the superficial, larger, Type II fibers in VL, while the EMG action potential of APB is measured from a greater

number of smaller fibers. As VL muscle fibers are longer than APB, there is also the possibility the electrode on the VL is not capturing all of the action potentials released[21]. EMG is a measurement of the total amount of electrical signals measured from the sum of all action potentials released at a given moment. Therefore, with a higher fiber density under the measurement electrode, the total number of action potentials executed will be larger, and overall electrical activity detection will be greater for a Type I muscle.

When we normalize both muscle EMG outputs to maximum units recruited, there remains a difference in electrical output between the muscles, indicating specific biomechanical differences. Our results show (Figure 17) that when normalized, VL exerts a greater force output per fibers recruited. We believe this is due to differences in fiber type composition. Type II motor units that are predominant in the VL muscle are not only thicker in diameter, but are also faster and stronger than Type I fibers. The fiber width of the APB muscle is much smaller, and requires a greater proportion of total fibers to perform at the same level of force.

Evident biomechanical differences are also reflected in the distribution of the signal frequencies. Traditionally, MDF values are used when investigating muscle recruitment, as MDF is a measurement of the frequency at which most fibers in the area become 'activated', or release an action potential. The EMG frequency spectrum is not dependent on the number and firing rate of motor units or changes in muscle force or load levels[78-81], but can be changed from muscles that have differences in fiber composition and distribution[82]. The range of values for MDF found in our experiment is consistent with instantaneous MDF values for the TA, another large leg muscle[83] , and the MDF values for the index finger[84], another small hand muscle. Several recent studies have reported that MDF increases as muscle fiber length decreases. This fiber length difference has only been studied using the same muscle, but the geometry application can be extended to separate muscles using the same concept. According to our results, there is no

change in MDF with increased activation and additional motor unit recruitment, but there is a difference between muscles with different fiber lengths. Although MDF has been examined among muscular recruitment of the same muscle[82], or group of muscles[85], it has not previously been used to quantify two muscles of different sizes and fiber distributions.

In addition to its use as an indication tool to display fiber type differences between muscles, MDF is one of the most commonly used outputs to show fatigue in a muscle[86,87]. Typically, when muscles fatigue, fibers at higher frequencies are recruited to supplement the exhausted fibers, increasing the MDF detected in the signal[88]. In our results, the unchanging MDF values per muscle regardless of the force output show there is no evidence of muscle fatigue with this procedure, although the significant differences between muscles suggest fiber type differences

Greater activation of muscle fibers during rehabilitation has been linked to progress toward recovery. Progressive resistance training has been used successfully to treat shoulder injuries, Achilles tendinopathy, and work-related neck and back pain[89,90]. Physical therapists often recommend strengthening exercises that involve the coordination of multiple muscles in a group[91], such as the quadriceps muscle or rotator cuff. Therefore, it is necessary to understand the recruitment and electrical output differences for each individual muscle.

In previous research on biofeedback applications, all muscles had been grouped together for analysis[92]. According to our results, when APB and VL are both contracting with the same proportional force (eg. 75% MVC), the APB must recruit a greater proportion of total fibers to engage in the task. Therefore, an injured player exhibiting 75% MVC force output with the VL does not necessarily engage the same proportion of functional motor units as an APB also contracting at 75% MVC. Based on our results, the recruitment patterns of muscles with different

fiber-types should be appraised independently. In order to expand on the results of our study, larger sample sizes testing additional muscles of varying fiber type compositions should be explored.

Evaluation of effectiveness of stimulation artifact removal

The addition of the EMD processing increased removal of the stimulation artifact, as demonstrated by increasing values of SNR, high values for PR, and decreased EIs. Each of these trends were predicted with reduction of 'noise', or 'artifact'. These three measurements indicate EMD is effective in reducing the effect of the stimulus pulse in the signal, regardless of the stimulation frequency.

