Modulation of Soleus H-Reflex across different levels of voluntary isometric contractions

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PREFACE

This master's thesis marks the culmination of my research on the Modulation of Soleus H-reflex across different levels of isometric voluntary contractions. My journey toward this topic began during my Bachelor's in Mechanical Engineering in India, where a course Biomechanics sparked my interest in the field. This eventually led me to pursue a master's degree in Mechanical Engineering with a focus on Biomechanical Design at TU Delft, Netherlands.

The motivation for selecting this topic stems from my deep interest in understanding the neural mechanisms that underlie muscle reflexes and motor control, which have applications in clinical and rehabilitation strategies. This thesis provided a significant departure from my undergraduate work, which centered on design. It gave me the opportunity to explore and conduct human experiments, an experience that not only broadened my technical skills but also greatly enhanced my confidence in interacting with and handling participants. Additionally, I gained valuable experience in data acquisition and analysis.

This thesis would not have been possible without the support of my supervisors, **Winfred Mugge** and **Mark van de Ruit**, to whom I owe my deepest gratitude. Their invaluable guidance, insightful feedback, and unwavering support were crucial to the completion of this work and my personal development throughout the process. I would also like to thank our lab technician, Christian, who endured more electrical stimuli than anyone and, his assistance in designing the experimental protocol and for helping me navigate various technical challenges.

I would also like to express my gratitude to my academic advisor, Benjamin Jansen, for keeping track of my well-being and academic progress. My heartfelt thanks go to my psychologists, Jordy de Kleijn and Nick Kerckhoffs, for their involvement in my personal development. I am deeply thankful to my parents for their financial support throughout my studies, and to my friends for the wonderful memories we created together. Finally, I extend my sincere appreciation to the 10 volunteers who generously contributed their time to participate in this study.

I hope that the findings presented in this thesis will make a meaningful contribution to the field of neuromuscular research and inspire further studies in this area.

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Modulation of Soleus H-Reflex across different levels of voluntary isometric contractions

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Abstract— **This study investigates "How does the modulation of the Soleus H-reflex vary across different levels of isometric voluntary contractions?" The hypothesis that the Soleus H-reflex amplitude follows a biphasic response, with an initial increase at lower contraction levels, followed by a plateau or decrease at higher levels of contractions. To test this, ten healthy subjects were recruited to perform isometric voluntary contractions at different levels of maximal voluntary contraction (MVC) (0%, 20%, 40%, 60%, and 80% MVC). The H-reflex peak-to-peak amplitude was measured and normalized as a percentage of the maximum M-wave (H/Mmax). The data were analyzed using a repeated measures ANOVA to assess the effect of MVC level on the Soleus H-reflex amplitude, followed by pairwise comparisons to identify significant differences between contraction levels. A repeated measures ANOVA revealed a significant effect of MVC level on Soleus H-reflex amplitude (H/Mmax) (F(4,32) = 4.82, $p < 0.05$). Pairwise comparisons showed a significant increase in H-reflex amplitude between 0% and 20% MVC (p = 0.013), while no significant differences were observed between higher contraction levels (20%, 40%, 60%, and 80% MVC). This pattern is indicative of the biphasic hypothesis, suggesting that the reflex amplitude significantly increases initially but plateaus as contraction intensity increases. These findings suggest that the initial increase and subsequent plateau in reflex amplitude may be influenced by changes in spinal excitability and inhibitory mechanisms as contraction intensity increases.**

Keywords: Soleus H-reflex, voluntary isometric contractions, excitatory, inhibitory.

I. Introduction

The modulation of the soleus H-reflex across different levels of isometric voluntary contractions is a substantial area of research in neurophysiology, particularly in understanding the mechanisms of motor control and reflexive responses. The Hoffmann reflex, or H-reflex, is an electrically induced reflex analogous to the mechanically induced spinal stretch reflex and thereby provides insights into spinal cord excitability and the functional integrity of the neuromuscular system [7] [12]. The H-reflex in Soleus is elicited by stimulating the Ia-afferent fibres of the tibial nerve, which in turn activates the motoneurons responsible for innervating the soleus muscle. The H-reflex is especially important because it provides insights into the excitatory and inhibitory processes that govern muscle activation during various physical activities [5] [8].

The Soleus muscle, located in the lower leg, plays a crucial role in maintaining posture and performing locomotor activities such as walking and standing. The muscle is primarily composed of slow-twitch, type I fibers, which are well-suited for endurance activities and are often activated during sustained, isometric contractions. Voluntary muscle contraction involves the activation of motor neurons via descending cortical input, modulating the excitability of the motor neuron pool and reflex pathways. As muscle contraction increases, neural mechanisms adjust reflex excitability to control motor output effectively. Previous studies have demonstrated that the H-reflex amplitude can vary significantly with different levels of voluntary contraction, suggesting an interplay between voluntary and reflexive mechanisms [20].

The research question guiding this thesis is: "How does the modulation of the soleus H-reflex vary across different levels of isometric voluntary contractions?" This inquiry is essential for elucidating the relationship between voluntary muscle activation and reflex excitability. The hypothesis is that the Soleus H-reflex amplitude demonstrates a biphasic response to increasing levels of isometric contraction, characterized by an initial increase at lower contraction levels followed by a decrease or plateau at higher levels. This expected biphasic behavior arises from the recruitment of motor neurons at lower contraction levels, which leads

to an initial increase in reflex amplitude and, the decrease or plateau in H-reflex amplitude at higher contraction levels may be explained by central inhibitory mechanisms, such as presynaptic inhibition and recurrent inhibition, which modulate reflex excitability as muscle contraction intensifies. This hypothesis is supported by findings [6] [27] that indicate an interplay between excitatory and inhibitory mechanisms at the spinal level.

By addressing this research question, the study aims to provide deeper insights into the neural control of reflex excitability and contribute to our understanding of motor control and reflex modulation during voluntary muscle activity.

II. Materials and Methods

A. Participants

The study involved 10 male participants (-aged 22 to 30 years), whom self-reported no neurological deficits. All participants provided written consent, and the study was approved by the Human Research Ethics (HREC) committee of Delft University of Technology (Approval number: Application number: 4308).

B. Experimental Setup

The study utilized the Achilles device (Moog, Nieuw-Vennep, the Netherlands), a single-degree-of-freedom haptic ankle manipulator capable of applying mechanical perturbations such as position and torque perturbations. The device's angular encoder measured the footplate's angular position and velocity, which corresponds with the ankle angle and angular velocity, while the force transducer measured the rotational torque.

Muscle activities (electromyography, EMG) of the tibialis anterior, soleus, gastrocnemius lateralis, and gastrocnemius medialis were recorded using the Porti EMG device (Porti7-8b8at) (TMSi, Oldenzaal, the Netherlands). Surface electrodes (Ag/AgCl, 0.8 cm diameter) were placed approximately 2 cm apart over the corresponding muscle belly and aligned with the assumed direction of the muscle fibers to record EMG signals. The ground electrode was positioned on the bony landmark of the lateral malleolus.

