



HUMAN ELECTRO-MUSCULAR INCAPACITATION (HEMI) SIMULATION STUDY

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EXECUTIVE SUMMARY

This task description encompassed two (2) distinct tasks the collective objective of which was to further elucidate and better inform the modeling of HEMI applications. Crucial in this effort was identifying where gaps might be found that would require future animal studies related to the mechanisms of action of HEMI devices and their relationship to alternate duty cycles, which would then feed back into the modeling effort. The strategic vision of the Joint Non-Lethal Weapons Program (JNLWP) is to enhance development of non-lethal technologies, and specifically testing of HEMI technologies with human effects modeling and simulations. In order to support such models, the studies at Penn State are being coordinated with specific simulations under development in parallel efforts and are assisting in identifying gaps that might be filled with empirical data from existing animal studies. Additionally, it is important to remain informed of developing research related to electrical stimuli in general, physiological responses, and underlying causes leading to less than desirable outcomes.

The first major task was examining the needs related to the development of human models (**“physics” aspects**) and the extent to which existing animal data could form a basis for the advancement of the model. This included examples of analysis of the existing animal data to determine the electro-mechanical time delays and time constants needed for modeling and simulation of HEMI stimuli. The HEMI data set includes animal data where the HEMI probes were placed in several different anatomical positions. This data is being used to determine the **“volume of activation”** of nerves, as well as the extent to which spinal segments (up and down the spinal cord) were activated.

We also examined the needs related to **physiological aspects**. Vital signs, breathing gas concentrations, work/power signals, and other physiological parameters were examined to determine and discuss the physics parameters needed for modeling and simulation of HEMI stimuli.

Additionally, the HEMI data set has demonstrated its usefulness by enabling the development of pilot examples of signal processing of existing data. This may lead to more accurate calculations of physical and physiological values enabling **mathematical fitting of envelopes** of the parameters for more detailed determination of physics aspects such as rise time, peak duration, and decrement time, for implementation in the mathematical model.

Finally, this task included direct bi-weekly discussions with contractors developing human models and simulations for the JNLWP in order to understand and implement the existing data set. This included the full digital data set of HEMI-4 accelerometer A-D data acquisition being provided to the modeling group. The data set will enable the modeling group to extract multiple parameters needed for the modeling effort.

A literature study was conducted as a second major task to enhance and support the work above, as well as explore new directions of research.

Multiple new methods of quantifying contraction time, relaxation time, onset and the time course of tetanization were found. Also found were several new physiological monitors, which would be better able to monitor and quantify the physiological changes in an environment of marked muscle and limb movements, which cause artifacts in the existing typical physiological monitors. Human volunteer studies on muscle fatigue due to tetanic stimulation were found. This led to the concept that changes in

the first five seconds of tetanic stimuli were not due to fatigue, but probably due to a limitation of the range of movement of the limbs due to the onset of tetanization.

Studies on isolated neuro-muscular preparations *where the effect of biochemical derangements are excluded*, help to elucidate the sites of the fatigue. Further such studies are suggested.

Further studies, also being proposed, will be based on results from the modeling projects that could examine a wide variety of combinations and permutations of variations of the duty cycle of the existing HEMI devices. For instance, the duty cycles of existing implanted medical devices as well as devices used for electroporation could be examined using the simulation model.

This study completed several important objectives which will be useful for the development of the simulation model as well as for quantifying deleterious effects:

- Calculated examples of the **electro-mechanical delay (EMD)**. This involved characterizing limb movements caused by HEMI stimuli to be able to differentiate from passive movements (e.g., gravity) and oscillations after HEMI induced movements. The electro-mechanical delay (EMD) is necessary for insertion into the simulation model.

The natural frequency of the oscillations will be useful as an indicator of deleterious effects, as natural frequency is postulated to increase as edema forms in muscles performing HEMI imposed supra-maximal work, resulting in such muscles becoming “stiffer.” Such stiffness should be explored as a potential new measure identifying and quantifying deleterious effects of HEMI stimulation.
- Identified methods of analyses of contraction and relaxation as possible measures of deleterious effects. Based on the literature search, the existing HEMI data set will enable the calculation of the characteristics of **active contraction** of muscle which will be useful for the modeling project. The characteristics include: duration of contraction, peak rate of active contraction, etc. which will be useful for the modeling project. We postulate that the existing HEMI data set could be used to show that fatigued muscle has a **slower rate of contraction**, and therefore a **longer duration to travel** the same distance of contraction. Both of these newly identified characteristics could be used as indicators of deleterious effects. **Relaxation of muscle** is an active process requiring energy (ATP) to re-absorb ionic Calcium from the cytosol into the endoplasmic reticulum. Characteristics of relaxation (e.g., duration and peak rate of relaxation) can be calculated from the existing data set. The literature search provided some examples of characteristics and measurements that could be used as a starting point. Alterations in such characteristics of relaxation are postulated to be useful as indicators of deleterious effects.
- Identified quantification of tetanization as a possible measure of deleterious effects. **The onset of tetanization** is a gradual process, not an event with an immediate onset. The existing HEMI data set provides a large number of instances to calculate the rate of onset of tetanization that will be useful for the modeling project. The literature search provided a variety of methods to quantify the degree of tetanization including the concept of “Fusion Index.” The literature also provided methods of quantifying the increase in contraction strength after tetanic stimulations, called Post Tetanic Facilitation (PTC) or Post Tetanic Potentiation (PTP). The quantification

method has been published under the term “Boost and Sag.” This methodology could be applied to the HEMI data sets. After the onset of tetanization, the **time course of tetanization** due to continued tetanic stimuli will be evident from the HEMI data sets, and can therefore be incorporated into the simulation models. Based on the literature, the decrease in the accelerometer signals within five seconds most likely does not represent fatigue, but rather is the result of a decreased range of potential movement due to the partially tetanized muscle which did not fully relax between the HEMI stimuli.

- Examined the effects of the **interruption of HEMI stimuli** leading to an increased contraction strength due to post tetanic facilitation. The increased contraction strength could be included in the modeling project. For instance, the increased muscle movement might cause enhanced incapacitation, while the interruption has been shown to enable (or even “force”) a respiratory movement, a so-called “Good Breath.”
- Correlated the changes in the **cardio-vascular system (CVS)** caused by HEMI stimuli. Understanding the increased blood pressure (BP) at the onset of the HEMI stimuli, as well as the marked decrease in BP after discontinuation thereof is critical to take into consideration when analyzing a series of duty cycles.
An important series of articles found in the literature discussed in-depth the “Heart-Lung Interactions” based on the concepts described under the “Valsalva Maneuver.” Due to newer monitoring techniques such as cardiac ultra-sound, a more in-depth understanding is being developed with important differences being identified due to hypovolemia, in contrast to “stunning” of the heart.
Based on improved signal processing, several newer monitoring concepts have been explored and implemented in clinical practice, including the “Blood Flow Index (BFI)”, “Peripheral Intra-Venous Analysis (PIVA)” and “Systolic-Dichrotic Notch Pressure Differential.” All of these should be explored as potential monitors of deleterious effects of HEMI stimulation.
Alternative explanations for post-HEMI hypotension were found, such as the “Contraction Induced Vaso-dilation.”
- Explored the **respiratory effects** of HEMI stimuli to differentiate inhalation from exhalation. Due to the 3-D nature of the eight accelerometers used, differentiation of inhalation and exhalation should be feasible. Some investigators found inhalation occurred with the onset of HEMI stimuli, while others found exhalation. We developed a new working hypothesis to be explored: the probe positions would have a major influence on the phases of respiration. For instance, stimulation of the phrenic nerve would cause inhalation via the diaphragm, while stimulation primarily of the internal intercostal muscles would cause exhalation.
- Found experimental **biochemical parameters** in the literature. Such biochemical measurements would be useful to indicate and quantify deleterious effects. For instance, **micro-vesicles** are sections of cellular membranes that are formed by cells under stress, including supra-maximal exercise. These micro-vesicles may contain DNA or RNA whereby the cells of origin can be determined.
- Provided a large number of **duty cycles** for the modeling project. The HEMI data sets contain duty cycles with varying durations of interruptions, as well as varying durations of total applications. These data sets provide a basis for the development of a robust simulation model. Such a model would be useful to examine a large number of combinations and permutations of

HEMI frequencies, waveforms, interruptions, total durations, etc. which would not be possible with human surrogates.

The findings and recommendations outlined above are described in more detail in the full report.

We believe that the HEMI data sets would provide important information related to multiple aspects of physics for the modeling project, while further detailed analysis of the physiologic aspects will be able to enhance and highlight understanding of the physiology, which would feed back into the simulation and modeling.

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INTRODUCTION

Background

The abundance of, and reliance upon, HEMI (Human Electro-Muscular Incapacitation) devices continue to generate scrutiny and examination of these technologies – and properly so. While the body of knowledge continues to grow regarding the mechanisms of effect, these mechanisms remain unclear as do the relative physiological effects of these electrical devices.

The application of non-lethal (NL) technologies provides military forces flexibility by allowing the application of a measure of force with reduced risk of serious injury or fatalities, but still in such a manner as to affect compliance. The strategic vision of the Joint Non-Lethal Weapons Program (JNLWP) is to enhance development of less-than-lethal technologies, and specifically testing of HEMI technologies with human effects modeling and simulations. To ensure these technologies achieve a repeatable human effect, and at the same time do not reach a threshold of serious injury or death, the Department of Defense conducts rigorous testing leading to the development of mathematical models of HEMI applications based on human surrogate testing, to gain insight into the effects and their thresholds. The development of mathematical models to simulate the application of HEMI stimuli, are expected to minimize the need for and use of human surrogate testing.

This effort included two (2) distinct tasks to further elucidate and better inform the modeling of HEMI applications by identifying where gaps exist, requiring future animal studies related to the mechanisms of action of HEMI devices and their relationship to alternate duty cycles, which would feed back into the modeling effort.

In order to support such models, the studies at Penn State were planned to be coordinated with specific simulations under development under parallel efforts and would assist in identifying gaps that might be filled with empirical data from existing animal studies.

The second task was aimed at a literature study to remain informed of developing research related to electrical stimuli in general, physiological responses, and underlying causes leading to less than desirable outcomes, as well as checking for updates on methods of identifying unwanted physiological effects of supra-maximal exercise.

A prior data human surrogate data set **HEMI 4**¹, funded by the JNLWP, was available as a backdrop to be used in the development of the models. (Murray et al. 2020) The report of the study demonstrated that the HEMI data set was useful as it enabled the development of pilot examples of signal processing of the existing data. This was expected to lead to more accurate calculations of physical and physiological values using the mathematical models. Examples of pilot data included analyses of the existing animal data to determine the electro-mechanical time delays and time constants needed for modeling and simulation of HEMI stimuli.

¹ Murray WB, Werner JR, Hughes EL, Welker ML, Lovell TD, Yuan AH, DiLella BJ
Human Electro-Muscular Incapacitation (HEMI) Mechanisms Study 4: An Examination of the Deleterious Physiological Effects of Variations in the Duty Cycle Parameters
30 June 2020 Available at: <https://apps.dtic.mil/sti/pdfs/AD1116553.pdf>

The HEMI 4 data set also includes animal data where the HEMI probes were placed in several different anatomical positions. This data was expected to be useful to determine the “**volume of activation**” of nerves, as well as the extent to which spinal segments (up and down the spinal cord) were activated. This task was to include direct discussions with contractors developing human models and simulations for the JNLWP, in order for the contractors to understand and implement the existing data set. This included the full digital data set of HEMI-4 accelerometer A-D data acquisition being provided to the modeling group. The data set was expected to enable the modeling group to extract multiple parameters needed for the modeling effort.

The second task would be based on a literature study to be conducted to enhance and support Task 1, as well as explore new directions of research.

Examples of literature planned to be searched included:

- new methods of quantifying contraction time, relaxation time, onset and time course of tetanization, etc.
- new physiological and clinical monitors, which would be better able to monitor and quantify the physiological changes in an environment of marked muscle and limb movements, which cause artifacts in the existing typical physiological monitors
- new biochemical substances which would **not** be limited by upper levels/ranges of measurement which would be exceeded by the effects of HEMI stimulation
- human volunteer studies on muscle fatigue due to tetanic stimulation

The successful completion of the tasks would enable the identification of gaps in the existing HEMI data sets and the literature. Such data would need to be collected in a future human surrogate study and fed back to enable the contractors to build a more robust and generally applicable simulation.

A. ELECTRO-MECHANICAL DELAY (EMD): IDENTIFYING THE ONSET OF HEMI STIMULATED MOVEMENT

The electro-mechanical delay (“latency”) is the time from the administration of the electrical stimulus to the onset of mechanical movement due to contraction of the muscles as recorded by the accelerometers. (Laakso et al. 2014, Smith et al. 2018, Smith et al. 2020)

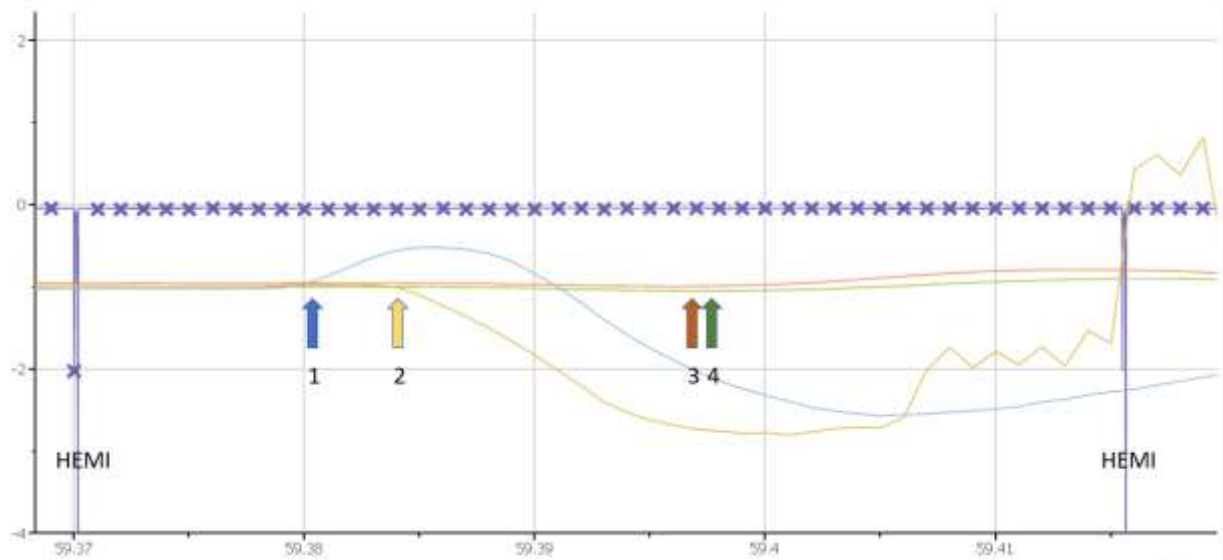
The electro-mechanical delay will be useful for modeling as it indicates how the HEMI stimuli affect various parts of the body. The usefulness derives from the data collected with applications of a variety of HEMI probe positions, and therefore a variety of distances between the probes and the accelerometers. (See Appendix for more details of probe and accelerometer positions.) The “**recruitment volume**” affected by the HEMI stimuli, and the distances that the stimuli spread up and down the spinal cord may be determined from this data set.