The validation for the reconstruction with EMD has been conducted previously[66], showing EMD can isolate the muscle response even when the stimulus artifact and muscle output overlap. Some advantages with EMD as a removal process are that it is data-adaptive, computationally fast, and not template-based, and so additional inputs are not required[65]. EMD is more comprehensive than time, FFT, or wavelet filtering by itself, as decomposition preserves elements in each mode, instead of separating the artifact and EMG frequencies and then filtering them.

As previously mentioned, Qiu et al. recently developed an adaptive matched filter based on a genetic algorithm[64]. Although this method addresses the inconsistencies with artifact and M-wave placement, the method uses fixed parameters to remove stimulus pulses via a modified template extraction. Conversely, EMD does not need initial inputs on parameters such as artifact durations and other signal properties. The extraction is completely driven by the data itself. Additionally, our power reduction values were all higher than the ones reported by Qiu[64] with the genetic algorithm adaptive filtering technique and comb filter. PR is a good evaluation tool for

examining the efficiency of the removal process, as it is not affected by control models, or voluntary response. For all stimulation levels, the PR values shown in Table 3 suggest EMD is more effective in removing the artifact than the adaptive filter proposed by Qiu.

In addition to adaptive filters, other removal techniques have been compared with EMD. EMD is more effective in reducing the power of noise when compared with wavelet analysis[93], mainly because of its adaptive nature. Wavelet techniques fit the signal outputs to a pre-determined waveform, or 'mother wavelet', and attempts to process the signal linearly with wavelet filters[94]. Distortion post-processing has been analyzed in frequency and time domains, and the EMD method presents the best option in decreasing noise of the signal, but preserving signal information[93]. Preserving the signal energy is important because the sEMG output is a direct representation of muscle activity, and eliminating the least amount of non-stimulus data as possible during processing would give the most accurate presentation of actual muscle response. EMD can do this more effectively than filter or wavelet-based methods, two techniques that are most commonly used currently.

However, there are known limitations with the EMD method. High frequency components are extracted by the algorithm into the first few IMFs[65]. In this investigation, the first four IMFs were dominated with stimulus artifact, when they were eliminated, some higher frequency components were also eliminated from the analysis, potentially removing some muscle response data.

Scale-mixing, or 'mode-mixing' is an additional limitation that can be present in EMD-processed signals. During decomposition, signals are separated based on an algorithm that combines frequency and time domains. This causes different IMF components to have differences in intermittency, where some have widely disparate time scales and others have

similar time scales. Intermittency means a component comes into existence or disappears from the signal entirely based on the particular time scale, altering the frequency of the IMF. IMFs are combined during reconstruction, regardless of intermittencies, causing 'mode mixing', and components may not add together properly, particularly in the frequency domain. Several solutions have been proposed to this issue. These options include creating a scale limit to the envelope of extrema which allows a component to pass through, or adding then subtracting a masking signal[95] or white noise[96]. However, these options are limited for cases when the components are not well separated in frequency, or are embedded in high frequency artifact. A standardized method for dealing with mode mixing has not been established.

Additionally, the bandwidth used for this process (Butterworth filter: 20-500Hz) is the range typically used for muscle data analysis[97] and is a native feature of the physiological recording software and could not be changed. However, this range could cut off the stimulus artifact. The bandwidth is not wide enough to capture data at the fast rate of the biphasic stimulus, particularly at higher stimulus rates such as 35 and 50Hz. Based on Nyquist frequency calculations and a pulse width of 300us, to get better reconstruction, it is advised to have an increased sampling frequency and wider bandwidth in future studies.