H-reflexes and direct muscle (M) responses were elicited by electrical stimuli delivered via a Micromed bipolar surface stimulator, using 6 mm diameter felt electrodes spaced 25 mm apart. The posterior tibial nerve was stimulated with bipolar electrodes placed longitudinally on the skin above the tibial nerve in the popliteal fossa, positioned at the point where the weakest stimulus intensity elicited an H-reflex. The M-wave is a direct muscle response caused by the activation of alpha motor neurons through direct electrical stimulation of the motor axons, while the H-reflex originates from

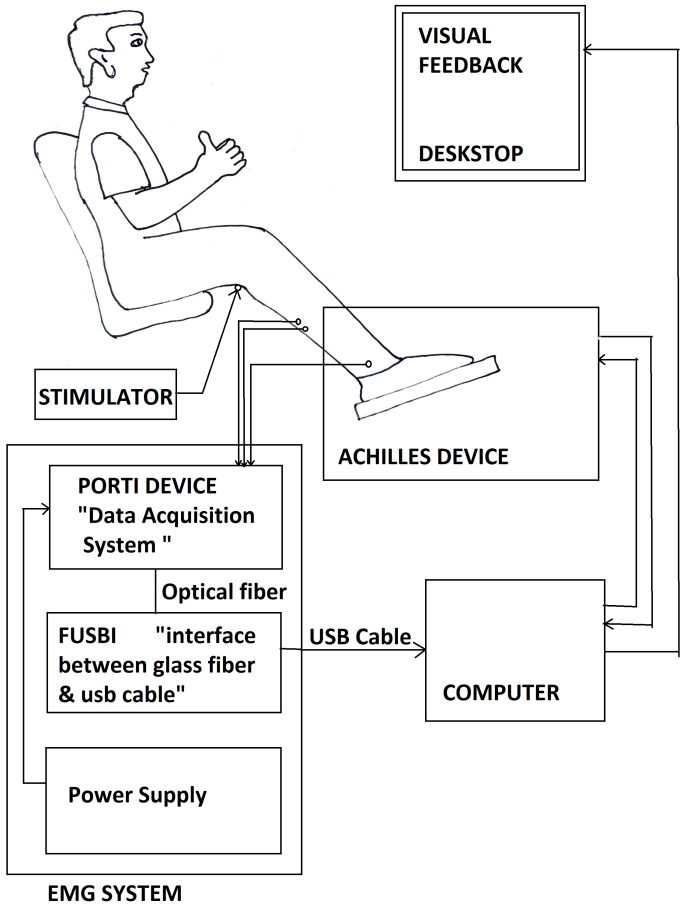

Fig. 1. The figure illustrates the experimental setup. A seated participant places right leg on an "Achilles Device," which stabilizes the foot or ankle during the experiment, likely to set joint angles and, provide support and resistance. Electrical stimulation is delivered to the leg via a stimulator, targeting the tibial nerve to elicit the Soleus H-reflex . The EMG signals are captured by the Porti EMG device (Porti7-8b8at) , a data acquisition system connected to the participant through electrodes. An optical fiber links the PORTI to the FUSBI, an interface that facilitates data transfer to the computer via USB. The EMG system, consisting of the PORTI, FUSBI, and power supply, processes the muscle's electrical activity. A real-time torque feedback to the participant is provided through a desktop display, helping participant to maintain specific contraction levels during voluntary isometric contractions.

the activation of Ia-afferent fibers, which produce a reflexive activation of motor neurons via the spinal cord. All data were stored using MATLAB (MathWorks, Natick, MA, USA) .

C. Procedures

The participant was seated in an adjustable chair as shown in Figure 1, with the right leg semi-flexed at the hip to 80 degrees (where 0 degrees represents full hip extension), the knee flexed to 60 degrees (with 0 degrees representing full knee extension), and the ankle positioned at 10 degrees of plantar flexion (with 0 degrees representing the neutral position of the ankle, where the foot is perpendicular to the leg). The participant's right foot was placed and secured using Velcro straps on the footplate of an Achilles device, aligned with the axis of rotation of the ankle joint. Electrodes were placed on the soleus, tibialis anterior, gastrocnemius lateralis, and gastrocnemius medialis muscles to record muscle activity. Additionally, stimulation electrodes were placed at the back of the knee to allow for electrical stimulation of the posterior tibial nerve. This setup was designed to elicit an H-reflex in the soleus muscle with minimal stimulation intensity.

At the start of the experiment, the initial ankle joint angle and torque generated by the participant's right foot were recorded by the Achilles device. Electrical stimulation (a 1-ms rectangular electrical pulse) was applied using a Micromed bipolar surface stimulator to the posterior tibial nerve (at the back of the knee) to determine the maximum peak-to-peak amplitude of the H- and M-waves, as well as the intensity required to elicit 50% of the maximum peak-to-peak amplitude of the H-wave in the soleus muscle at rest. This was achieved by gradually increasing the stimulation intensity from 0 mA in 2 mA increments until the M-wave reached its maximum amplitude without further increase in response to higher stimulation intensities. A minimum gap of 10 seconds was maintained between stimuli to minimize the effects of post-activation depression [21].

Maximal plantar flexion torque was measured under isometric conditions, where the participant was instructed to push against the footplate with maximal effort for 5 seconds. The highest value recorded from these three trials was taken as the participant's MVC. The main experiment consisted of 5 sets (with rest periods of 1-5 minutes between sets), and each set comprised 5 trials (with rest periods of 30-60 seconds between trials). During each trial, the participant was instructed to maintain a specific torque level, corresponding to 0%, 20%, 40%, 60%, or 80% of their maximal plantar flexion torque, using visual feedback displayed on a screen. The torque exerted during plantar flexion was presented on a computer screen placed in front of the participant as visual feedback. Once the participant reached the required torque level and maintained it for at least 2 seconds, a submaximal electrical stimulus (a 1-ms rectangular pulse at the intensity required to elicit 50% of the maximum peak-to-peak H-wave amplitude in the soleus at rest) was delivered to evoke H-reflex responses.

D. Data Acquisition

The post-processing of the recorded Soleus EMG data for each trial was carried out to identify the H-wave and M-wave. The data were first converted to the time domain using a sampling frequency of 2048 Hz. This signal was filtered using a Butterworth band-pass filter with a low cutoff frequency of 30 Hz and a high cutoff frequency of 400 Hz. To calculate the root mean square (RMS) value of the Soleus background EMG (BEMG), an EMG window of 1000 ms duration, including a 1000 ms pre-stimulus period, was used.This signal was then rectified, and the RMS envelope was computed using a 100 ms window size. An EMG window of 100 ms was extracted, comprising a 30 ms pre-stimulus period and a 70 ms post-stimulus period.

The peak-to-peak (P-T-P) amplitudes of the H-reflex and M-waves were automatically determined based on their respective latencies. The peak-to-peak (P-T-P) amplitude was calculated as the absolute difference between the maximum positive and negative values of the response as shown in Figure 2. The M-wave and H-wave magnitudes were defined as the P-T-P amplitudes of the unrectified EMG signal within the 3 ms to 25 ms window for the M-wave, and the 25 ms to 60 ms window for the H-wave, with 0 ms representing the time of stimulus elicited.

The H-reflex peak-to-peak amplitude values obtained under all conditions were normalized

to each participant's maximal M-wave (M-max) recorded at rest to account for variations between participants due to anatomical differences, nerve conduction, and electrode placement. Similarly, the M-wave peak-to-peak amplitude values obtained under all conditions were normalized to each participant's maximal M-wave (M-max) recorded at rest. For one participant, the maximal M-wave at rest could not be determined; therefore, this participant's data was excluded from the statistical analysis.

E. Statistical Analysis

H/Mmax, M/Mmax and RMS data of soleus were collected for each subject across different levels of voluntary isometric contraction (0%, 20%, 40%, 60%, and 80% MVC). Descriptive statistics (mean and standard deviation) were calculated to summarize H/Mmax, M/Mmax and RMS at each contraction level both within each subject and across all subjects. Before selecting a statistical test, normality checks were performed to assess whether the data were normally distributed at each MVC level. If the data were found to be normally distributed, a parametric test—Repeated Measures ANOVA (Analysis of Variance) was chosen; if not, a Friedman test would be applied. Upon finding a significant effect, a post-hoc test was performed to conduct pairwise comparisons between the different contraction levels.