Defining the upper and lower limits of the electro-mechanical delay will enable the existing HEMI 4 data set to be used to differentiate active contractions due to HEMI stimulation, from passive movements (e.g., post stimulation oscillations and/or transmitted movements from other parts of the body.)

We also expect that calculations of the electro-mechanical delay in the data set will enable early detection of the onset of fatigue. The data set contains a variety of durations of HEMI applications: from a single twitch, through 6-10 single twitches 1 second apart, 5-35 seconds of continuous and interrupted applications of HEMI stimuli, up to HEMI applications of 360 seconds (6 minutes.) Similarly, we believe the data set could be used to inform on the recovery process, as the recovery intervals in the data set vary over a wide range (1 to 60 minutes).

The number of synapses involved (mono-synaptic versus poly-synaptic) may be estimated from the electro-mechanical delay, as the chemical processes in the synapses are much slower than the conduction in a nerve. (Figure 1)

Figure 1: Electro-mechanical delay for limbs closer to HEMI probes compared to limbs at a more distant site.



The figure shows the accelerometer detected movements of the four limbs when the HEMI probes are placed on the left (LA) and right (RA) forelimbs.

The x-axis is in seconds, the y-axis is in “G” (gravity acceleration) units

Where the start of each limb movement is annotated as follows:

1: left forelimb (LA)

2: right forelimb (RA)

3: left hind limb (LL)

4: right hind limb (RL)

Note the large difference between the electro-mechanical delay (EMD) for the forelimbs (1, 2) compared to the much longer EMD for the hind limbs (3, 4).

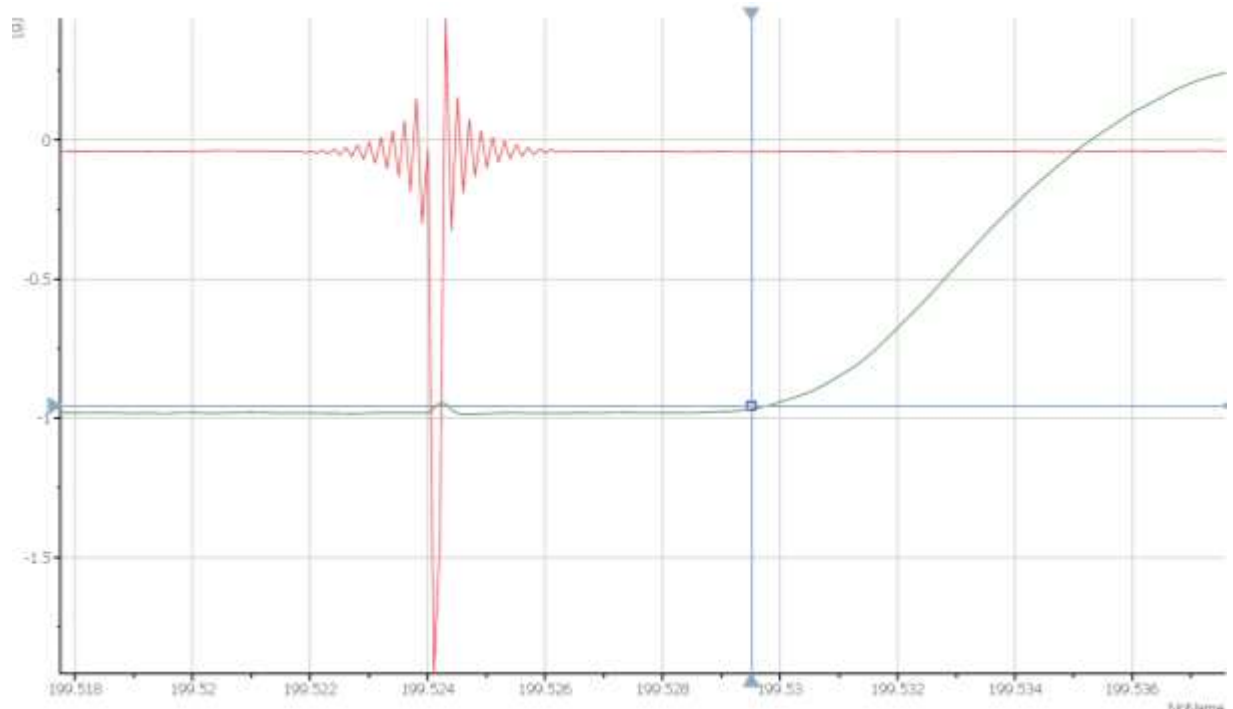
There are several methods to measure the electro-mechanical delay, also known as the “Phase Delay” or “Latency” of the onset of movement. Both the time of the HEMI stimulus and the time of the onset of movement need to be objectively and accurately determined. As mentioned, this accuracy is important for modeling.

Determining the timing of the HEMI stimulus is relatively clear: it is considered to be delivered at the time of the lowest point of the current signal (nadir). (See Figure 2)

However, the onset of movement is quite challenging to identify. There are multiple methods to identify the onset of movement, for instance, a.) continued movement in a given direction greater than two

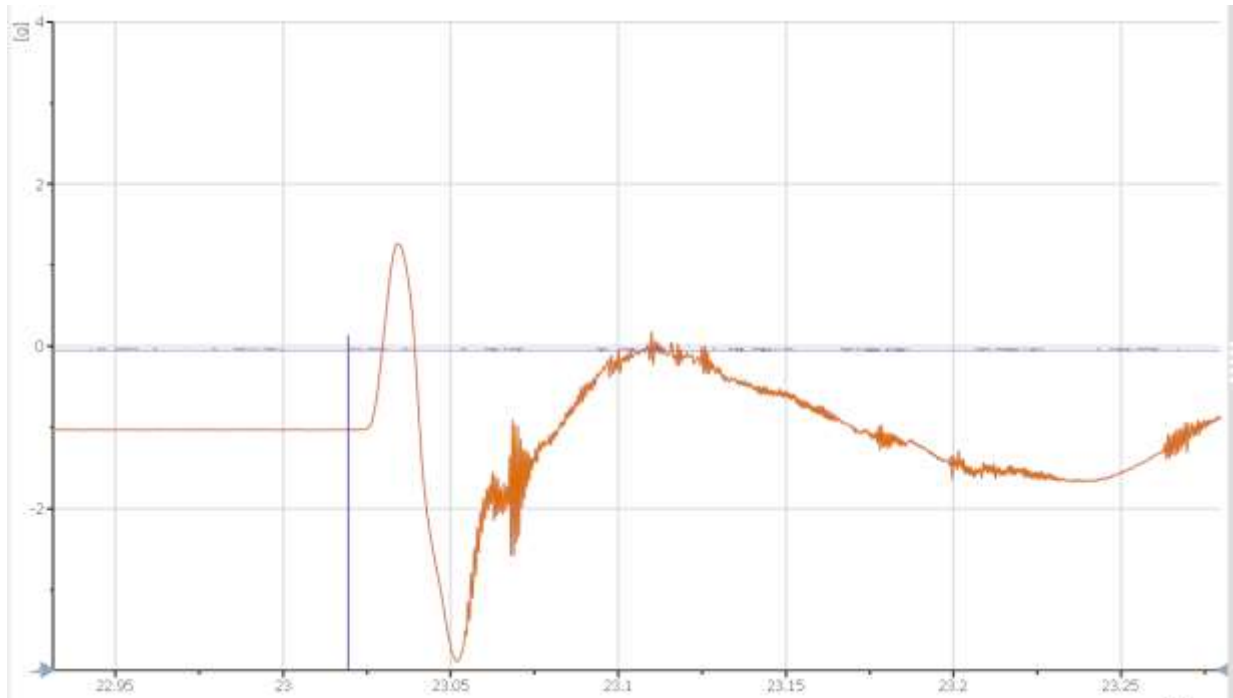
standard deviations of the baseline variance of the A-D converter, b.) movement greater than a specified percentage of the total movement, etc. A self-calibrating, automated method has been described. (Murray et al. 2020)

Figure 2: Phase delay between HEMI stimulus and muscle movement

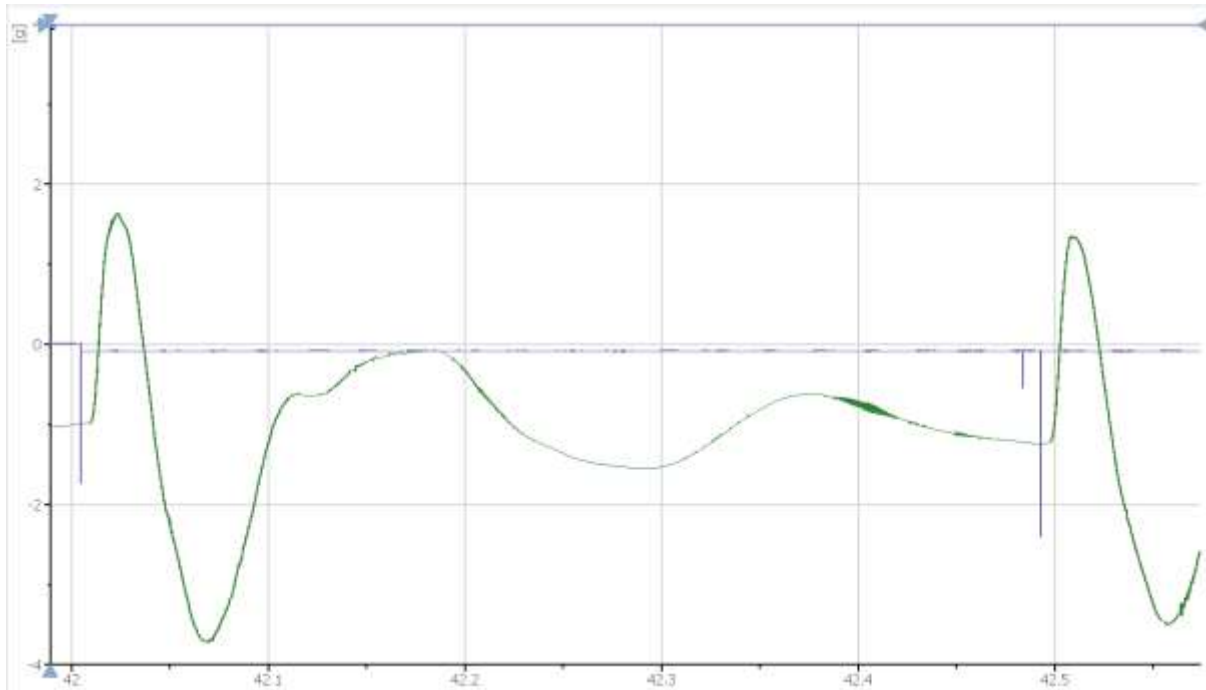


The figure shows the Phase delay between HEMI stimulus and onset of muscle movement. The red line (upper) is the current signal. The HEMI stimulus is delivered at the time of the lowest point of the current signal (nadir). The green line (lower) is the Z direction of the accelerometer placed on the left forelimb (LA). The x-axis is in seconds, the y-axis is in "G" (gravity acceleration) units

Any automated method should be able to clearly identify the difference between a movement that was caused by a HEMI stimulus from a passive movement or oscillation. (Figures 3 and 4.)

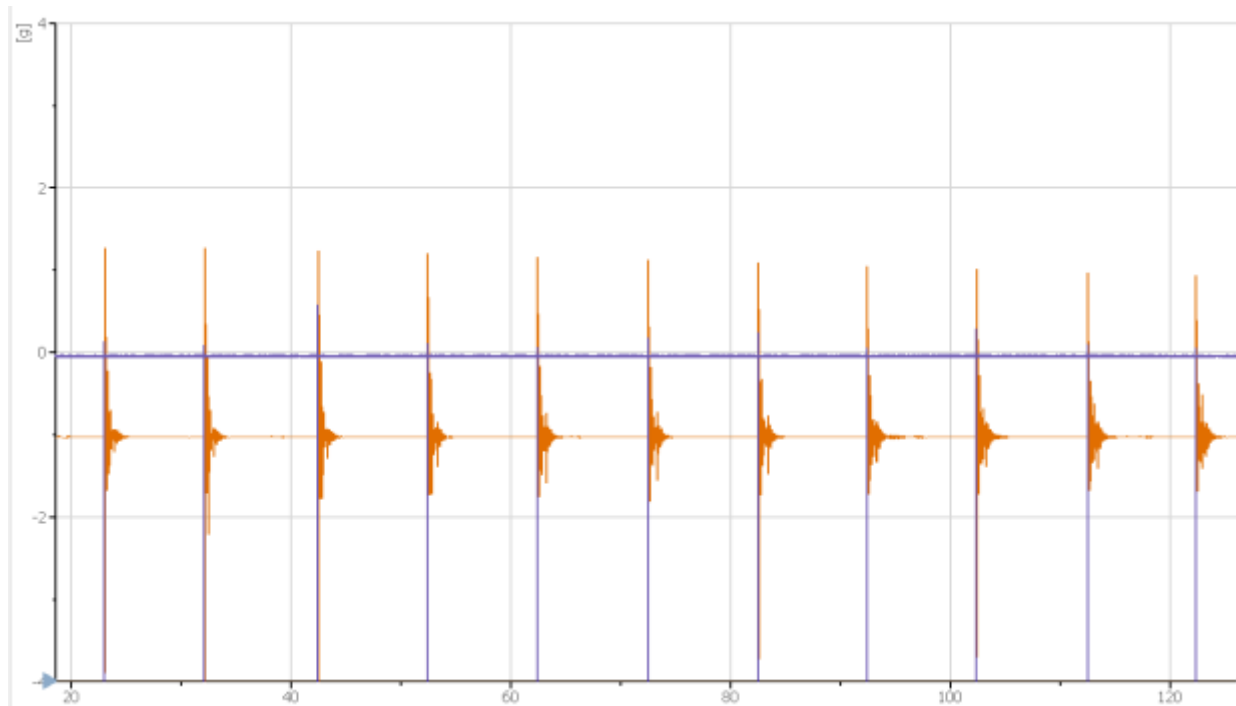
Figure 3: An example of a movement due to HEMI stimulation in contrast to an oscillation

The figure shows a HEMI stimulus (blue) followed by an upward and downward movement (extension and relaxation of the left forelimb in the Z direction.) The second upwards and downwards movements represent an oscillation of the limb. The x-axis is in seconds, the y-axis is in "G" (gravity acceleration) units

Figure 4: Differentiating HEMI twitches from passive movement (“oscillations”)

The figure shows two HEMI stimuli (blue) 500 msec apart. Both HEMI stimuli are followed by upwards and downwards movements (extension and relaxation of the left forelimb in the Z direction representations of accelerometer signals of limb movements - green.) The upwards and downwards movements between the HEMI stimuli represent passive oscillations of the limb. An automated program should be able to identify HEMI induced movements, due to the proximity of the movements to the HEMI stimulus. The x-axis is in seconds, the y-axis is in “G” (gravity acceleration) units

As shown in the previous figures, there are multiple causes of movements during HEMI studies. It will be essential to test various automated methods to find a method that reliably differentiates between the causes of the movements. The first attempts to test the automated programs should be in a “clean” data set, such as a single twitch, as shown in Figure 3. Once this method is shown to be reliable, the method can be tested in a more complex data set, such as in Figure 4 or Figure 5. It would be important to manually examine the results of the automated program in a limited data set to ensure accuracy, before running the program on a data set of 19 Hz for 30 or more seconds. The existing data set contains HEMI stimuli of increasing complexity due to a decreasing interruption time between the HEMI stimuli: Figure 5 has an interruption time of 1,000 msec, while Figure 6 has an interruption time of 500 msec.