Previously, muscle activity during ES has been difficult to monitor due to the contaminating stimulus artifact. Based on our results, it appears the artifact can been reduced using EMD, and this decomposition process is more effective in reducing the corrupting artifact than other alternatives. To accurately compare ES-induced and voluntary muscle activity to determine fiber recruitment progression, it is essential to examine only the activity from the muscle. It is therefore vital to remove as much artifact from the signal as possible. EMD alone reduced the power of the signals in this protocol 26 to 30 times (Table 3). Because of the difference in amplitude scale between artifacts and m-waves, some stimulation remaining in the

signal could dramatically increase the electrical output detected with EMG, and provide misinformation. EMD is the best way to preserve signal information while eliminating corrupting artifacts.

Comparison of muscle responses at different frequencies

Required intensity to reach 25% MVC

First of all, the smaller muscle (APB) required significantly less intensity to reach the 25% MVC force threshold than VL. VL required greater stimulation to activate the muscle most likely due to its size. Based on the force measured with the dynamometer in this study, there was a greater total force output by the large muscle. According to the S-shaped relationship between frequency and force output discovered by Binder-Macleod et al.[28] (Figure 8), as stimulation frequency increases, so does the normalized force generated by the muscle. In fact, the progressive relationship between increasing stimulation intensity and force output of the leg muscle has been established previously by Frigo[63]. Frigo engaged in a similar protocol, increasing intensity during knee extension until 40% MVC, and then supplementing voluntary contraction until 80% MVC was reached.

Frigo, however, did not examine differences in intensity required to activate different muscles. We found VL force outputs were significantly higher than APB, caused by greater current into the muscle to induce contraction. Additionally, when stimulated at 10 Hz, participants generally required higher intensities of stimulation in order to reach the same force level, similar to the trend found by Frigo with 16.67 and 25 Hz stimulation frequencies[63]. There was no difference between 35 and 50Hz for both muscles. This difference can be attributed to biomechanical differences in muscle fibers, discussed in the following section.

ES-induced muscle response at 25% MVC

According to our post-EMD processed results, there appear to be differences in electrical activity output between low and higher frequencies of stimulation. In the VL, there are significant differences in electrical activity measured between 10Hz and higher rates ($p<0.05$), but none between the higher frequencies, 35 and 50Hz.

For VL at 10Hz, a slower pulse is delivered to the muscle and less total recruitment is required for the muscles to generate 25% MVC. However, higher levels of intensity are required to be delivered to reach the same force level with 10Hz than with 35 or 50Hz- activated muscle. Overall, this indicates greater muscle activation occurs when the muscle is subject to faster stimulation rates. Consistent with our results for both muscles, a recent study exploring the effects of single motor unit stimulation of the human tibial nerve found that higher stimulation frequencies recruited more units at shorter latencies than lower frequencies[98]. This is most likely due to a changing contraction mechanism when an electrical pulse is delivered to the muscle at fast rates. Motor units are recruited as their motor axons are depolarized and then relax after the action potential has fired and the axon is repolarized[99]. However, if activation is delivered so frequently it does not allow for full depolarization, tetanic, or fused contraction occurs. This creates a cumulative effect involving multiple stimuli, causing greater muscle recruitment and higher EMG amplitudes. Higher stimulation frequencies such as 35 and 50Hz could cause tetanic contractions, forcing additional motor units to be recruited to complete the task. The EMG electrical activity results reported in this study indicate 35 or 50 Hz would be better to induce higher muscle fiber activation.

Further evidence of a changing contraction mechanism at faster stimulation rates is shown with the non-significance between 35 and 50Hz, which indicates that muscle is activated equally when stimulated at either frequency rate. Clinicians can use 35 and 50Hz to generate

tetanic contractions, although 50Hz is more common[20]. In fact, most clinical treatment protocols use frequencies between 20-50Hz[5,32], as higher frequencies produce stronger muscle contractions and are helpful to increase muscular strength[28]. There have been debates as to what stimulation frequency is most effective in a rehabilitation setting. 35 and 50Hz are two of the most common rates available on stimulation treatment machines, and according to the results presented here, both levels have a similar effect on the muscle. Therefore, clinicians can focus on the level which is more comfortable for the patient, rather which causes the most increased activation.