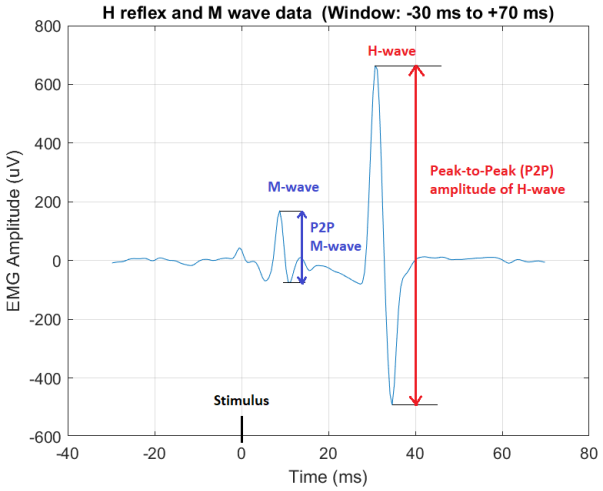

Fig. 2. A graphical representation of the M-wave and H-wave of Subject 2 at 0% MVC

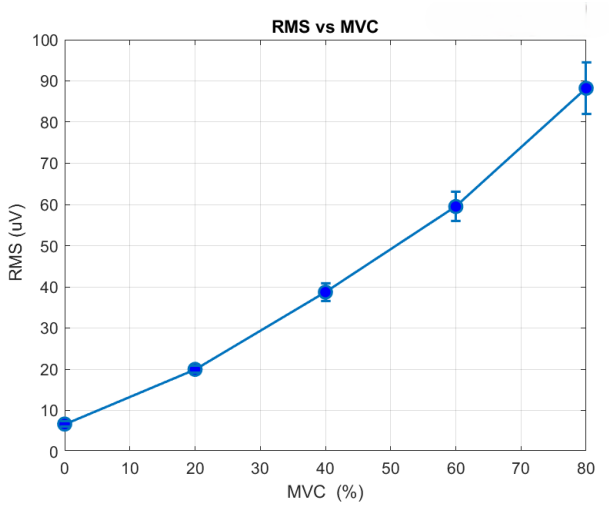

Fig. 3. A graphical representation of the Soleus BEMG RMS value with error bars (SEM) across % MVC

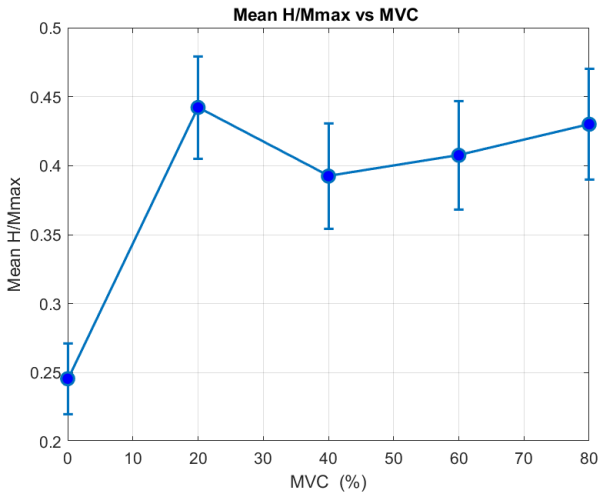

Fig. 4. A graphical representation of the mean H/Mmax with error bars (SEM) across different levels of MVC (%)

TABLE I

PAIRWISE COMPARISONS BETWEEN MVC LEVELS FOR H-REFLEX AMPLITUDE (H/MMAX)

MVC Level 1 (%)	**MVC Level 2 (%)**	**pValue**
0	20	0.0039
0	40	0.0039
0	60	0.0039
0	80	0.0078
20	40	0.8203
20	60	0.4258
20	80	0.3594
40	60	0.7344
40	80	0.7344
60	80	0.8203

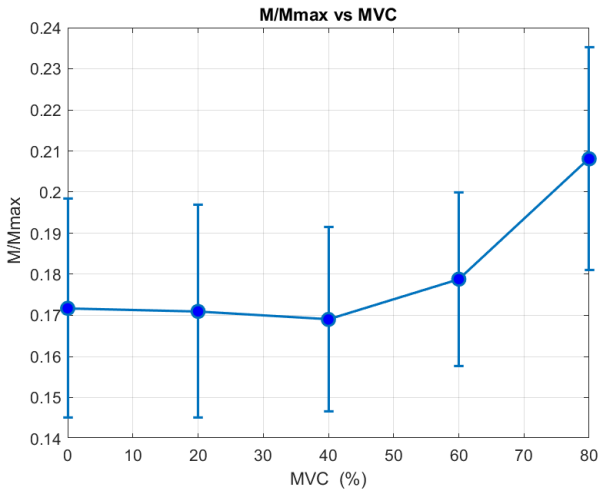

Fig. 5. A graphical representation of the mean M/Mmax with error bars (SEM) across different levels of MVC (%)

III. Results

Normality checks were performed on RMS data and found out that the RMS data was not normally distributed. Friedman test was used. Upon finding a significant effect, pairwise comparisons using Wilcoxon Signed-Rank test was performed. The Root Mean Square (RMS) of the Soleus background EMG (BEMG) was significantly increased with the increase in contraction level as shown in Figure 3. This was supported by Friedman test which returned a significant result with a p-value of 2.8937e-07, indicating that the MVC levels show highly significant differences in RMS value. Thus, the participants may have successfully executed the task, as indicated by the background EMG data of Soleus muscle.

A graphical representation of the mean H/Mmax with error bars is shown in Figure 4. Normality checks were performed on H/Mmax data and found out that the H/Mmax data was not normally distributed. Friedman test was used. Upon finding a significant effect ($p = 0.0014194$), pairwise comparisons using Wilcoxon Signed-Rank test was performed. The direction of the effect indicated a significant increase in H-reflex amplitude between 0% and 20% MVC. Pairwise comparisons revealed a significant difference between these two levels. No significant differences were observed between higher contraction levels (20%, 40%, 60%, and 80% MVC) as shown in table II, suggesting that the H-reflex amplitude plateaued after the initial increase. The initial significant increase from 0% to 20% MVC, followed by non-significant differences across higher MVC levels (20%, 40% , 60% , 80% MVC), suggests a biphasic response.

A graphical representation of the mean M/Mmax with error bars is shown in Figure 5. Normality checks were performed on M/Mmax data and found out that the M/Mmax data was not normally distributed. Since the M/Mmax data were not normally distributed, a Friedman test was used. A Friedman test indicated no significant effect of MVC level on M-wave amplitude ($p = 0.28053$). This result confirms that the M-wave remained stable regardless of the increase in MVC levels.

IV. Discussion

This study aimed to investigate how the modulation of the Soleus H-reflex amplitude varies across different levels of isometric voluntary contractions. Specifically, the study hypothesized that the Soleus H-reflex amplitude would exhibit a biphasic pattern, with an initial increase at lower contraction levels, followed by a plateau or decrease at higher levels. The main finding was that the H-reflex amplitude significantly increased from 0% to 20% MVC, followed by a plateau at higher contraction levels (20%, 40%, 60%, 80% MVC), with no further significant changes. This indicate a interplay between excitatory and inhibitory mechanisms at the spinal level.