Figure 5: Repetitive twitches 1 second apart

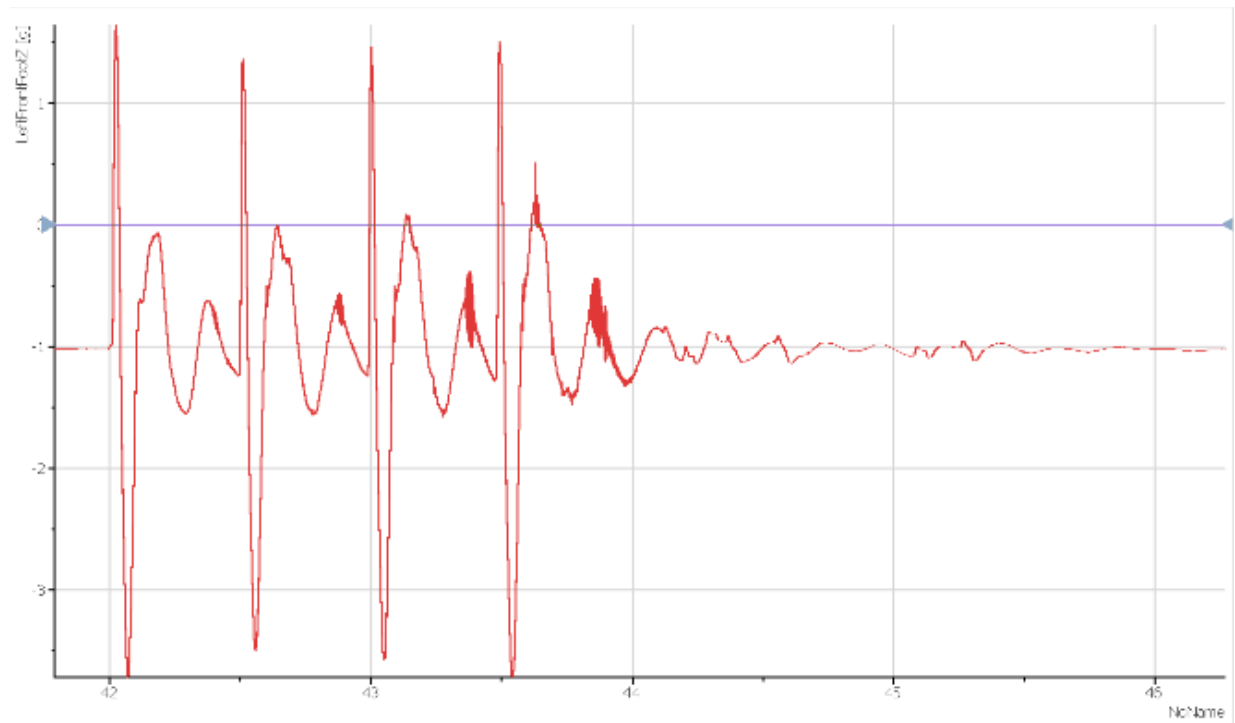
The figure shows an example of a series of “1 second twitches.” The blue line represents the current signal. The orange line shows the movements of the left forelimb (LA) in the Z direction. The EMD can be calculated for each twitch to check for consistency and accuracy of an automated detection algorithm. The x-axis is in seconds, the y-axis is in “G” (gravity acceleration) units

As mentioned, the HEMI data set can be used to test for accuracy and rejection of artifacts in a series of more closely spaced HEMI stimuli, such as the Train-of-Four (TOF) neuro-muscular testing application.

Figure 6 shows a TOF twitch series which is used to test for presence of muscle relaxants or failure of the neuro-muscular junction to produce sufficient acetyl-choline. The size of the 4th twitch is expressed as a percentage of the first twitch. This example shows the TOF after 35 seconds of HEMI stimulation. The Post-HEMI TOF shows all 4 the stimulated twitches as being essentially the same size, which is a similar pattern to the TOF before the HEMI stimulation. A decrement of the size of the 4th twitch as a percentage of 1st twitch would be seen as a deleterious effect, specifically as a deleterious effect on the neuro-muscular junction, due to a decrease in the available acetyl-choline neuro-transmitter. Further studies would be indicated to determine if HEMI stimulation has an effect on the neuro-muscular junction, which might then need to be included in the mathematical model.

We programmed the HEMI device to administer four single twitches which were 500 msec apart. This represents the so-called Train-of-Four (TOF) stimulation method.

Figure 6: Train of Four (TOF) twitch



The figure shows an example of a Train of Four (TOF) twitch with twitches 500 msec apart. The blue line represents the current. The red line represents the movement of a limb. The EMD can be calculated for each twitch to check for consistency and accuracy of an automated detection algorithm, as well as the exclusion of non-HEMI stimulated movement and/or oscillations. Two oscillations are noted between each of the HEMI stimuli, while 12 oscillations (decreasing size) are noted after the last stimulus. The x-axis is in seconds, the y-axis is in “G” (gravity acceleration) units

Once the algorithm for detecting the HEMI stimulus and onset of movement has been developed for the Z directions in the limbs (the largest and most easily identifiable direction), the algorithm can be tested:

- For X and Y directions in the limbs
- For X, Y and Z directions in the chest and abdominal accelerometer data
- For testing different probe positions
- For testing of the development of fatigue

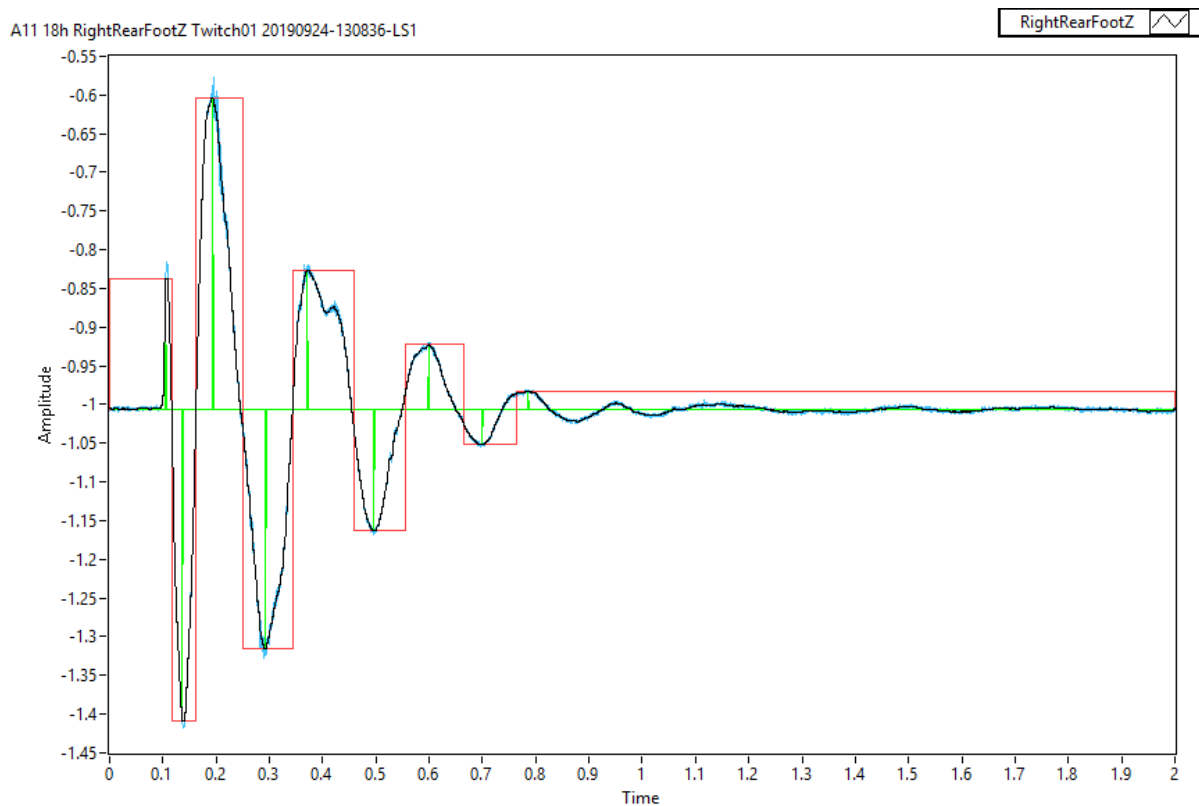
All these data points would be needed for the modeling project

Value and uses of oscillations occurring after a HEMI stimulus

As mentioned earlier, the oscillations are considered artifacts when calculating the electro-mechanical delay. However, the oscillations could be useful in their own right to quantify changes in physiology during and after HEMI exposures, as will be shown in this section.

We show, as a pilot, the results of a method of automated data analysis for identifying characteristics (maximum, minimum, duration, frequency, etc.) of the oscillations, which can then be used for the calculation of the natural frequency of the oscillation of a limb after a single twitch. (Figure 7)

Figure 7: Oscillations of the Right hind limb (RL) after a single twitch.



The figure shows the oscillations of a right hind limb (RL) after a single HEMI stimulation, where oscillations are present and characteristics of each oscillation (maximum and minimum peak, zero crossing, duration, etc.) were identified by an automated program. The blue line represents the accelerometer signal. The red boxes identify individual positive and negative excursions. The green lines identify the maximum and minimum of each individual excursion. The value of calculating the natural frequency of oscillation after a single twitch, or at the end of a run of HEMI stimuli, is that

there are more waveforms available to analyze, enabling a more robust answer. In this case, there are up to 12 oscillations, in contrast to Figure 4 where there are only two oscillations, before the next HEMI stimulus occurs. As expected with the decrement of oscillations, the decrement seems to follow an exponential decline. The x-axis is in seconds, the y-axis is in “g” (gravity acceleration) units

It will be important to be able to **differentiate** movements due to HEMI stimulation from other artifactual oscillations, e.g., transmitted movements from other limbs, etc., when analyzing multiple sequential HEMI stimuli at 19 Hz, as the artefactual movements will still be occurring when the next HEMI stimulus is administered, as in Figure 6.

It will be important to have a reliable, **automated** method for analysis, as 19 Hz for 35 seconds represents more than 600 stimuli to be analyzed.

Signal processing and curve smoothing of the peaks and valleys will be required. For instance, the 3rd positive excursion in Figure 7 shows a double peak, which might indicate a transmitted movement from another limb. Similarly, Figure 7 shows several artifacts after the 4th twitch.

We had noticed clinically, that the limbs of some animals tended to be quite stiff with post-mortem examination. We postulated that there would be an exponential decay of the magnitude of the oscillations (as shown in Figure 7) that could potentially be used to characterize the stiffness of a given limb, (e.g., before and after HEMI stimulation say, of 35 seconds,) and that the stiffness could be used as a measure of deleterious effects. We postulate that stiffness would produce a higher frequency of oscillation, as well as a larger number of oscillations. As expected with the decrement of oscillations, the decrement seems to follow an exponential decline. This needs to be tested in future research. The existing data set has pre-HEMI as well as post-HEMI stimulus values which will enable robust statistical comparisons.

Several existing data sets could be used as a start for developing the automated programs. The value of calculating the natural frequency of oscillation after a single twitch, or at the end of a run of HEMI stimuli, is that there are more waveforms available to analyze, enabling a more robust answer. In this case, there are up to 12 oscillations, in contrast to Figure 4 where there are only two oscillations, before the next HEMI stimulus occurs. (See Appendix for a list of the data sets and suggested steps in the analysis and curve fitting of the oscillations)

A related postulate is that the stiffness is caused by fluid leaking into the muscle cells from the plasma and the extra-cellular fluid, leading to an increase in the limb circumference, as well as an increase in the Hct. (See section on Biochemical aspects for further details.)