Biologically, tetanic or progressive voluntary contractions can cause an increase in muscle size and cross sectional fiber area, called 'muscle hypertrophy'. To induce muscle hypertrophy, progressive loading causes intermittent levels of stress to skeletal muscle, forcing adaptation to occur by increasing the size and amount of contractile proteins in each muscle fiber[100]. This leads to an increase in size in the individual muscle fibers and their resulting force production. Previous research shows that ES can cause hypertrophy in individuals with spinal cord injuries[101,102], enhancing regeneration of injured muscle tissue. Musculoskeletal issues are common with neuromuscular disorders like spinal cord injuries, as atrophy often occurs due to the inability to control the muscles without operational nerve connections, although a recommended stimulation frequency has not been determined yet.

Based on the results presented here and the evidence from additional sources, more stimulation causes greater activation, which ultimately leads to greater recovery. However, there is a limiting variable to this relationship, known as muscle fatigue. With regeneration of muscle tissue, there is often a trade-off between greater muscle activation and fatigue in the muscle, potentially hindering recovery. It has been well-established that muscle fatigue occurs faster with ES than with voluntarily-activated muscle[103]. This is most likely due to a difference in muscle fiber recruitment patterns, as discussed earlier. One study examined fatigue on progressively

increasing intensities[43]. According to the preferential selection principle of voluntary activation, more fatigue-resistant slow fibers should be recruited at lower intensities followed by fast fibers at higher levels. However, this study saw no differences in fatigue progression as stimulation intensity increased, indicating a non-selective, rather than preferential recruitment order occurred.

Furthermore, higher ES frequencies lead to greater muscle fatigue[104-106] and the optimal frequency depends on the type of muscle[107]. Preliminary investigations showed ES at a frequency below 40-50 Hz excited more slow-twitch, fatigue resistant fibers, and higher frequencies elicited fast-twitch, fatigable units[108,109]. Fatigue progressed with the increase in stimulation frequency. Overall, higher intensity and higher frequency induce greater muscle activations that can lead to recovery, but also more quick-setting muscle fatigue. 35 and 50 Hz stimulation appear to activate muscles similarly, but clinicians should keep fatigue in mind when developing rehabilitation protocols.

Increased muscle activation has been linked to progress toward recovery [89,90] and greater muscle hypertrophy. Accordingly, higher frequency stimulation, such as 35 or 50 Hz, should cause greater recruitment during a rehabilitation protocol than lower frequency activations, and potentially greater recovery.

Comparison of muscle responses voluntarily and electrically-elicited

Hybrid activation muscle response at 50% and 75% MVC

It is important to supplement stimulated muscle with voluntary activation for several reasons. First, the maximum tolerated intensity in human subjects is lower than that induced by voluntary contractions, as the electrical current is more targeted and limits the spatial arrangement of the motor units recruited[59]. Secondly, according to the significant differences

seen in ES and voluntarily activated muscle at 25% MVC, there is a difference in recruitment, as ES appears to select fibers for recruitment non-preferentially, and more likely based on position to the stimulating electrode. Additionally, a voluntary movement will activate synergistic and stabilizer muscles to complete a task, an arrangement not replicated by ES[60]. Therefore, ES cannot complete muscle movements with equivalent strength, as intermuscular coordination is not employed. Finally, voluntary contractions induce less fatigue than ES contractions[103]. During a long voluntary contraction, slow fatigue-resistant fibers are recruited first, and once they fatigue, neighboring motor units are recruited to complete the task. There is evidence ES recruits fibers non preferentially, and so the same types are recruited throughout the stimulation[56,57]. Specifically for larger, faster motor units such as the VL, fatigue is more intense and selective when electrically activated[60,61,69], and can cause more damage than voluntary activated muscle[110]. Therefore, supplementing stimulated contraction with voluntary should delay the effects of fatigue[111], another key advantage with hybrid stimulation.