A. Neural Mechanisms

The change in the Soleus H-reflex amplitude with the level of voluntary contraction can be explained by the interplay of multiple neural mechanisms:

1) Motor Neuron Excitability: As the levels of voluntary contraction increases, the motor neurons become progressively more depolarized, bringing them closer to the activation threshold. At lower contraction levels, this results in a higher probability that excitatory postsynaptic potentials (EPSPs) generated by the H-reflex stimulation will reach threshold, leading to an increase in reflex amplitude [1]. This is the mechanism underlying the initial increase in

H-reflex amplitude with increases in low-level contractions [11]. This is because with further increase in low-level voluntary contraction, the motor neurons become further more excitable. At around 20% MVC, enough motor neurons are brought near their activation threshold,leading to maximal recruitment from the reflex pathway.

2) Motor Neuron Recruitment and Saturation: At higher levels of contraction, many motor neurons are already firing as a result of the voluntary input. Fewer motor neurons are thus available to be recruited by the reflex pathway, leading to a peak in reflex amplitude followed by a reduction or plateau in reflex amplitude, since motor neurons already fired may become refractory or hyperpolarized and less responsive to further stimulation [9]. Already at 20% MVC, a considerable portion of the motor neuron pool is activated due to voluntary contraction. During higher levels of contraction-say, 40% MVC-most of the motor neurons are already recruited by the voluntary input and thus fewer are available to be excited by the reflex, which in turn has a tendency to plateau or decrease.

3) Inhibitory mechanisms: These inhibitory processes may also contribute to the modulation of the H-reflex amplitude across different contraction levels.

(a). Presynaptic Inhibition:

Presynaptic inhibition is a mechanism that controls the amount of neurotransmitter released at the synapse between an afferent neuron (sensory neuron) and a motor neuron. This reduces the efficacy of synaptic transmission to prevent overexcitation of the motor neuron due to excessive sensory input [16]. The Ia afferent fibers (which carry sensory information from muscle spindles) synapse on alpha motor neurons in the spinal cord to produce the H-reflex.In presynaptic inhibition, an interneuron (typically a GABAergic interneuron), forms a synapse on the axon terminals of the Ia afferent fibers, not the motor neuron directly. The interneuron then releases gamma-aminobutyric acid (GABA), which binds to GABA-A receptors on the presynaptic terminal of the afferent fibre [14]. Activation of these receptors increases chloride (Cl) conductance, leading to a shunting effect that reduces the amplitude of action potentials arriving at the terminal. Thus, less calcium (Ca^2) enters the terminal, reducing neurotransmitter (glutamate) release into the synapse. Eventually, the motor neuron would get an overall weakened excitatory signal and hence eventually decrease the H-reflex. Presynaptic inhibition fine-tunes sensory input to prevent excessive reflex responses. It plays a role in tasks requiring smooth movement, allowing selective modulation of reflexes depending on the motor task or posture [24].

As voluntary contraction increases, the nervous system requires greater fine-tuning of motor neuron excitability to prevent excessive reflex responses and ensure smooth and controlled movements. Presynaptic inhibition allows for this fine control by adjusting the amount of sensory input from Ia afferents reaching the motor neurons. This inhibition increases with voluntary contraction in order to modulate reflex excitability and prevent over-activation of the motor neurons. At lower levels of voluntary contraction (0-20% MVC), the amount of presynaptic inhibition is relatively low, thus, allowing the Ia afferents to transmit strong excitatory signals to the motor neurons. Because strong inhibition does not occur, amplitude of the H-reflex can increase as more motor neurons are recruited and excited by the afferent input. As voluntary contraction level increases, he activation of GABAergic interneurons increases, leading to increasing presynaptic inhibition, which in turn decreases the amount of excitatory input reaching the motor neurons, contributing to the decrease or plateau in H-reflex amplitude at higher contraction levels which is observed in between 20% to 80% MVC in figure. The increased presynaptic inhibition prevents the reflex pathway from over-exciting motor neurons, thus dampening the reflex despite the stronger voluntary contraction.

(b) Recurrent Inhibition

Recurrent inhibition is a feedback mechanism that involves the firing of the alpha motor neurons to stimulate a specialized group of inhibitory interneurons called Renshaw cells in the spinal

cord. In return, these Renshaw cells inhibit the same alpha motor neurons or other nearby motor neurons, preventing excessive and prolonged motor neuron firing using a form of negative feedback [10] [19]. This mechanism has great significance in the limitation of the duration and intensity with which the motor neurons can fire during sustained contractions. Recurrent inhibition therefore plays a very significant role in the stabilization of the activity of motor neurons and in the prevention of overexcitement during voluntary contractions. As the motor neurons fire more rapidly with increased contraction, the Renshaw cells become more active, exerting greater inhibitory influence on the motor neurons to prevent them from becoming over-excited. This increase in recurrent inhibition helps regulate motor output and prevents excessive motor neuron activation, ensuring smooth and controlled contractions without excessive reflex activity.

At low levels of voluntary contraction,motor neuron activity is relatively low, and recurrent inhibition is minimal since the frequency of motor neurons firing is not high enough to fire the Renshaw cells at any significant rate. This minimal recurrent inhibition allows motor neurons to be more excitable, contributing to the increase in the H-reflex amplitude which is observed, as motor neurons are more readily recruited by the reflex pathway. As levels of voluntary contraction increase, alpha motor neurons fire more frequently to sustain the increasing muscle contraction. The increase in firing causes Renshaw cells to become active that provide feedback inhibition either to the same or surrounding motor neurons [28]. This recurrent inhibition decreases motor neuron excitability, thereby restricting the responses of the motor neurons to reflexive input from the Ia afferents, which contributes to the reduction or plateau in the H-reflex amplitude at higher contraction levels.

(c) Reciprocal Ia Inhibition

Reciprocal inhibition is a neural mechanism that involves the contraction of an agonist muscle, causing the inhibition of its antagonist muscle. This promotes smooth, coordinated movements and helps prevent the co-contraction of opposing muscles.the spinal cord sends signals via Ia inhibitory interneurons to inhibit the motor neurons that activate the antagonist muscle [29]. For example, during plantar flexion (when the Soleus contracts), reciprocal inhibition reduces the activity of the tibialis anterior (dorsiflexor) [18].

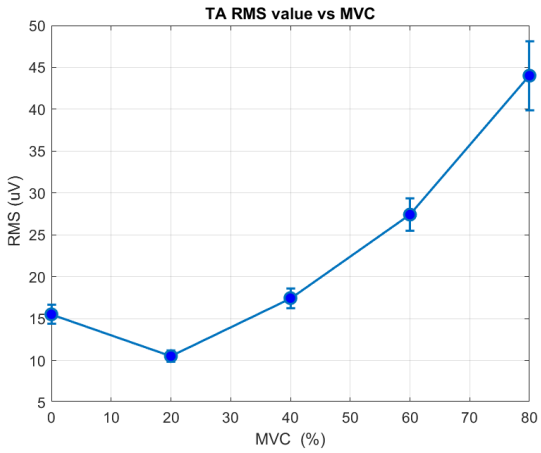

Fig. 6. A graphical representation of the Tibialis Anterior (TA) BEMG RMS value with error bars (SEM) across % MVC

At lower levels of contraction, the agonist muscle, Soleus, is being activated while its antagonist, Tibialis Anterior, is relatively at rest. Minimal reciprocal inhibition occurs because there is little or no activation of the tibialis anterior [18]. This allows for strong activation of the Soleus and a higher Soleus H-reflex amplitude. As the level of voluntary contraction increases (beyond 20% MVC), the tibialis anterior (antagonist to the Soleus) may begin to activate as shown in Figure 6. This would therefore increase the reciprocal inhibition of the Soleus due to the activation of the tibialis anterior through the Ia inhibitory interneurons [27]. The Ia inhibitory interneurons inhibit the motor neurons of the Soleus. This reduces the amplitude of the H-reflex despite the increasing voluntary contraction of the Soleus. Reciprocal inhibition becomes more relevant at higher levels of contraction where the activation of the antagonist muscle (tibialis anterior) increases [18]. This activation leads to greater inhibition of the Soleus motor neurons, contributing to the decrease or plateau in the H-reflex amplitude. Therefore, reciprocal inhibition works alongside presynaptic and recurrent inhibition to regulate the reflex response as voluntary contraction levels

increase.