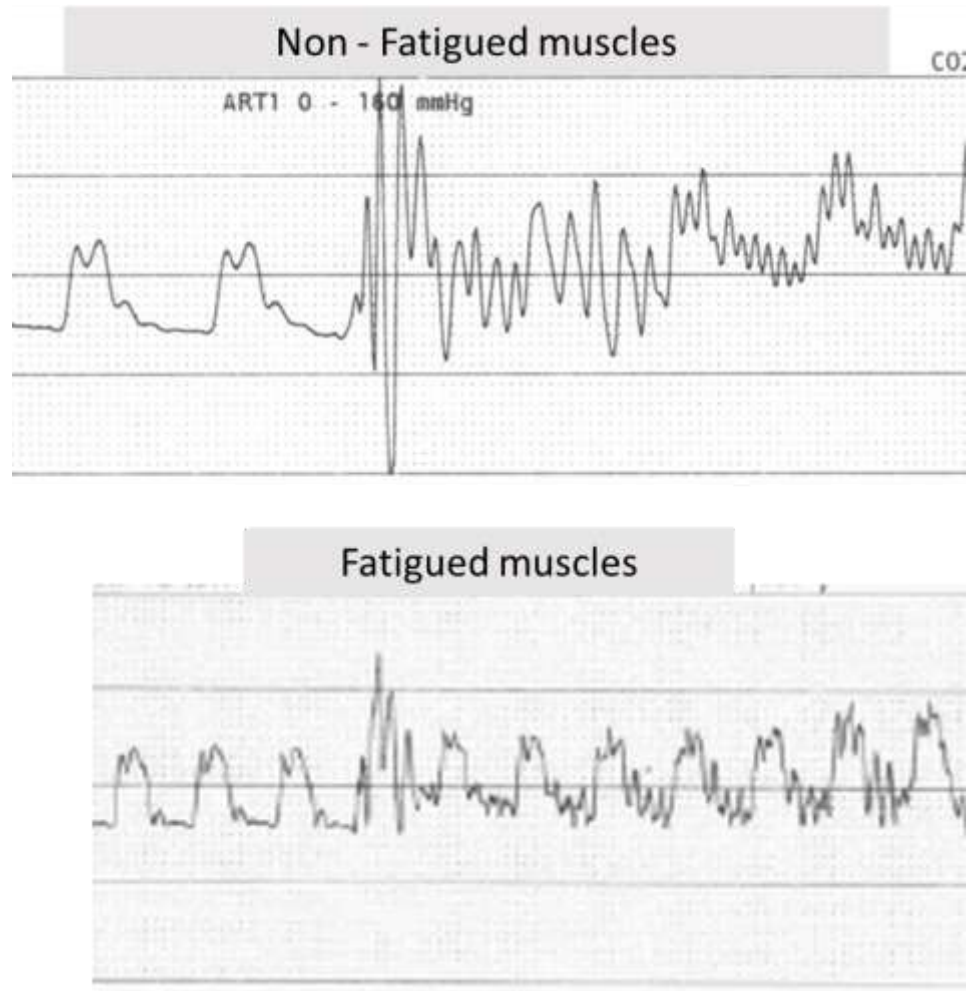
The oscillations are caused by, and follow after, the HEMI stimulations. The limb movements as well as the oscillations have effects on other physiological monitors, for instance causing artifacts on the signal of the invasive arterial blood pressure waveform. The movements caused by the HEMI stimulation, and the exponentially decreasing oscillations are reflected in the invasive arterial blood pressure signal. (Figure 8)

Based on clinical observations, the larger muscles movements (e.g., initial “clonic” or “jerking” early in a “train” when the HEMI stimuli start) cause larger artifacts in the arterial waveform compared to the later smaller (“tonic”) contractions, once fatigue is initiated. (Figure 8.) Also noted, is that larger muscle movements and larger oscillations lead to a larger number of oscillations before dissipating (after the end of the HEMI stimuli).

All these movements create artifacts in the physiological signals, in this case the arterial waveform signals. Signal processing, specifically filtering, will be needed to remove the movement artifacts and oscillations from the arterial waveform signal. The filtering will need to be done without a phase shift, to enable comparison with other signals.

The magnitude (extent) of the filtering required would most likely be an indicator (inversely proportional) of the extent of fatigue. This could be tested in future research with the existing data set.

Figure 8: Arterial blood pressure: Non-fatigued muscles cause larger artifacts than fatigued muscles



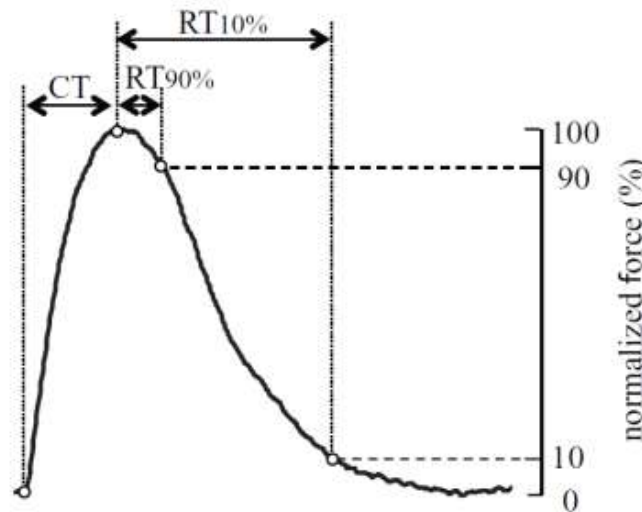
The figure shows that, with the onset of HEMI stimuli, non-fatigued muscles cause larger artefacts in the arterial waveform, compared to fatigued muscles. Note the increasing BP signal as tetanization ensues in the non-fatigued muscles (top panel).

B. CONTRACTION: CHARACTERISTICS AND MEASUREMENTS

Multiple characteristics of the contraction phase have been described to objectively characterize the contraction phase. These characteristics can be used to determine when, and quantify, any changes that may occur, such as after HEMI stimulation. Characteristics that may be measured include **duration**, **rate**, **force (correlated with the distance from the probe)**, **3-D movement (X, Y, X and total distance)**, **ratio of 3-D movements**, etc.

The **duration** of the contraction of muscles (Contraction Time – CT) is the period from the onset of muscle movement until the peak force has been achieved (Figure 9), or in the case of an accelerometer, the direction of the movement reverses (Figure 4), indicating the onset of relaxation. Therefore, this definition is applicable to the HEMI 4 data set where movements were detected via accelerometers, and the peak force can also be mathematically derived from the existing data set.

Figure 9: Illustration of contraction time (CT)



The figure shows the determination of the contraction time (CT) as it is defined as the time duration from the initial rise of a twitch to the peak of a twitch. In this case, the graphic shows the measurement of force versus time.

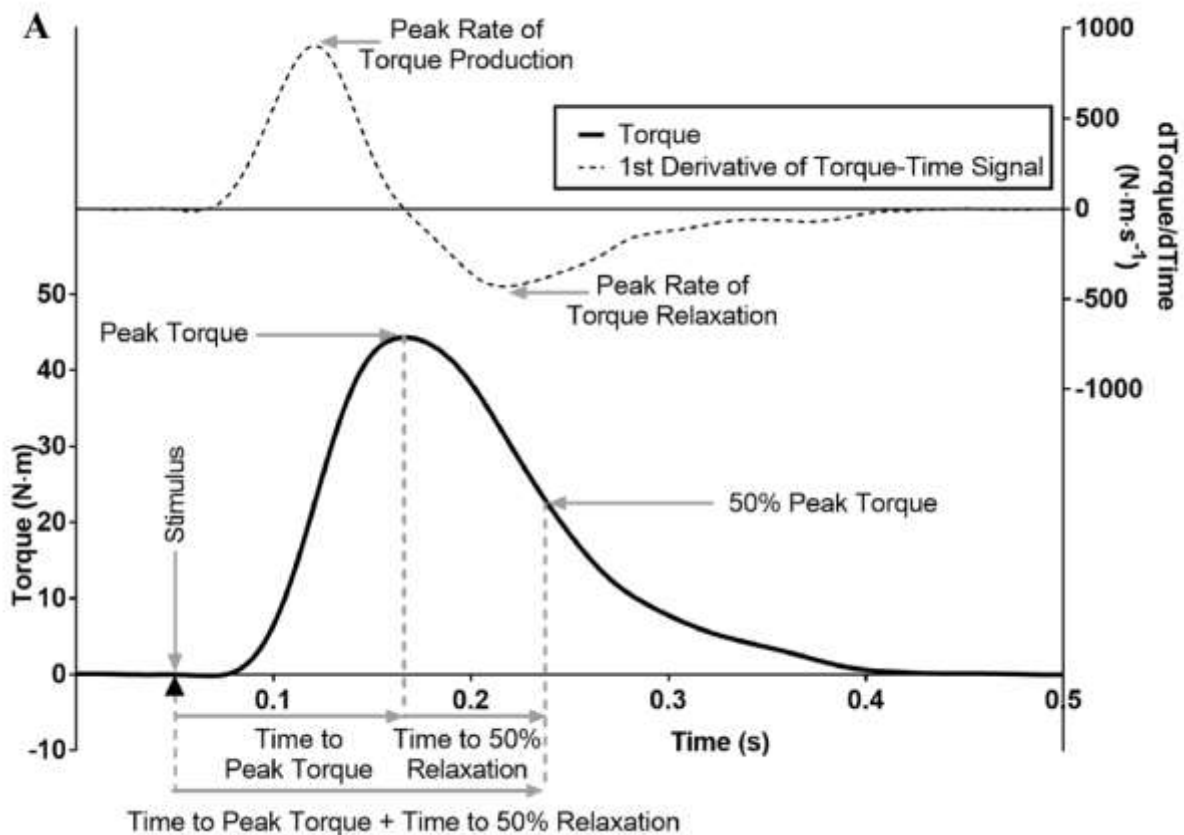
The analysis of the contraction characteristics of the muscles will be useful for the modeling as well as physiological purposes:

- Modeling: overall and peak rates of contraction (dP/dt), duration, maximum calculated forces of limbs and individual muscles, etc.
- Position of the limb in 3-D space (distance moved): onset of tetanization (see following sections), as well as calculation of power, work, and energy generated
- Effect of distance from HEMI probe on limb movements: distance moved, timing, force, etc.

- Determination of deleterious effects of HEMI by comparing contraction characteristics of the first run with decrements during subsequent runs and/or longer runs
- Comparison of nerve pathway stimulating the muscles by analyzing electro-mechanical delay and the effect on contraction time
- Comparison of animal and published human data
- Comparison of various types of muscles

Figure 10 shows a graphic depiction of the calculation of the “**Peak Rate of Torque Production**” which is noted as the “first derivative of the torque time signal.” This characteristic of the contraction of muscle is postulated to be one of the first parameters to decrease with muscle fatigue, and would therefore be an early indicator of the decrement of muscle function.

Figure 10: Peak Torque (maximum dP/dt)



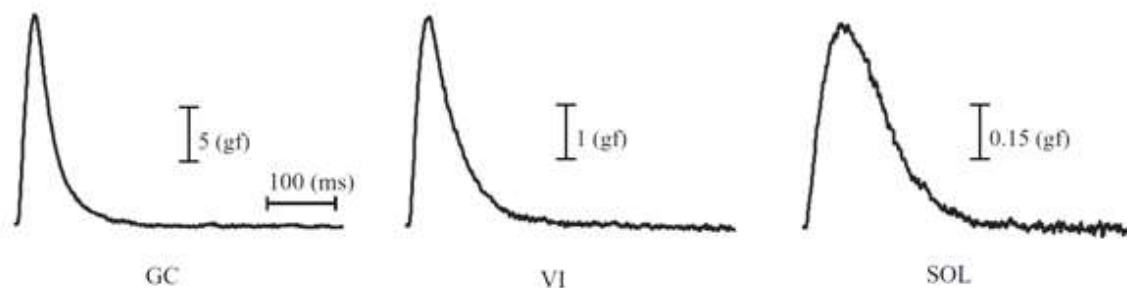
The figure shows a graphic of the first derivative of the force time signal and the calculation of the peak torque.

An understanding of the contractions of different types of muscle (Figure 11) is necessary for the development of a mathematical model as various types of muscles might have to be modeled differently.

Nakayama et al (1970) describe muscles with different kinetic and tonic components which can be detected by analyzing M-waves (via EMG). Furthermore, they showed that the strength-duration curves are different: the tonic muscles have a higher threshold for electrical stimuli compared to the kinetic muscles. Specifically relevant to the HEMI effort, is that the muscle types perform differently after repetitive stimulation. The duration of the recovery (resting) periods also had an influence that varied per muscle type. (Nakayama et al 1970.) The **recovery periods** of 100 and 300 msec are relevant to the HEMI stimuli which are typically administered at ± 50 msec intervals (19 Hz.) This difference in recovery periods may explain some of the early decreases in force (“fatigue”) noticed in repeated runs of stimulation of mixed muscle types in the whole body.

Watanabe et al (2017) showed typical twitch waveforms for gastrocnemius (GC), vastus intermedius (VI) and soleus (SOL) muscles which differed from one another – relevant to modeling.

Figure 11: Muscles have different contraction characteristics



The figure shows typical contraction (“twitch”) waveforms for three types of muscles: gastrocnemius (GC), vastus intermedius (VI) and soleus (SOL) muscles.

- **Gastrocnemius: small, very superficial, posterior leg**
- **Vastus lateralis: small, lateral leg**
- **Soleus: lower leg - part of Achilles tendon, posterior, largest calf muscle**

No previous 3-D data was found on chest and abdominal muscles undergoing HEMI stimulation. There is no clear relationship between HEMI stimulation’s effect on “breathing muscles” (e.g., intercostal muscles, diaphragm, and abdominal muscles.) -Inhalation and/or exhalation might be caused by different HEMI probe positions. Due to the 3-D nature of the eight accelerometers used in the existing HEMI 4 data set, differentiation of inhalation and exhalation should be feasible.

Some investigators found inhalation occurred (even “forced”) with the onset of HEMI stimuli, while others found exhalation. We developed a new working hypothesis to be explored: the probe positions would have a major influence on the phases of respiration. For instance, stimulation of the phrenic

nerve would cause inhalation via the diaphragm, while stimulation primarily of the internal intercostal muscles would cause exhalation.

It should be possible to use the 3-D movements of chest and abdominal muscles in the existing data set to determine whether inhalation or exhalation occurs with HEMI probes in the standard positions for HEMI 4 (left forelimb-LA and right hind limb-RL). It should also be possible to determine if different probe positions (e.g., **left hind limb-LL** and right hind limb-RL) lead to different relationships in terms of inhalation and exhalation, as this will be due to different levels of spinal cord being stimulated, and different pathways (up or down the spinal cord) that the signal is transmitted.

Another interesting aspect of the existing data set would be to compare the effect of HEMI stimulation on different types of muscles as represented by the X, Y, and Z directions of muscle movements. The Z direction consists of a mixture of muscle types: postural to keep the animal upright, as well as dynamic used for motion. The Y and Z directions consist of smaller muscles to control direction and motion. It is postulated that HEMI stimulation would have different effects on the different types of muscles. This could be examined by calculating the **ratios of the 3-D movements**.