Because of the many favorable outcomes with hybrid activation, it is crucial to first evaluate the effectiveness of the combined therapy. We were able to successfully activate muscles with electrical stimulation and then supplement with voluntary activation to employ high recruitment levels up to 75% MVC. Another study examined reconstruction of the anterior cruciate ligament (ACL) post-surgery with standard isometric training with and without percutaneous ES during the recovery period (1 to 5 weeks) [112]. The electrically-stimulated group had better muscle function and greater oxidative enzyme activity after the intervention, suggesting ES prevented muscle atrophy after ligament reconstruction surgery. Another study reported quadriceps strength improved more quickly than traditional rehab when adding ES to a rehabilitation plan after a total knee arthroplasty[113]. Overall, a combined treatment, including voluntary and electrically-activated components, restores strength post-surgery, particularly in

the early phases of rehabilitation[24,114]. Petterson and Snyder-Mackler suggested that strengthening solely through voluntary training might not be enough to overload the muscle to increase strength so early in the recovery process, as patients are unable to contract their muscles for several days post-surgery[115]. During the initial recovery periods, ES is often the only source for muscle activity or contraction, and ES-activation alone is enough to stimulate neuromuscular adaptations[24]. As the muscle recovers, supplementation of voluntary activation may expedite the recovery process and prevent fatigue. When fibers regain more activation, treatment with ES can be reduced, and voluntary training can be phased-in during later sessions as strength is improved[59]. This treatment protocol is similar to the one done in this investigation. A similar investigation could be conducted to determine the efficacy of ES and voluntary activation with other difficult-to-treat areas such as the rotator cuff.

Isolated voluntary muscle response at 50% and 75% MVC

With hybrid activation, it is important to evaluate the individual contributions from both components of the signal in terms of muscular response. By separating the signal into voluntary and ES-induced muscle behavior, it is possible to further examine recruitment differences. For measurements recorded at 50 and 75% MVC, voluntary muscle activity was extracted from activity caused from ES. Due to the difficulty in effectively eliminating the artifact, no other study has specifically examined the muscle activity output of the voluntary component during combined activation. The effectiveness of our removal procedure and accuracy of the isolated voluntary response data were evaluated in the same manner as normal voluntary contraction. For isolated voluntary response post-extraction, there was an effect on the electrical activity output when changing muscle type and varying contraction levels. Based on these calculations, isolated voluntary muscle behavior followed the same trend as progressive normal voluntary contraction

behavior (K.S., unpublished data, 2015), although the relationship between electrical output and increasing contraction was stronger with normal voluntary than isolated voluntary.

Fortunately, normal voluntary behavior has been extensively researched and Henneman's progressive size recruitment principle states more fibers are recruited with increased contraction, visible through increased amplitudes with sEMG. This progressive increase was true for voluntary responses (K.S., unpublished data, 2015) with small hand and large leg muscles, indicating both muscle responses follow the same type of recruitment pattern. This is particularly important for hybrid activation, as the isolated voluntary component has not been analyzed previously, and this evidence suggests the orderly voluntary mechanism for recruitment continues, even after ES has selected its fibers for recruitment without preference toward a specific type.

Although the overall progressive relationship between normal voluntary and isolated voluntary response was similar, the electrical activity magnitude between 50% and 25% MVC was significantly larger for voluntary contraction than isolated. Figure 24 showed the isolated voluntary response (shown in Figure 24 as 'increase from 25% MVC' for isolated voluntary response). Therefore, although there is a similar pattern of recruitment between both voluntary types of contractions, the component that is part of the hybrid activation releases less muscle activity detected by the electrode. One possibility for this marked reduction in electrical activity measured with the EMG could be due to fiber regionalization in the muscle. EMG surface electrodes detect signals of the closest muscle fibers to the surface. If ES evoked the closest muscle fibers to the stimulating electrode, the most superficial muscle units would be recruited, resulting in large activities detected by the EMG. This high measurement is demonstrated in Figure 22 and 23 at 25% MVC. 50 and 75% MVC contractions then required voluntary activation, a preferential recruitment of specific types of fibers in order. However, because all the