(d) Ib Inhibition

Ib inhibition, also called Golgi tendon organ (GTO) inhibition, also may contribute to the depression of the Soleus H-reflex with increasing voluntary contraction levels. Such inhibition would be mediated by Ib afferents from the Golgi tendon organs which monitor muscle tension rather than muscle length (like muscle spindles). Ib inhibition occurs when sensory information from the Golgi tendon organs (located in the tendons of muscles) activates Ib afferents, which then synapse onto inhibitory interneurons in the spinal cord [2]. These interneurons inhibit the alpha motor neurons that control the same muscle (autogenic inhibition) [3]. This system prevents excessive force production and protects muscles and tendons from damage due to excessive tension.

At lower levels of contraction, the tension in the muscle is relatively low, and Golgi tendon organs are not highly activated. This reflects a minimal Ib inhibition, freeing more motor neurons to be excited via the H-reflex pathway, resulting in the initial increase in H-reflex amplitude with increased voluntary contraction [19]. As contraction levels increase, the tension in the muscle rises, leading to more activation of the Golgi tendon organs [17]. The Ib afferents activate inhibitory interneurons that decrease the excitability of the alpha motor neurons in the Soleus, contributing to a reduction in the H-reflex amplitude. This Ib inhibition is increased when the level of muscle contraction becomes higher-for example, beyond 20 percent MVC-wherein the reflex response begins to decline or plateau.

The demand for inhibition is low at low levels of contraction because the motor neurons are not highly excited. Hence, presynaptic inhibition, as well as recurrent, reciprocal and Ib inhibitions, are weak or minimal. This allows the H-reflex to increase with increasing levels of contraction. At higher contraction levels, as more motor neurons are recruited and muscle activity increases, these inhibitory mechanisms are activated to prevent excessive muscle activation, ensure coordinated movement, and protect muscles from damage due to over-tension. This leads to a decrease or plateau in the H-reflex as voluntary contraction increases.

B. Peak at 20 percent

Where precisely this peak (according to results H-reflex peaked at 20% MVC) occurs might vary between individuals. It is observed that those subjects with larger H-reflex amplitudes at rest (0% MVC) tend to peak at lower contraction levels, while individuals with smaller reflex amplitudes at 0% MVC peaked at higher contraction levels, which was also noticed in previous study [27]. For example in this study, H-reflex amplitude peaked closer to 20% MVC in subjects who exhibited larger H-reflex amplitudes at 0% MVC. Considering such variability, it seems reasonable to suggest that the H-reflex peak-to-peak amplitude around 20% MVC is influenced by individual neural excitability and muscle recruitment patterns. Thus, 20% MVC is a general value where the balance between excitation and inhibition is optimized for most subjects, but this can shift depending on the individual's baseline reflex excitability.

The H-reflex peaked at 20%-30% MVC is specifically observed in the Soleus muscle for most investigations [27], but for other muscles, such as the medial gastrocnemius, this may be quite different due to differences in muscle function, neural control, and motor unit recruitment. The Soleus muscle is primarily composed of slow-twitch, type I muscle fibers, which are recruited early during voluntary contractions. These fibers are suitable for endurance and very sensitive to reflex pathways such as the H-reflex. This makes the Soleus more likely to show a peak in the H-reflex amplitude around moderate contraction levels at around 20 - 30% MVC. For instance, medial gastrocnemius contains a greater proportion of fast-twitch, type II fibers, which are recruited during higher levels of voluntary contraction than the Soleus. Thus, the 20-30% MVC peak is more characteristic of the Soleus muscle, while muscles like the medial gastrocnemius may have their peak H-reflex amplitude at different MVC levels due to distinct functional roles and neural recruitment patterns.

C. No significant change from 20% MVC

The H-reflex typically plateaus at higher levels of voluntary contraction instead of continuously declining, even though inhibitory mechanisms (presynaptic, recurrent, reciprocal, and Ib inhibition) increase. This plateauing behavior occurs due to a balance between excitatory and inhibitory influences on the motor neurons. While inhibition does increase at higher contraction levels, other factors limit the extent of this inhibition, allowing the reflex to stabilize at a certain level instead of completely declining.

1. Saturation of Inhibitory Mechanisms

Inhibition does not continue to increase indefinitely. Each of these inhibitory mechanisms such as presynaptic inhibition, recurrent inhibition, reciprocal inhibition, and Ib inhibition, has a maximum level of effectiveness [23]. Once this maximum is reached, these inhibitory influences cannot further reduce the excitability of the motor neurons [25]. As a result, once these mechanisms reach their saturation point, the reflex can no longer be significantly reduced by inhibition, leading to a plateau in the H-reflex amplitude.

2. Persistent Excitatory Drive

The excitatory inputs from the voluntary contraction continue to drive the motor neurons, even at higher levels of contraction. This means the descending drive from the brain (corticospinal input) remains strong to ensure that motor neurons are still activated to maintain the voluntary contraction [13]. This represents a balancing excitatory input to the inhibitory mechanisms that are trying to reduce motor neuron excitability. This persistent drive from voluntary contraction help to sustain the motor neuron activity at the same level, leading to plateauing rather than continuous decline of the H-reflex. This persistent excitatory input means the motor neurons still respond to some extent to the reflex pathway, even though they are under inhibitory control.

3. Reduced Motor Neuron Recruitment Pool

At high contraction levels, most of the motor neurons are already recruited and actively firing due to the voluntary contraction. Thus, there are fewer available motor neurons left for recruitment via the reflex pathway. With so many motor neurons already firing, the additional contribution of the reflex pathway becomes limited, leading to a plateau in reflex amplitude as there are fewer motor neurons that can be reflexively recruited [4]. Thus, when maximal recruitment of motor neurons is combined with inhibitory inputs, the H-reflex may stabilizes at plateau level.

4. Nonlinear Recruitment of Motor Neurons

The motor neurons are recruited in a nonlinear fashion [26] such that for low levels of contraction, a moderate increase in excitatory drive may recruit many additional motor neurons and result in a large increase in the H-reflex. However, at higher levels of contraction, most of the low-threshold motor neurons are already recruited and further excitatory drive recruits only a small number of high-threshold motor neurons [15]. This nonlinear recruitment provides a basis for diminishing returns and thus causing the H-reflex to plateaus.

5. Reduced Sensitivity of Motor Neurons to Reflex Input

At higher contraction levels, the motor neurons are less sensitive to input from the reflex pathway because, they have already been activated by voluntary contraction. This limiting effect, due to the reduced sensitivity [22], prevents further increases in H-reflex amplitude and thus contributes to the plateau. In other words, the motor neurons are less sensitive to the reflexive input due to previously occurring strong excitatory input of the descending motor commands.