The ratios of 3-D movements could be used:

- to compare limbs: e.g., closer to the HEMI probe versus at a further distance
- development of fatigue overall, but also for each individual direction (X, Y and Z), which represents different muscle types
- possibly to check for tetanization during the 1st 5 sec of the contraction for each individual direction (X, Y and Z),
- possibly help to explain the double peaks found in the 1st five seconds of HEMI application
- to differentiate active (stimulated) and passive movements
- to check for fatigue by a change in the ratio: muscles moving the largest distance, will most probably show the most fatigue, due to both more work and decreased perfusion during the contraction
- to explore the contribution of local muscle fatigue (due to work), versus “fatigue” (poor muscle function) due to systemic physiological effects (hypoxia, acidosis, etc.)
- distance moved at the beginning of run compared the end of run

The value of using the **ratios of the 3-D movements** includes the ability to standardize (or “index”) each group of muscles for variations in muscle size in a given animal (e.g., forelimbs versus hind limbs), as well as to standardize across animals for size, etc.

The ratios would also provide a window into the role that central biochemical abnormalities (pH, Na⁺, K⁺, Hct, as well as other yet to be named items) play in the development of fatigue, compared to the role of supra-maximal contractions of a given muscle. The reasoning is as follows: muscles performing the most work in the Z direction will be subject to both central biochemical abnormalities as well as muscle cell fatigue. Muscles working less vigorously (Y and Z directions) will mainly be exposed to central biochemical abnormalities. Any decrease in the distances moved in the Y and Z directions could then be attributed to the central biochemical abnormalities. Any greater difference in the onset and

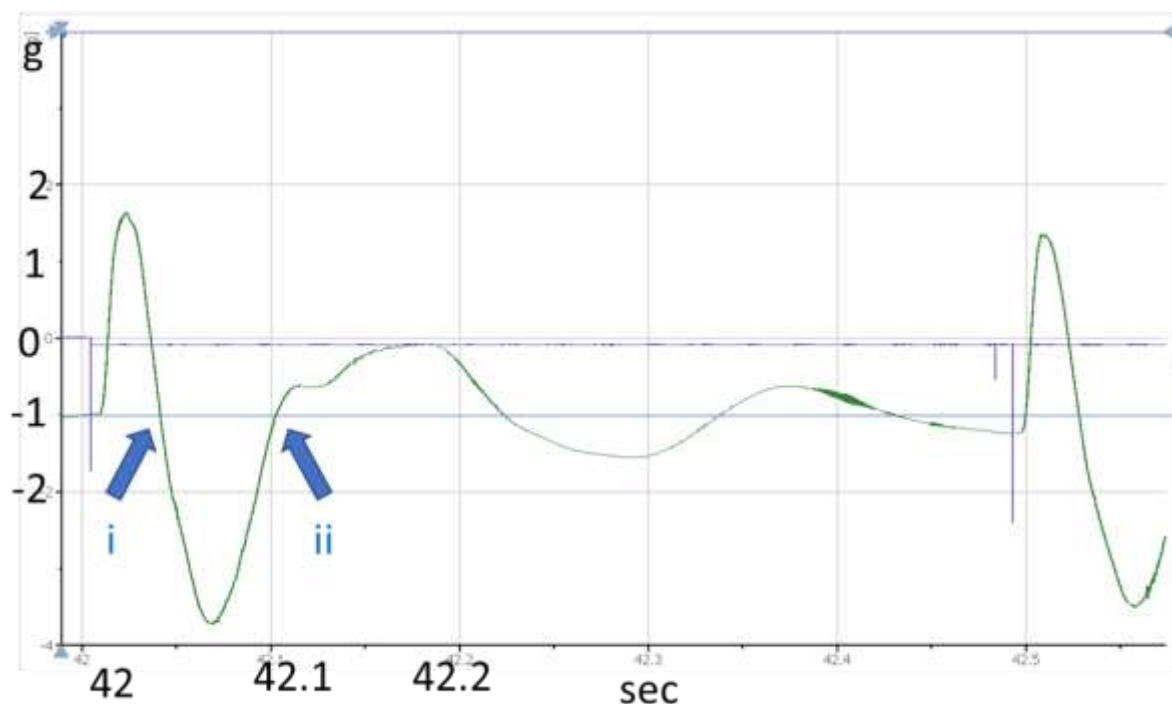
extent of the fatigue in the Z direction could then be contributed to the muscle contractions and work (which includes decreased perfusion during a supra-maximal contraction – a normal physiological effect, magnified due to the tetanic nature of the contractions.) Similar reasoning would apply to probe positions at such a distance from a distant limb that the limb is performing only minimal work. Please see the Appendix for suggestions to present the data in table and figure formats, as well as suggestions for statistical comparisons.

C. RELAXATION: CHARACTERISTICS AND MEASUREMENTS

Relaxation, in the Z direction, starts when the upwards (“positive”) 3-D movement of contraction is reversed. Relaxation ends when the downwards (“negative”) 3-D movement is reversed to a movement in the opposite (upwards/positive) direction. (Figure 12)

Note: relaxation is an active process requiring energy (ATP) to move calcium ions back into the sarcoplasmic reticulum. An analysis of the development and extent of changes in the characteristics of relaxation might provide an insight into the role of Calcium re-uptake as a cause of fatigue. If the data indicates such a mechanism, this would be relevant to the development of the simulation modeling effort as well.

Figure 12: Start and end of relaxation

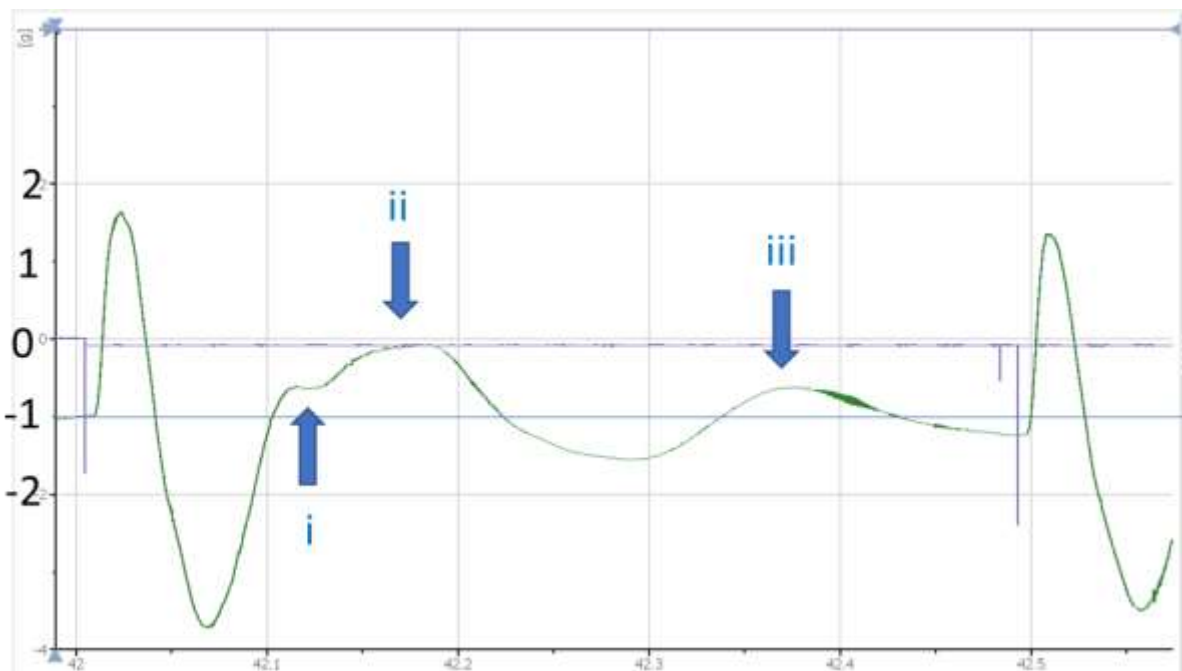


The figure shows two HEMI stimuli (blue.) The first HEMI stimulus is followed by an upwards movement representing contraction in the Z direction of the left forelimb (green.) The contraction is followed by a downwards movement representing relaxation. The arrows represent the start (i) and end (ii) of relaxation when the accelerometer signal crosses the “baseline.” The baseline for the Z direction is minus 1 (“-1”), which represents the constant “negative”, downwards gravity signal. The x-axis is in seconds, the y-axis is in “g” (gravity acceleration) units.

The 3-D movement in the opposite (upwards/positive) direction may follow an electrical impulse (HEMI), an oscillation movement or a period of quiescence (given a non-stimulatory period of sufficient duration.)

Therefore, the end of relaxation period may be obscured by non-HEMI induced movements, such as oscillations or transmitted movements from other limbs. It is critical for the simulation project to identify such artefactual movements. The automated detection algorithms should initially be manually checked for accuracy. (Figure 13.) The existing HEMI 4 data set has multiple data sets to achieve this goal.

Figure 13: Artefactual movement



The figure shows two HEMI stimuli (purple.) The first HEMI stimulus is followed by an upwards movement representing contraction in the Z direction of the left forelimb (green.) The contraction is followed by a downwards movement representing relaxation. The arrows (i, ii and iii) represent three artefacts with different amplitudes and periods (frequencies), therefore from different sources. The artefacts occur during the period between HEMI stimuli. Such artefacts need to be identified by an automated signal processing algorithm. The baseline for the Z direction is minus 1 ("−1"), which represents the constant "negative", downwards gravity signal. The x-axis is in seconds, the y-axis is in "g" (gravity acceleration) units.

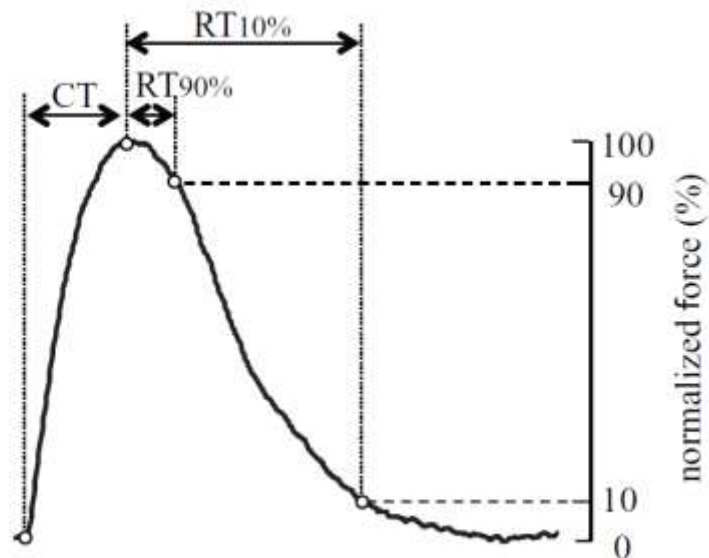
Analysis of the relaxation characteristics of the muscles will be useful for many of the purposes as outlined in the previous section under “Contraction” except that the nerve pathways do not have a known role in relaxation

- Modeling: overall and peak rates of relaxation, duration, etc.
- Position of the limb, after relaxation, in 3-D space (distance moved): onset and quantification of tetanization, i.e., when there is insufficient time for complete relaxation and the muscles do not return to the starting position
- Determination of deleterious effects of HEMI by comparing relaxation during the early part of a run with changes in relaxation during the latter part of a (longer) run
- Determination of deleterious effects of HEMI by comparing relaxation during the first run with changes in relaxation during subsequent runs and/or longer runs: calculating distance moved, timing, etc.
- Comparison of animal and published human data
- Comparison of various types of muscles (Watanabe et al. 2017)
- No previous data found on 3-D **movements (distances moved)** during relaxation
- No previous data found on 3-D **power or work** during relaxation
- Analysis of **fatigue during relaxation**: the description and ideas mentioned previously with contraction are relevant here

There are several methods of describing and **quantifying the extent and duration of relaxation**.

Watanabe et al. (2017) defined relaxation time as the period to decrease to 90% and to 10% of the peak of a twitch. (Figure 14)

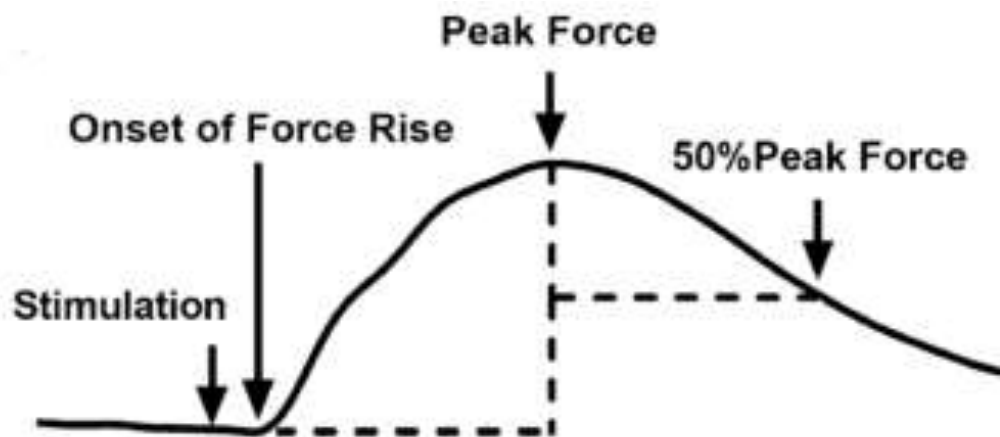
Figure 14: Definition of Relaxation Time: Decrement to 90% and to 10% of “peak of a twitch.”



The figure shows a proposed definition of “Relaxation Time” (RT) as being 10% (RT10%) and 90% (RT90%) of the time durations from the peak of a twitch to the time the twitch decreases to 10% and 90% from the peak. Watanabe et al. (2017)

Smith et al. (2018) used the time when the relaxation had reached 50% of the Peak Force. (Figure 15)

Figure 15: Definition of Relaxation: Decrease to 50% of “Peak Force”



The figure shows a method of characterizing relaxation as 50% of peak force. (Smith et al. 2018)

Investigating active, energy consuming relaxation processes: Our previous analysis of the data **combined** active (AGAINST gravity) work and relaxation (WITH gravity) work (Z direction) to calculate “total work, i.e., did not calculate work separately for contraction and relaxation. Given that gravity is constant, we should be able to separate out, during the relaxation phase, the passive work by gravity and the active work of relaxation. We also have the 3-D data for X and Y axes, which are gravity independent. For instance, the thoracic and abdominal accelerometer (“Work”) measurements are essentially expected to be gravity independent (in all 3 axes: X, Y and Z).

The next study of work should be designed to negate the effects of gravity on the limbs, for instance, by having the animals in the lateral position. The limbs, being the main muscles for “work” will then extend in the horizontal direction which is gravity independent thereby being “free floating.”