fibers closest to the surface of the skin were already recruited by the ES, it is possible deeper motor units then needed to be called upon to engage the muscle up to 50 or 75% MVC. In this case, the preferential recruitment caused by voluntary activation would still occur with the fibers that were available (the deeper units), but the far location from the recording electrode would cause less electrical detection by the EMG. Only small amounts of increased activity were measured at 50 and 75% MVC, although the muscle output two to three times the amount of force, supporting this theory.

Furthermore, effect sizes suggested strong and medium effects from different contraction forces and muscle types in terms of electrical output from the muscle during isolated muscle response. The increased contraction effect displays the same trend suggested by Henneman for voluntary activity, as 75% activity values were higher than 50% MVC. However, the increase is not proportional, and does not fit a linear model for either muscle, highlighting again the differences in recruitment between electrically-elicited and voluntary contractions.

Regardless if muscles were electrically stimulated or voluntarily contracted, the small thumb muscle consistently output higher electrical activities than the large leg muscle[21,83,84] (also K.S., unpublished data, 2015). This is most likely due to the fiber differences in the muscle, as the signal amplitude is directly related to the number of fibers clustered in one area, specifically the area detectable by an electrode. For a predominately Type I muscle such as the APB, many smaller fibers are distributed closer to the surface of the skin, causing greater action potentials to be detected by the surface electrodes[12].

Overall, muscle response with ES has previously been unexplored due to the overwhelming stimulus artifact. Based on the evidence presented here, EMD is an effective mechanism for eliminating the artifact post-data collection and should be used to analyze more

muscle response data. Although hybrid activations have been established as effective in improving muscle performance and recovery, the electrical activity has never been examined in terms of contributions from ES-induced and voluntary components. This information is important when developing treatment regimens to regenerate muscle, create rehabilitation protocols that will start with ES and gradually progress toward voluntary-controlled activation, and finally aid in the development of neuroprosthetic devices.

VII. CONCLUSION AND CLINICAL IMPLICATIONS

Overall, this investigation addressed several knowledge gaps in terms of muscle response when exposed to stimulation. Results from this study helped to reveal differences in recruitment patterns during voluntary activation of two different muscles. Smaller, predominately Type I muscles require a higher muscle performance target during rehabilitation as more electrical activity can be generated during voluntary activation.

Additionally, isolating muscle response data from stimulations that are contained in the same spectra is a continuous challenge. It appears that using decomposition can effectively reduce the muscle response from the stimulus artifact without using templates or additional parameter inputs, making this method of filtering non-linear and non-stationary data more generalizable to users who are not experts in the field of signal analysis.

Using this method, we were able to compare muscle activity from three separate frequencies and discovered stimulation at higher frequencies such as 35 and 50 Hz generated greater electrical activity, and correspondingly greater fiber recruitment, from the muscle. Greater muscle activation during treatment is related to greater recovery of muscle tissue, and so the use of higher frequency stimulation during rehabilitation is expected to lead to more functional improvement.

Finally, to obtain even greater muscle activation, we supplemented voluntary contractions on top of stimulated muscle, creating ‘hybrid activation’. Hybrid-activated muscle is a good option for clinicians to generate higher force outputs and greater muscle activity, while minimizing the likelihood of fatigue or tissue damage. However, the mechanism of recruitment is not the same for stimulated muscle and additional voluntary muscle supplemented on top, as each component contributes different levels of activity to the overall electrical activity.

Overall, information generated with this procedure will give valuable knowledge regarding motor unit recruitment with stimulation, and with a combination of voluntary and electrically-elicited contractions.

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