6. Compensatory Mechanisms to Maintain Functionality

The nervous system is designed to optimize movement and control, not to completely suppress reflexes. The plateau in the H-reflex amplitude at high contraction levels likely reflects a balance that allows the nervous system to maintain functional motor output without causing excessive inhibition that could interfere with motor performance [27]. The nervous system fine-tunes reflexes to prevent reflexive overactivity while ensuring that volun-

tary movement remains smooth and controlled, contributing to the stabilization of the H-reflex response at higher contraction levels [27].

V. Limitations

While this study provides valuable insights into the modulation of the Soleus H-reflex across different levels of isometric voluntary contraction, several limitations must be acknowledged. First, the sample size is relatively small, and was limited to 10 male participants between 22 and 30 years of age, reducing the generalizability of the findings. Future studies should entail even larger and more varied populations in an effort to observe the relationship between the H-reflex and MVC more closely. Second, the use of surface electrodes introduces variability in stimulation and recording, particularly at higher contraction levels, where slight movements of the electrodes can affect consistency. Employing more precise methods, such as intramuscular electrodes, in future research could reduce this variability. Additionally, this study only measured MVC levels up to 80%, leaving uncertainty about whether higher levels (e.g., 90% or 100%) would produce a plateau in reflex amplitude. Future studies should also look at the influence of larger levels of MVC on the H-reflex. Fatigue was also not explicitly measured in this study, even though participants were given designated breaks. Fatigue, especially at higher contraction levels, may have influenced reflex excitability and should be accounted for in future research. It was also observed (Figure 6) that the TA RMS increased significantly with increasing MVC levels, which indicated co-contraction of the muscles, especially at higher contractions; hence, participants were unable to maintain constant TA activation. Future experiments should be designed in such a way that the activation of TA remains consistent throughout the contraction levels inorder to study about Soleus H-reflex.

VI. Conclusion

In conclusion, this study demonstrates that the modulation of the Soleus H-reflex across different levels of voluntary isometric contraction follows a biphasic response, with a significant increase in reflex amplitude between 0% and 20% MVC, followed by a plateau at higher contraction levels. These findings suggest that motor neuron recruitment and inhibitory mechanisms play critical roles in modulating the excitability of the neural components of the reflex arc, during voluntary contraction.

The present study also shows that M-wave amplitude (M/Mmax) remained significantly unchanged across different contraction levels. As such, it also indicates that the M-wave itself does not significantly influence H-reflex modulation in this context. Besides this, the present study infer that H-reflex amplitude, especially from the Soleus muscle, becomes independent of contraction strength at higher levels of contraction, meaning that the H-reflex may reliably reflect motor neuron excitability only below 20% MVC. This finding agrees with previous studies [6] [27] that has proposed that the H-reflex is less representative of motor neuron behavior during higher intensities of contraction. Therefore, when the H-reflex is used as a tool in experimental settings or for clinical purposes, it is important to consider that it provides a more reliable measure of the excitability of the neural components of the reflex arc, below 20% MVC.

References

[1] L. Batista-Ferreira, N. F. Rabelo, G. M. d. Cruz, J. N. d. A. Costa, L. A. Elias, and R. A. Mezzarane. Effects of voluntary contraction on the soleus h-reflex of different amplitudes in healthy young adults and in the elderly. *Frontiers in Human Neuroscience*, 16, 2022.

[2] M. E. Bechler and W. J. Brown. Pafah ib phospholipase a2 subunits have distinct roles in maintaining golgi structure and function. *Biochimica Et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 1831:595–601, 2013.

[3] M. E. Bechler, A. M. Doody, E. L. Racoosin, L. Lin, K. H. Lee, and W. J. Brown. The phospholipase complex pafah ib regulates the functional organization of the golgi complex. *Journal of Cell Biology*, 190:45–53, 2010.

[4] R. Borzuola, L. Labanca, A. Macaluso, and L. Laudani. Modulation of spinal excitability following neuromuscular electrical stimulation superimposed to voluntary contraction. *European Journal of Applied Physiology*, 120:2105–2113, 2020.

[5] A. Botter, I. Vazzoler, and T. Vieira. High density emg investigation of h-reflex distribution over the soleus muscle. *2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, 2015.

[6] A.J. Butler, G. Yue, and W.G. Darling. Variations in soleus h-reflexes as a function of plantarflexion torque in man. *Brain Research*, 632(1):95–104, 1993.

[7] M Cerrato, Claudia Bonell, and Carolina Beatriz Tabernig. [factors that affect the hoffmann reflex as a tool used in neurophysiological examination]. *Revista de neurologia*, 41 6:354–60, 2005.

[8] J. Clair, J. Anderson-Reid, C. Graham, and D. Collins. Postactivation depression and recovery of reflex transmission during repetitive electrical stimulation of the human tibial nerve. *Journal of Neurophysiology*, 106:184–192, 2011.

[9] X. Hu, K. Tong, and L. Hung. Oscillations in the power spectra of motor unit signals caused by refractoriness variations. *Journal of Neural Engineering*, 1:174–185, 2004.

[10] Hans Hultborn and Emmanuel Pierrot-Deseilligny. Changes in recurrent inhibition during voluntary soleus contractions in man studied by an h-reflex technique. *The Journal of Physiology*, 297, 1979.

[11] M. Kalc, J. Škarabot, M. Divjak, F. Urh, M. Kramberger, M. Vogrin, and A. Holobar. Identification of motor unit firings in h-reflex of soleus muscle recorded by high-density surface electromyography. *Ieee Transactions on Neural Systems and Rehabilitation Engineering*, 31:119–129, 2023.

[12] Maria Knikou. The h-reflex as a probe: Pathways and pitfalls. *Journal of Neuroscience Methods*, 171:1–12, 2008.

[13] S. Li, R. J. Triolo, and H. Charkhkar. Neural sensory stimulation does not interfere with the h-reflex in individuals with lower limb amputation. *Frontiers in Neuroscience*, 17, 2023.

[14] Y. Li, Z. Lei, and Z. C. Xu. Enhancement of inhibitory synaptic transmission in large aspiny neurons after transient cerebral ischemia. *Neuroscience*, 159:670–681, 2009.

[15] Carlo J. De Luca and Emily C. Hostage. Relationship between firing rate and recruitment threshold of motoneurons in voluntary isometric contractions. *Journal of neurophysiology*, 104 2:1034–46, 2010.

[16] M. Mende, E. V. Fletcher, J. L. Belluardo, J. P. Pierce, P. K. Bommareddy, J. A. P. Weinrich, Z. D. Kabir, K. C. Schierberl, J. G. Pagiazitis, A. I. Mendelsohn, A. Francesconi, R. H. Edwards, T. A. Milner, A. M. Rajadhyaksha, P. J. v. Roessel, G. Z. Mentis, and J. A. Kaltschmidt. Sensory-derived glutamate regulates presynaptic inhibitory terminals in mouse spinal cord. *Neuron*, 90:1189–1202, 2016.

[17] K. Miller and J. A. Burne. Golgi tendon organ reflex inhibition following manually applied acute static stretching. *Journal of Sports Sciences*, pages 1–7, 2014.

[18] M. Mirbagheri, L. D. Duffell, D. Kotsapouikis, and L. M. Rogers. Reciprocal inhibition becomes facilitation after spinal cord injury: clinical application of a system identification approach. *2014 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 2014.

[19] A. Nagamori, C. M. Laine, and F. J. Valero-Cuevas. Cardinal features of involuntary force variability can arise from the closed-loop control of viscoelastic afferented muscles. *PLOS Computational Biology*, 14:e1005884, 2018.