Relaxation is an active process and requires energy stores (ATP.) “Slowing” of relaxation might also be used as a measure of deleterious effects.

Using the present HEMI data sets, it should be feasible to check if the relaxation phase contains two exponential components: a.) due to gravity, and b.) due to physiology. Gravity is constant and any difference between the exponential values of non-fatigued versus fatigued muscles could be attributed to changes in physiology, e.g., slowing of Calcium re-uptake.

D. ONSET OF TETANIZATION: USING ACCELERATION TO CALCULATE DISTANCE MOVED WITH TETANIZATION

“Tetanic contractions” are said to occur when a following stimulus occurs before the muscle has had time to fully “relax.” However, there is a range of “unfused” tetanic contractions before fully “fused” tetanic contractions become evident.

Watanabe et al. (2017) provided an excellent description of unfused and fused tetanic contractions:

- *“Tetanic contraction (tetanus) is evoked by the summation of several twitches when the skeletal muscle is stimulated at a high frequency. In particular, unfused tetanus has muscular relaxation between individual/successive stimulations, whereas fused tetanus has no muscular relaxation.”*

The **onset** of tetanization is a gradual process, not an event with an immediate onset. The existing HEMI data set provides a large number of instances to calculate the rate of onset of tetanization that will be useful for the modeling project.

The literature search provided a variety of methods to quantify the degree of tetanization including the concept of “Fusion Index.” The literature also provided methods of quantifying the increase in contraction strength after tetanic stimulations, called Post Tetanic Facilitation (PTC) or Post Tetanic Potentiation (PTP). The quantification method has been published under the term “Boost and Sag.” This methodology could be applied to the HEMI data sets.

Fusion index (FI)

As mentioned before, tetanic contractions are defined by the inability to fully relax before the next electrical stimulus occurs.

As the frequency of the stimuli increases, the unfused tetanic contractions gradually become fused, when there is minimal to zero relaxation between stimuli. The exact stages where unfused and fused contractions become evident, is difficult to pinpoint.

The concept of Fusion Index (FI) was introduced to describe the continuum from unfused to fused tetanic contractions.

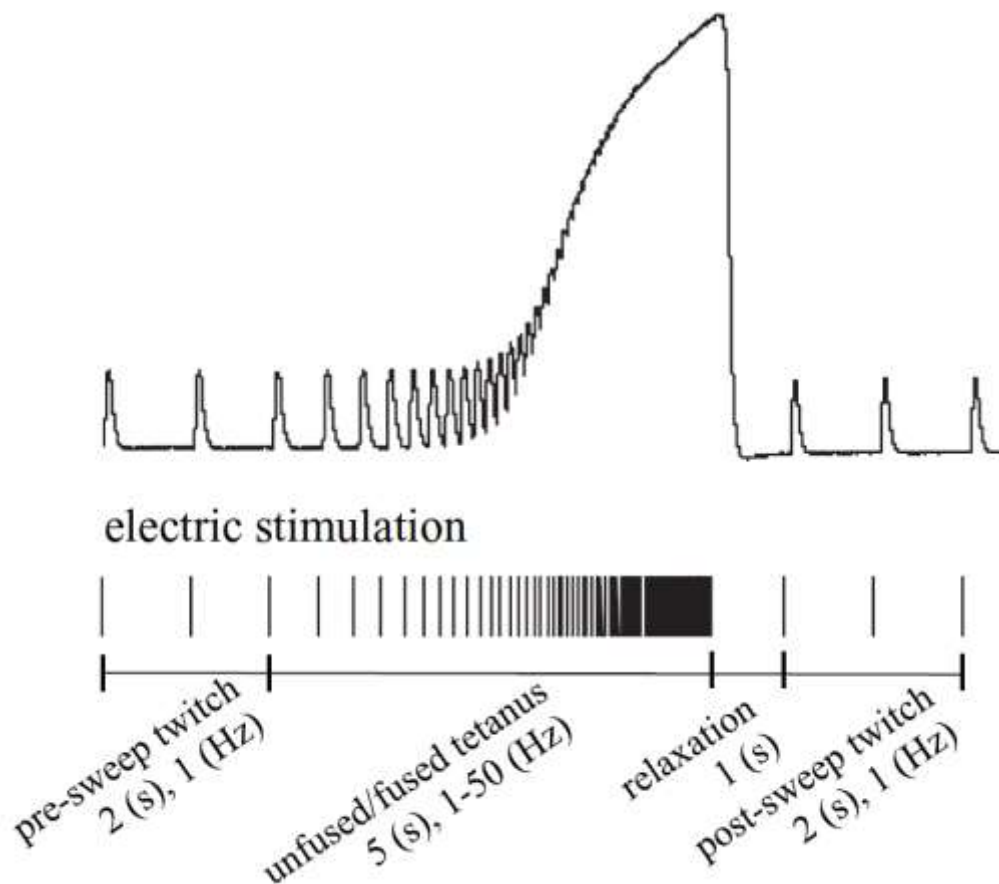
One method to generate the Fusion Index was described by Watanabe et al (2017.) They logarithmically increased the frequency of tetanic contractions from 1 Hz to 50 Hz over 5 second. They applied 1 Hz twitches pre- and post-tetanic stimulation as a measure of quality control.

The value of the Fusion index

- The fusion index will be useful for understanding and modeling the effects of HEMI on different types of nerves and muscles
- The extent to which tetanization occurs on limb muscles versus on thoracic and abdominal muscles will be important in terms of work performed and the resulting energy consumption (which most likely correlates with metabolic and physiological effects.)
- The distance in 3-D space to which the muscle/limb contracts and relaxes could be used to
 - correlate with the amount of work performed
 - correlate with arterial BP increases (for more details, see CVS section)
 - correlate with fatigue developing with longer HEMI stimulations

- The amount of tetanization occurring with “fresh muscles” versus “fatigued muscles”, should be able to be used as an indication of deleterious effects
- Fatigue might lead to earlier fusion – due to both slower contraction and especially slower relaxation, as relaxation is an active process requiring energy (ATP)
- Less force might be developed due to fatigue. In the HEMI 4 data set, less force will be shown as a smaller distance moved in 3-D space.
- The duration of the movements (contraction and relaxation) as discussed earlier would be expected to have a significant influence on the Fusion Index.

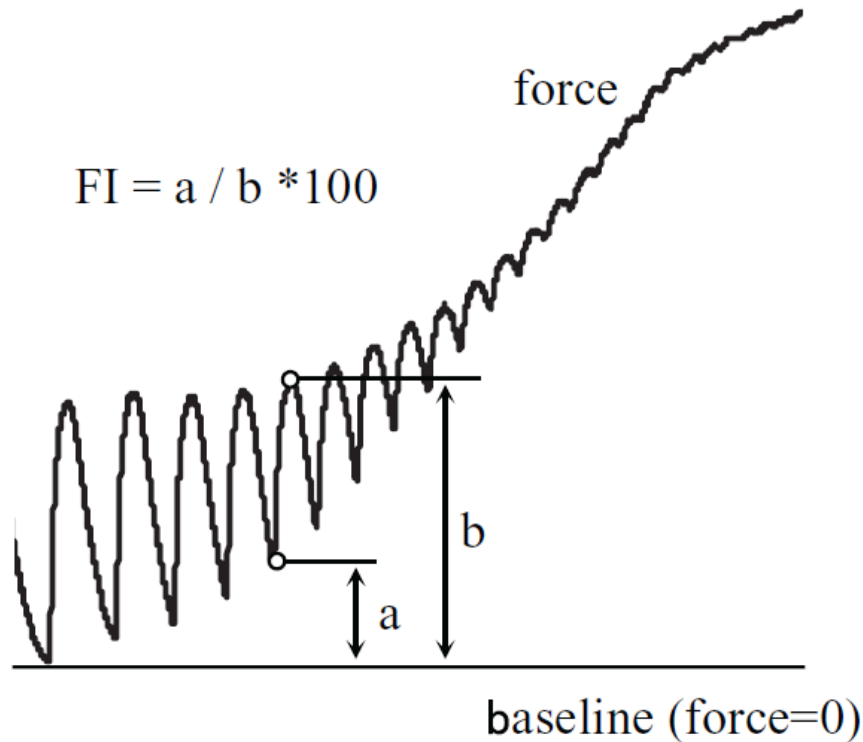
Figure 16: Increasing frequency of stimulation leading from unfused to fused tetani



The figure shows an electrical stimulation causing a typical force waveform. The stimulation frequency is increased in a logarithmically fashion from 1 to 50 Hz to induce unfused tetanus building up to fused tetanus.

A method of calculating the Fusion Index is shown in Figure 17. (Watanabe et al. 2017)

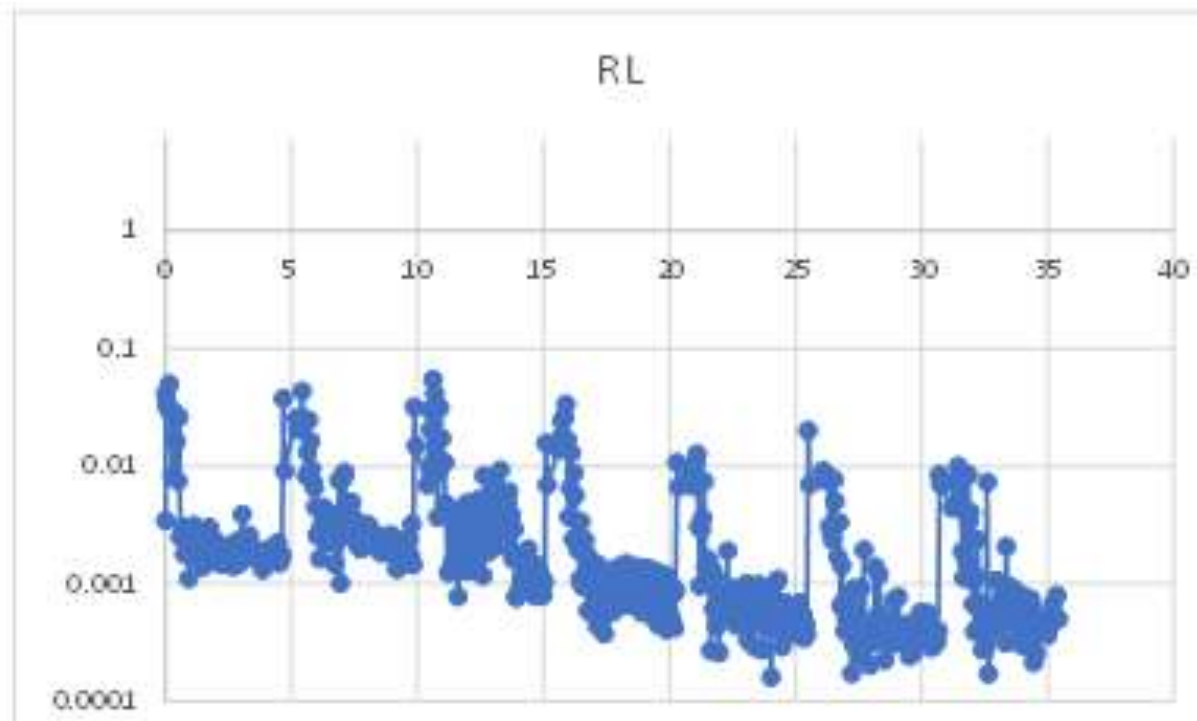
Figure 17: A method of calculating the Fusion Index



The figure shows a method for calculating tetanic progression from muscle twitches. The fusion index (FI) is calculated from the minimum force (a) and maximum force (b) for each individual stimulation and is calculated as $a/b * 100$ (as a %). See Figure 16 for more detail)

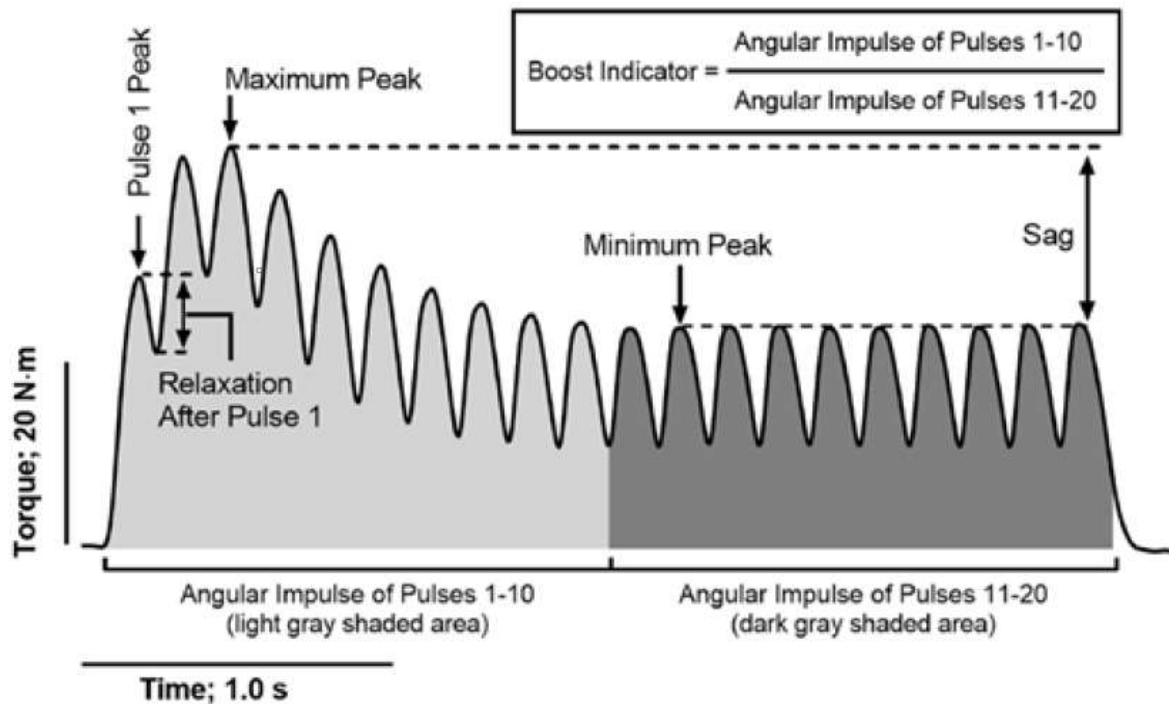
An increase in the strength of muscle contraction after a period of tetanic stimulations is well known, and used daily in human anesthesia practice to evaluate the residual effect of muscle relaxants (e.g., “curare”.) The phenomenon is called Post Tetanic Facilitation (PTC) or Post Tetanic Potentiation (PTP). This phenomenon is evident after the interruptions of HEMI stimuli (Figure 18), although the cause has not been clearly defined, nor has the extent of the increased muscle contractions been quantified.

Figure 18: Post Tetanic Facilitation (PTC) occurs after interruptions of the HEMI stimuli



The figure shows an accelerometer signal (right hind limb RL) which increases after each interruption of the HEMI stimulation. Each HEMI stimulation lasts 5 seconds and the interruptions are 200 msec. The x-axis is in seconds, the y-axis is in “g” (gravity acceleration) logarithmic units

The literature provided methods of quantifying the increase in contraction strength after tetanic stimulations, called Post Tetanic Facilitation (PTC) or Post Tetanic Potentiation (PTP). The quantification method has been published under the term “Boost and Sag.” (Smith et al. 2021.) This methodology could be applied to the HEMI data sets to quantify the “Boost” after the interruption, and follow any decrement of the boost as fatigue develops.

Figure19: Definition of “Boost and Sag”

The figure shows the calculation of the “Boost and Sag” as the ratio of the size of the first 10 twitches compared to the size of the following 10 twitches. (Smith et al. 2012)

While incomplete tetanization might cause loss of postural tone, causing the subject to "fall down," it might not cause loss of upper body (arms) which might enable belligerent activities (e.g., throwing objects) to occur. Future studies may include video analysis of applications of HEMI available on the internet to determine the effect of HEMI probe positions on upper and lower body tetanization.

E. TIME COURSE OF TETANIZATION RELATED TO TIME COURSE OF OTHER PHYSIOLOGICAL EFFECTS

Tetanzation is not instantaneous – it takes several stimulations to build up to the maximum tetanzation status. The progression and maintenance of tetanzation is also not linear, nor constant.

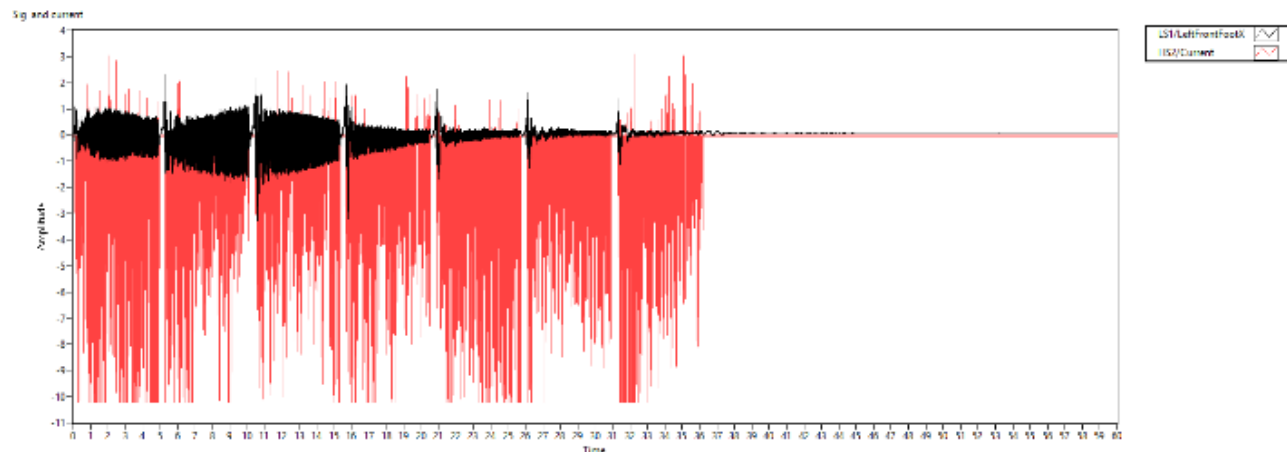
The HEMI 4 data set contains multiple data sets that would be useful for the modeling project.

There are at least three phases of the tetanzation progression which are clinically visible:

- i. The first 0--10 stimulations – major “jerking” movements (representing post tetanic facilitation – PTF – see previous section)
- ii. The next 10 seconds – large “clonic” movements – similar to large epileptic movements – which decrease over a further 10 seconds to the next more quiescent phase
- iii. Stimulations from +- 20 seconds onwards: the muscle movements become much smaller – similar to “shivering” due to being cold. During this phase, the limbs are clinically noted to be quite stiff. It will be important to use the data set to calculate the position of the limbs in 3-D space to determine if the decrease in movement (noted as “shivering”) is due to fatigue, or due to “fused tetanzation” with limited distance available to move.

The variations of the progression of tetanzation, as clinically visible, is demonstrated by the accelerometer signal in Figure 20.

Figure 20: Progression of tetanzation over 35 seconds showing variability



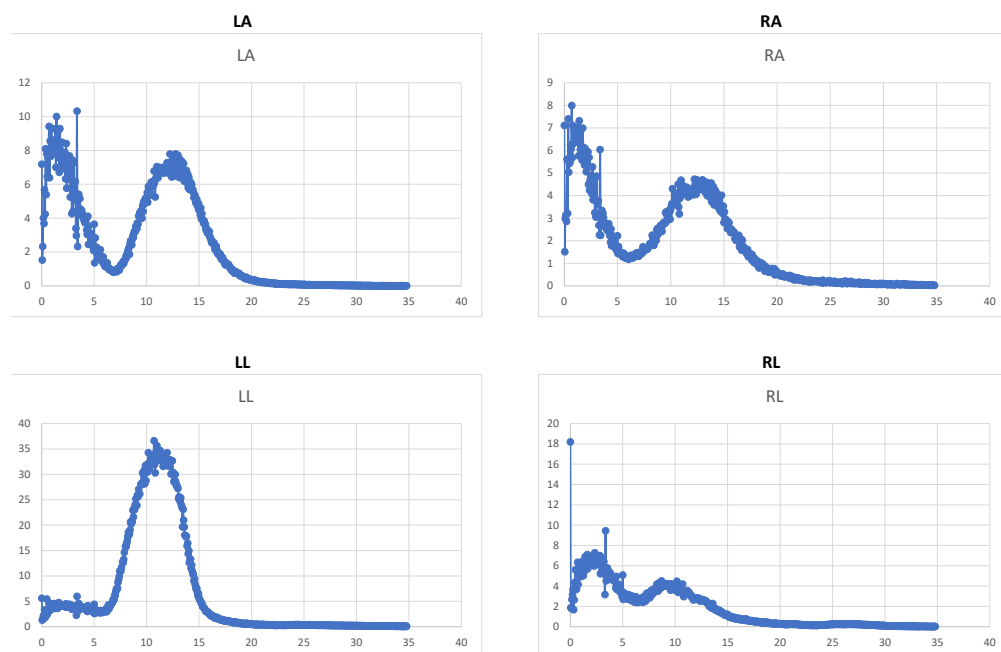
The figure shows the non-linear progression (variability) of the acceleration signal due to tetanic stimulation over 35 seconds. The acceleration signal of the hind limb (Z Direction - black) varies in amplitude throughout the 35 second period of HEMI stimulation. The High-Speed Sampling (HS2) current is shown in red. The x-axis is in seconds, the y-axis is in “g” (gravity acceleration) units

The variability of the time course of tetanization is also exemplified by the Generation of Single and Double Peaks of Work during the 35 second period of HEMI stimulation as shown in the HEMI 4 data set (Figure 21), where various patterns are shown of the timing of the periods of maximum work (“peaks”) in each of the four limbs of a single animal. The HEMI stimulus consisted of 35 seconds of continuous HEMI stimulation, without any interruptions.

The y-axis represents the work generated for each individual HEMI stimulus. Three of the limbs exhibited double peaks, where the second peak had a lower magnitude than the first: left (LA) and right (RA) forelimbs as well as the right hind limb (RL.) The second peak in these three limbs was of a lower magnitude than the first peak. The left hind limb (LL) showed a single peak at 10-12 seconds, which is well above the range of both peaks in the other 3 limbs.

Signal processing of these data sets, and calculation of the distances that the limbs moved, would be useful to elucidate the causes of the differences in the limbs and the runs.

Figure 21: Variability of “Work” generated over 35 seconds due to HEMI stimulation



The figure shows samples of the generation of single and double peaks of work. There are various patterns of the timing of the periods of maximum work (“peaks”) in each of the four limbs of a single animal. The x-axis is in seconds. The HEMI stimulus consisted of 35 seconds of continuous HEMI stimulation, without any interruptions. The y-axis represents the work generated for each individual HEMI stimulus. Three of the limbs exhibited double peaks, where the second peak had a lower magnitude than the first: left (LA) and right (RA) forelimbs as well as the right hind limb (RL.) The second peak in these three limbs was of a lower magnitude than the first peak. The left hind limb (LL) showed a single peak at 10-12 seconds, which is well above the range of both peaks in the other 3 limbs

The time to build up tetanization is also reflected in **physiological measurements**, e.g., in the **arterial waveform**. For instance, figure 8 shows the initial increase in blood pressure due to the onset of tetanization due to HEMI stimulation. (see CVS section for more examples of increases in the build-up of blood pressure.) A future study of the HEMI 4 data set could test (after signal processing and filtering of the 19 Hz artefacts) for the correlation on the onset of tetanization and the increase in the blood pressure measurements.

The progression of the tetanic state is modified by multiple parameters. For instance, by physiology, energy stores, (aerobic, anaerobic), Ca²⁺ availability and re-uptake from cytosol to endoplasmic reticulum, neuro-muscular junction (pre- and post-synaptic availability of acetyl choline), etc. After the onset of tetanization, the **time course of tetanization** due to continued tetanic stimuli will be evident from the HEMI data sets, and can therefore be incorporated into the simulation models.

Based on the literature, the decrease in the accelerometer signals within five seconds most likely does not represent fatigue, but rather is the result of a decreased range of potential movement due to the partially tetanized muscle which did not fully relax between the HEMI stimuli. Analysis of elements in the HEMI 4 data set should be able to elucidate this question.

F. DUTY CYCLES AND INTERRUPTIONS

The existing HEMI data sets contain a large number of **duty cycles** with varying durations of interruptions, as well as varying durations of total applications. These data sets provide a basis for the development of a robust simulation model. Such a simulation model would be useful to examine a large number of combinations and permutations of HEMI frequencies, waveforms, interruptions, total durations, etc. which would not be possible with human surrogates.

There are many biological examples of animals using electrical incapacitating stimuli such as electric eels (Catania 2019.) These animal derived duty cycles could be tested with the simulation program to identify candidate duty cycles to be tested in human surrogates.

There are multiple electrical stimuli used in human medical practice, each with different duty cycles

- TENS - trans-cutaneous-electrical stimulation for chronic pain
- Spinal cord stimulators – for chronic pain
- MRI - maximum permissible power of radio frequency signals
- ECT - electro-convulsive therapy, e.g., for depression in humans - multiple square waves
- DBS - deep brain stimulation, e.g., for Parkinson’s disease
- Implanted stimulator e.g., for vagal therapy, respiratory stimulation (phrenic nerve)
- Nerve stimulators as used in daily anesthesia practice to test the activity of muscle relaxants
- Nano-second stimuli DoD
- Nano-second stimuli - used for cancer therapy in solid tissue organs
- Defibrillators - external, AICD (automated-implantable-cardiac-defibrillator)
- Cardioversion of cardiac arrhythmias, e.g., atrial fibrillation
- Cardiac pacemakers - external, (internal – very small stimulus)
- magnetic stimulation of the brain - HEMI studies
- magnetic stimulation of the brain - medical therapy

Most of these duty cycles have been described in detail. (Reilly et al. 2011)

These duty cycles used in humans could be tested with the simulation program to identify candidate duty cycles to be tested in human surrogates with HEMI currents.

A future study examining the **volume of activation** could be performed by observing (high speed video) the effect of cardioversion on the limbs. The defibrillation pads are placed on the front and the back of the chest at the nipple line (T4.) Clinical observation shows movement of the arms and the legs, but this might just be due to activation of the chest and abdominal muscle, rather than due to the relevant spinal roots.

Previous HEMI studies have examined interruptions that enabled a “Good Breath” (Werner 2019) in a limited number of animals with a limited number of steps of durations of interruptions. The simulation program could be used to study a larger number of steps of interruptions range to identify an optimal range of interruptions for the maximum “Good Breath” while maintaining an appropriate level on incapacitation.

As mentioned in previous sections, post-tetanic facilitation (PTF) as well as “Boost and Sag” phenomena occur after interruptions. No literature was found related to the effect of the duration of interruptions

on these phenomena, nor on the amount of work caused by variations in the durations of the interruptions.

For instance,

- How long do larger contractions last after interruptions with varying durations?
Does this time/duration of potentiation change/decrease with fatigue?

Signal processing will be needed, using the characteristics outlined in previous sections to prepare the data for analysis.

The HEMI 4 data sets have the information for these studies to be performed. Signal processing will be needed, using the characteristics outlined in previous sections to prepare the data for analysis.

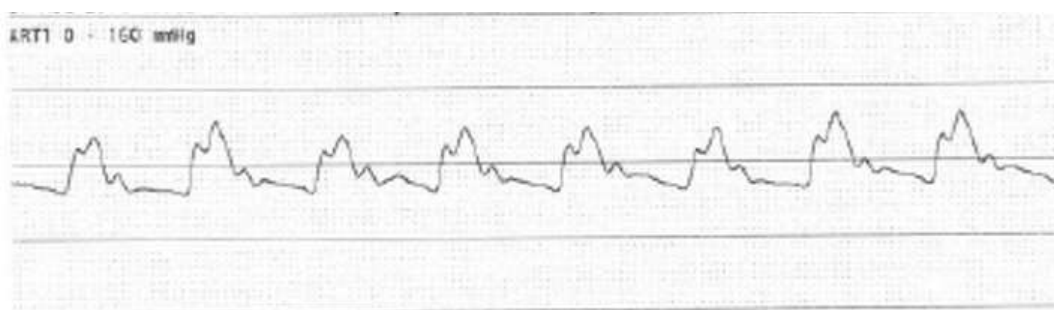
G. CARDIO-VASCULAR SYSTEM (CVS): EFFECT OF HEMI STIMULATION ON ARTERIAL AND VENOUS WAVEFORMS AND ECG

A major effort of **signal processing** is needed to understand the effect of HEMI stimulation on the cardio-vascular system, especially the arterial waveforms and BP, as well as on the ECG. We recommend that the first step is to analyze our existing digital data to create methods to "smooth out" the HEMI artifacts in the digital signals. Existing data includes digitized data sets which can be used by various sophisticated scientific programs. Existing data also includes analogue data on paper strips. (Figure 22.) Such analogue data will need to be digitized to enable digital data processing. Several stand-alone programs exist for this purpose. However, we advise using programs such as MatLab (National Instruments) so that the data is available for all the other powerful functions of these programs. (See Appendix for more details.)