[20] P. D. Oza, S. Dudley-Javoroski, and R. K. Shields. Sustained submaximal contraction yields biphasic modulation of soleus post-activation depression in healthy humans. *Scandinavian Journal of Medicine Amp; Science in Sports*, 29:944–951, 2019.

[21] Robert M Palmieri, Christopher D Ingersoll, and Mark A Hoffman. The hoffmann reflex: methodologic considerations and applications for use in sports medicine and athletic training research. *Journal of Athletic Training*, 39(3):268–277, Jul 2004.

[22] Manuela Pensini and A. N. Martin. Effect of voluntary contraction intensity on the h-reflex and v-wave responses. *Neuroscience Letters*, 367:369–374, 2004.

[23] Nicolas Place, Julien Duclay, Romuald Lepers, and Alain Martin. Unchanged h-reflex during a sustained isometric submaximal plantar flexion performed with an emg biofeedback. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, 19 6:e395–402, 2009.

[24] S. Satake, F. Saitow, D. A. Rusakov, and S. Konishi. Ampa receptor-mediated presynaptic inhibition at cerebellar gabaergic synapses: a characterization of molecular mechanisms. *European Journal of Neuroscience*, 19:2464–2474, 2004.

[25] O. Seynnes, N. A. Maffiuletti, A. M. Horstman, and M. V. Narici. Increased h-reflex excitability is not accompanied by changes in neural drive following 24 days of unilateral lower limb suspension. *Muscle Amp; Nerve*, 42:749–755, 2009.

[26] L Smith, Tao Zhong, and Parveen N. S. Bawa. Nonlinear behaviour of human motoneurons. *Canadian journal of physiology and pharmacology*, 73 1:113–23, 1995.

[27] Richard B. Stein, Kristen L. Estabrooks, Steven C. McGie, Michael J. Roth, and Kelvin E. Jones. Quantifying the effects of voluntary contraction and inter-stimulus interval on the human soleus h-reflex. *Experimental Brain Research*, 182:309–319, 2007.

[28] B. Tahayori, B. Tahayori, and D. M. Koceja. Characteristics of preceding ia activity on postactivation depression in health and disease. *Journal of Neurophysiology*, 113:3751–3758, 2015.

[29] U. Ş. Yavuz, F. Negro, R. Diedrichs, and D. Farina. Reciprocal inhibition between motor neurons of the tibialis anterior and triceps surae in humans. *Journal of Neurophysiology*, 119:1699–1706, 2018.

VII. Appendix

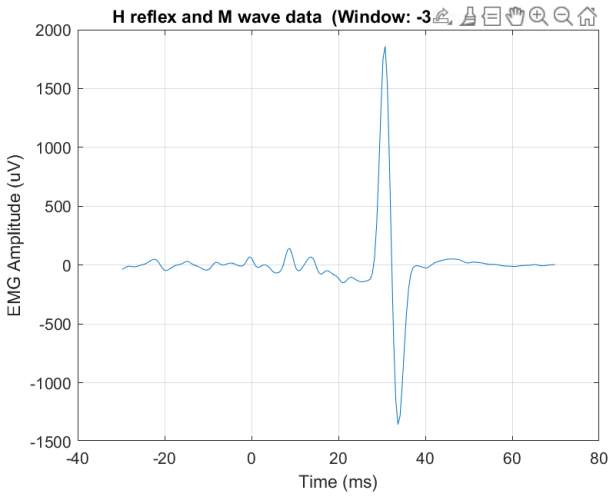

Fig. 7. A graphical representation of the M-wave and H-wave of Subject 2 at 20% MVC

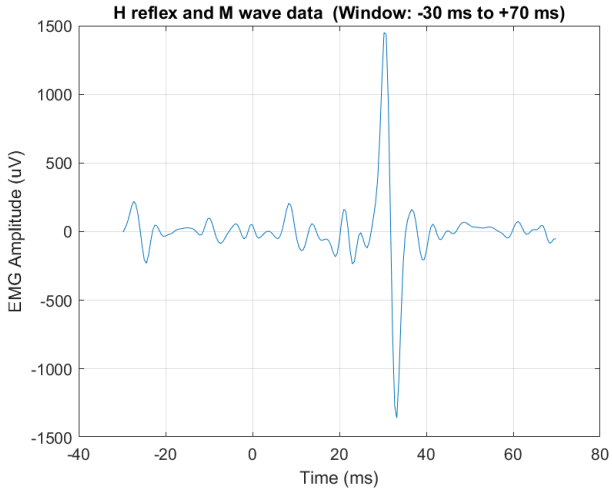

Fig. 8. A graphical representation of the M-wave and H-wave of Subject 2 at 80% MVC

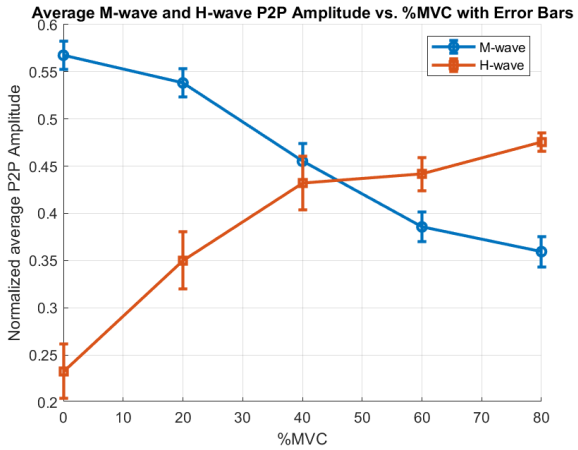

Fig. 9. A graphical representation of the mean H/Mmax with error bars across different levels of MVC (%) of Subject 1

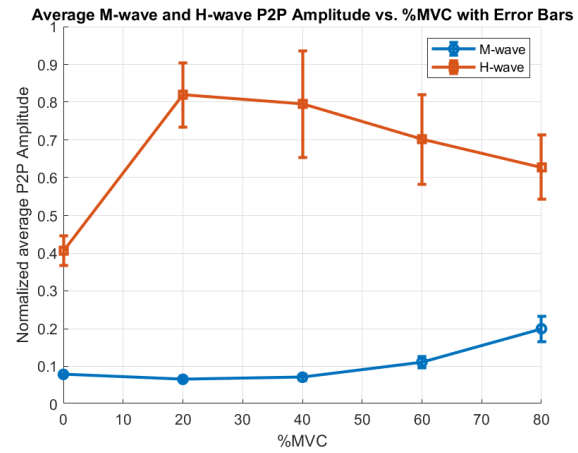

Fig. 10. A graphical representation of the mean H/Mmax with error bars across different levels of MVC (%) of Subject 2

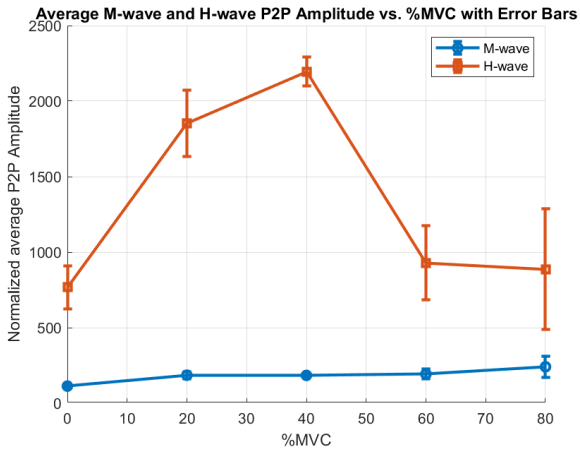

Fig. 11. A graphical representation of the mean H/Mmax with error bars across different levels of MVC (%) of Subject 3

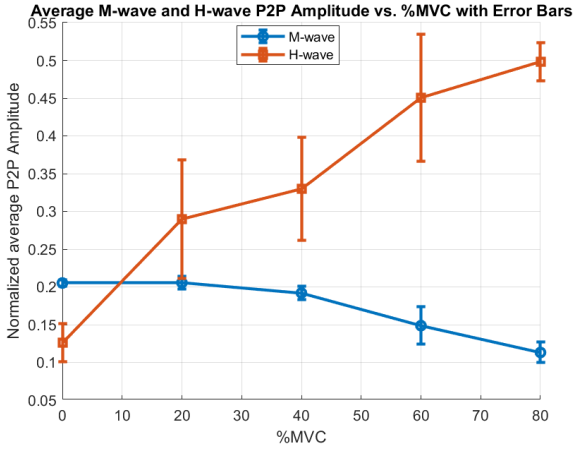

Fig. 12. A graphical representation of the mean H/Mmax with error bars across different levels of MVC (%) of Subject 4

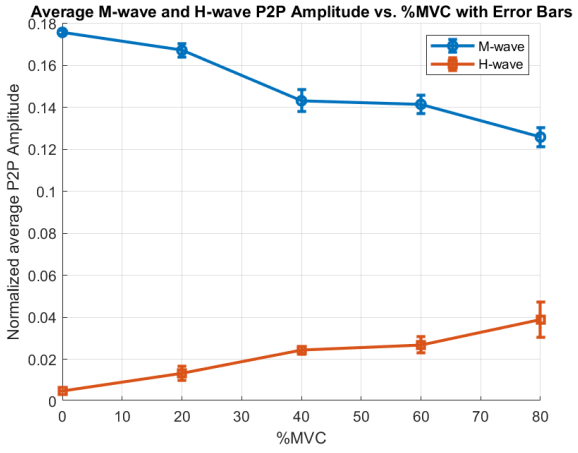

Fig. 13. A graphical representation of the mean H/Mmax with error bars across different levels of MVC (%) of Subject 5

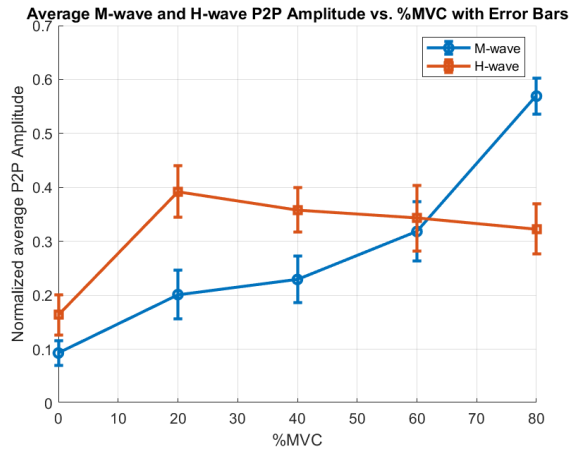

Fig. 14. A graphical representation of the mean H/Mmax with error bars across different levels of MVC (%) of Subject 6

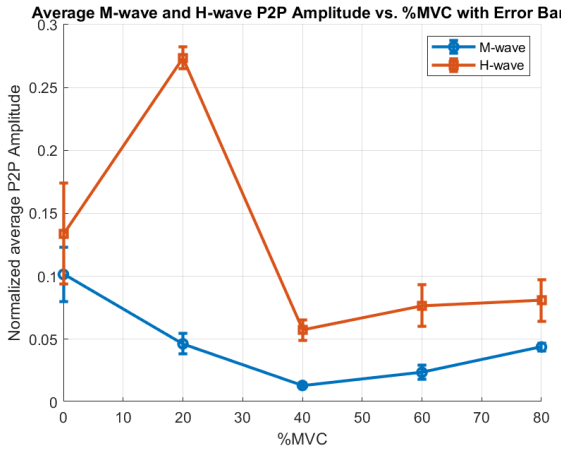

Fig. 15. A graphical representation of the mean H/Mmax with error bars across different levels of MVC (%) of Subject 7

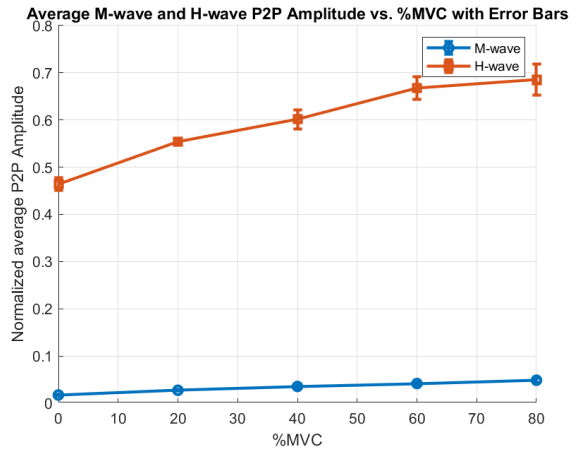

Fig. 16. A graphical representation of the mean H/Mmax with error bars across different levels of MVC (%) of Subject 8

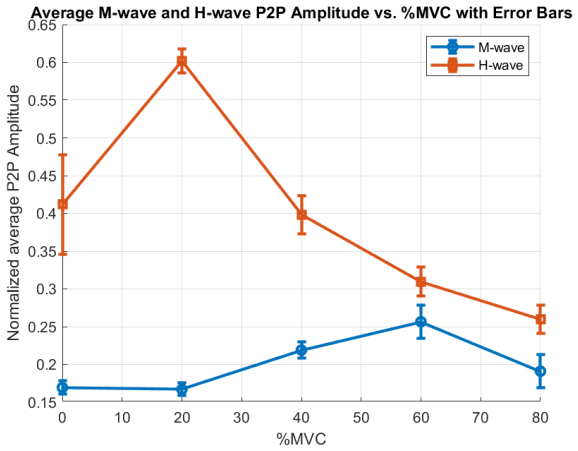

Fig. 17. A graphical representation of the mean H/Mmax with error bars across different levels of MVC (%) of Subject 9

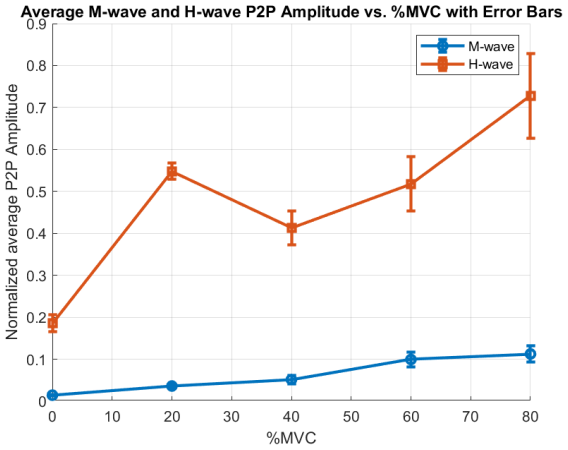

Fig. 18. A graphical representation of the mean H/Mmax with error bars across different levels of MVC (%) of Subject 10

TABLE II

PAIRWISE COMPARISONS BETWEEN MVC LEVELS FOR SOLEUS BEMG RMS

MVC Level 1 (%)	**MVC Level 2 (%)**	**pValue**
0	20	0.0039
0	40	0.0039
0	60	0.0039
0	80	0.0039
20	40	0.0039
20	60	0.0039
20	80	0.0039
40	60	0.0039
40	80	0.0039
60	80	0.0039