Furthermore, we need to determine and correlate the effects of intra-thoracic pressures on the extra-thoracic blood pressures (Valsalva phenomenon, also named "Sag and Boost".) This correlation is critical for physiological determinants of longer durations of HEMI applications, as well as perfusion of peripheral muscles and tissues.

As a baseline for comparison of this series of graphics, Figure 22 is presented as showing a normal BP waveform (analogue, as recorded on a paper strip.) The double systolic peak is termed an "Inotropic Pulse." The presence of an inotropic pulse is considered an indication of a vigorous, non-stunned myocardium. The "peak rate of rise" (dP/dt) may also be calculated and followed over the course of the HEMI application to determine the onset of, and follow the development of deleterious effects. The dichrotic notch is visible in the downslope of the arterial waveform. While the dichrotic notch measured close to the aortic valve is an indication of aortic valve closure, the dichrotic notch in the peripheral arteries is considered a "reflection" of the arterial waveform from the peripheral circulation and is mainly influenced by the arterial vascular resistance (peripheral vascular resistance – PVR or SVR) (Murray et al. 1996)

Figure 22: Normal invasive arterial BP waveform (analogue)



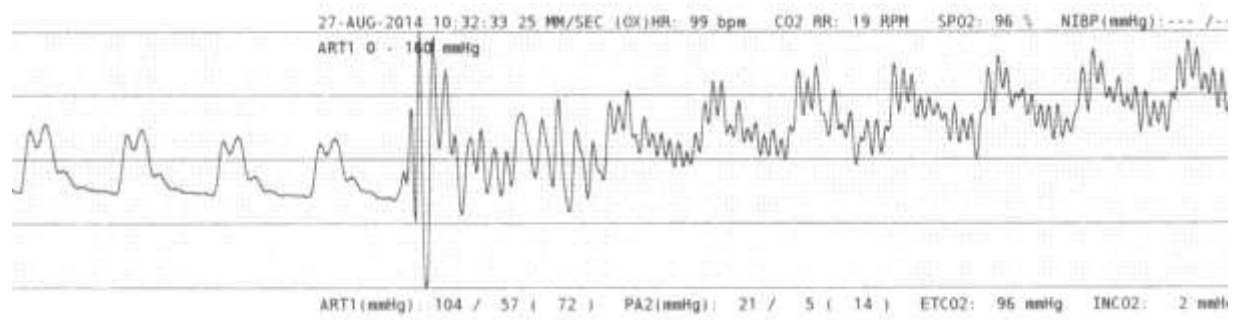
The figure shows a normal BP waveform (analogue) from a paper recording. The scale (y-axis) is 0 to 160 mmHg. The x-axis is time at 25 mm/sec. The peak is systolic BP. The nadir is diastolic BP. The area under the curve is used to calculate the mean BP where 50% of the area is above and 50% below the mean BP. Given the shape of the BP wave form resembling a triangle, the mean BP is close to one third the difference between the systolic and diastolic BP values.

Figure 23 shows an example of major interference with the arterial BP wave form due to the 19 Hz muscle movements caused by HEMI stimuli. The liquid (saline) in the arterial BP sampling line has inertia – this inertia leads to “fling” as the muscle contractions cause movement of the animal and creates inertial impulses in the liquid filled line.

After the start of the HEMI stimuli, the first two heart beats show large and irregular effect on the arterial waveform. This is due to post-tetanic facilitation (PTF) after an interruption. The amplitude and duration of the post-tetanic facilitation might be useful as an indicator of deleterious effects.

The last seven heart beats show more regular artifacts at 19 Hz. This occurs once tetanization sets in. Applying appropriate signal processing (“filters”) should be able to reproduce a “normal” arterial BP wave form. An alternative would be to measure the arterial BP waveform with a Millar catheter, which used a pressure sensor at the tip of the arterial catheter, thereby avoiding any inertial component.

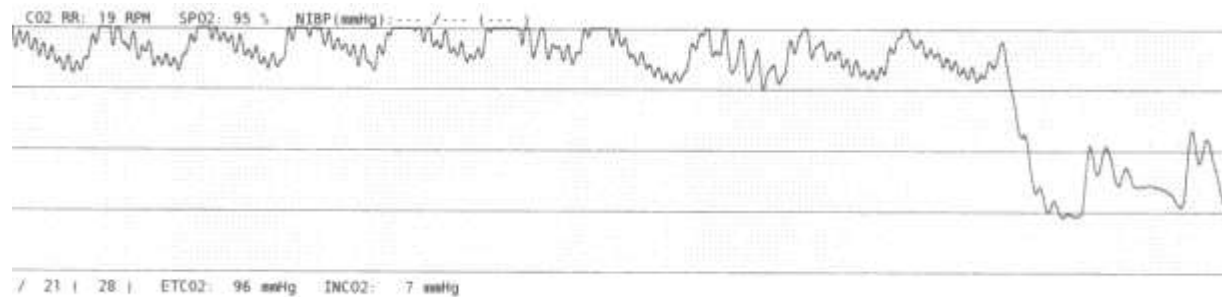
Figure 23: Two types of artefacts the arterial BP wave form due to 19 Hz HEMI stimuli



The figure shows four normal arterial line heartbeats, followed by very large excursions for two heartbeats. The excursions (“swings”) cover the whole scale of the blood pressure tracing (from 0 to 160 mmHg). These initial large movements (“clonic contractions”) are noted in the extremities of the subjects as soon as the HEMI stimulus is initiated. These large movements are quite short lived - in this case less than 2 seconds (less than two heart beats at 99 beats per minute; the paper speed is the standard 25 mm/sec, which is the standard for an EKG tracing). The last seven heart beats in this tracing demonstrate the typical 19 Hz “swings” in the arterial line tracings. These swings occur due to the inertia of the saline in the arterial pressure monitoring system. We have noted clinically that the size of the excursion correlate with the size of the muscle movements in the subjects. The tracing also demonstrates that even after about nine seconds (9 heartbeats) after the start of the HEMI application, the BP values are still rising, and have not yet reached a peak. This gradual increase in BP might be another indicator of tetanization, and may be used as a monitor for the onset of fatigue and deleterious effects/

Figure 24 shows the decreased effect that fatigued muscles have on the arterial waveform – only minor excursions. We propose that the degree/extent of filtering necessary to remove the artifacts may be used as an objective indicator of fatigue.

Figure 24: Arterial pressure tracing: Fatigue leading to minor excursions



The figure demonstrates the very minor excursions in the arterial line tracing towards the end of the HEMI stimulus after fatigue has set in. The typical major decrease in BP after the end of the HEMI stimulation is also clearly demonstrated. For further details, see Figure 22.

Figure 25 also demonstrates the rapid and precipitous decrease in all of the blood pressure values as soon as the HEMI stimulus is discontinued. The extent of the hypotension seems to be an indicator of deleterious effects. Of further note is that the blood pressure decreases during the ejection phase of the cardiac cycle (i.e., the heart is in systole, the HEMI stimulus is discontinued, and the blood pressure decreases while the left ventricle is ejecting). This could be due to an immediate decrease in systemic vascular resistance when the HEMI stimulus is discontinued or a phenomenon similar to Phase III of the Valsalva maneuver where a decrease in intra-thoracic pressure leads to a decrease in the blood pressure. This indicates the need to correlate intra-thoracic pressures and extra-thoracic arterial pressures to determine the role of the Valsalva effect on the peripheral BP.

Pinsky et al (2007, 2012) published a series of articles outlining the heart-lung interactions, including the increases in BP with increased intra-thoracic pressures, as found to occur with positive pressure ventilation (e.g., in ventilated patients in the ICU.) The background and physiology is relevant to the HEMI simulation effort.

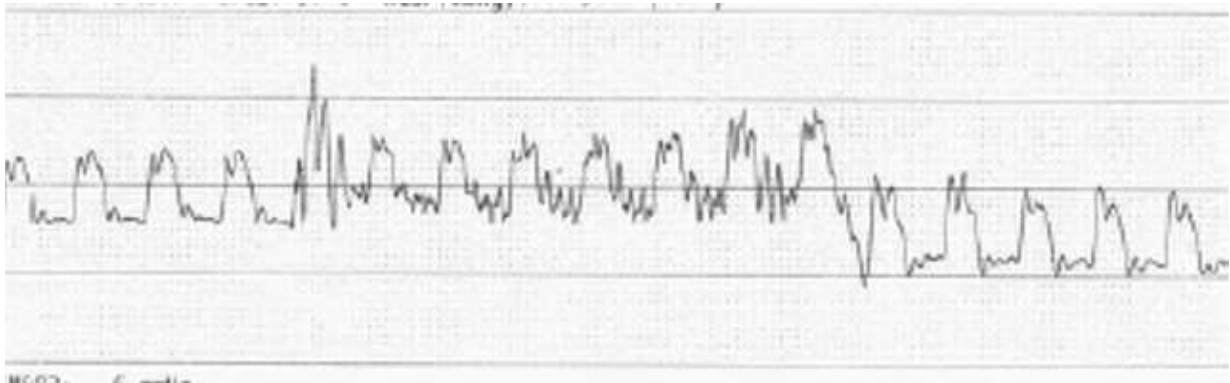
Figure 25 shows minimal 19 Hz movement interference with the wave form, due to fatigue of the muscles. Therefore, the artifact is relatively minor (compared to Figure 23.)

Due to the artifacts, the

- systolic BP is falsely high
- diastolic BP is falsely low

It is not clear what the effects of the movement artifacts are on the mean BP. It will be necessary to analyze the waveform using signal processing to determine the effect on the mean BP.

Figure 25: Fatigue leads to less post-tetanic Facilitation



The figure shows an arterial waveform in a subject with fatigued muscles. Note the minor increase in BP with the onset of the HEMI stimulation. Or further details see text and Figure 22.

The figure shows the minor increase in BP with the onset of HEMI stimulation. The irregular interference only lasts for a single heart beat in contrast to Figure 23, where the irregular interference lasted twice as long. We propose that signal processing of the existing HEMI 4 data set will enable utilization of the increase in BP as well as the duration of the irregular artifacts as indicators of fatigue, as well as being objective measures of deleterious effects.

In general, it seemed that instances of vigorous contractions (more “work”) leads to marked increases in blood pressure (Figure 23), as well as led to more marked decreases in BP at the end of the HEMI stimuli. A future study could be designed to investigate this correlation, as well as whether such animals also exhibited more severe deleterious effects, as evidenced by biochemistry and other measures.

Effects of HEMI on the ECG

Application of HEMI stimuli lead to rapidly changing elements of the ECG. For instance, the T-waves may be enlarged for a single beat due to respiratory effects, inverted due to the HEMI stimulation, or develop changes during the rest period, after the HEMI stimulation has been discontinued. One of the subjects had an inversion of the T-waves eight seconds after the end 360 seconds of HEMI stimulation. Another subject had greatly enlarged T-waves 6 minutes post-HEMI application in the rest period. To analyze such rapid changes, digitization of the ECG signal would be necessary. At 25 mm/sec, an inordinate amount of paper recordings would be generated, and need to be digitized. It would therefore be more productive to collect digitized ECG signal ab initio. A few examples of the rapid changes will be presented.

The upper trace in Figure 26 shows an EKG trace while the lower trace shows the arterial blood pressure. The graphic was produced 2 min after the end of a 1 min EMD exposure. Note the changes in the T-waves due to respiration.

The lower blood pressure trace shows a wide variation in the pulse pressure (definition of pulse pressure: difference between systolic and diastolic pressures.) Some of the pulse pressures are larger, and some are almost non-existent. The changes are in phase with (and caused by respiration.) Such pulse pressure variation (PPV) caused by respiration is considered a strong indicator of a low intra-vascular volume (low CVP, and low wedge pressure.)

Figure 26: Rapid changes of the ECG after HEMI stimulation



The figure shows an ECG tracing in the upper panel. Note the differences in the sizes of the T-waves (i and ii.) The lower panel shows an arterial waveform. Note the changes in the pulse pressure due to respiration (iii and iv.) In a future study, it would be more efficient and accurate to collect digitized signals, rather than digitize analogue signals.

Systolic-dichrotic notch pressure differential

Morelli et al. (2020) describe the value of measuring the difference in pressures

- a.) from the systolic peak pressure to
- b.) the pressure of the dichrotic notch.

A lower difference correlated with a lower dp/dT_{max} (“maximum rate of intra-arterial blood pressure rise”) thereby indicating a fixed and lower stroke volume which correlates with a “stunned” heart due to sepsis. They found that both a lower dp/dT_{max} and a lower systolic-dichrotic notch pressure difference was more sensitive than echocardiographic parameters.

Value to the HEMI projects: The dp/dT_{max} and the systolic-dichrotic notch pressure difference would be useful to elucidate the role of a stunned heart as a cause of the low BP after HEMI exposure. This is in addition to other causes of hypotension, such as hypovolemia. A possible cause for a stunned heart is cardiac (left ventricle) ejection against a markedly increased systemic vascular resistance (SVR) during the application of the HEMI stimuli – similar to the Tako-tsubo syndrome (Wittstein et al. 2008, Sclarovsky et al. 2010, Nef et al. 2010), as described in our previous HEMI 4 report.

Peripheral IV Analysis (PIVA) of venous wave forms (Hocking et al. 2017) for volume assessment was found to be more accurate as an assessment of intra-vascular fluid volume status than CVP, BP, pulse rate, respiratory rate, and peripheral venous pressure (PVP.) During renal dialysis in human subjects, Fast Fourier Transform (FFT) of the peripheral venous pressure signal was calculated in conjunction with known volumes of plasma removal. The Fast Fourier Transform changes correlated significantly ($R^2 = 0.77$) with the amount of fluid volume removed. This technique has been shown to detect a little as 6% blood volume loss in a human model (Sileshi et al. 2015.)

Peripheral IV Analysis (PIVA) of venous wave forms for volume assessment has been used successfully in swine to detect both hemorrhage and volume overload (Hocking et al. 2016)

Peripheral venous waveform analysis (PIVA) would be a useful additional technique to elucidate any volume changes, and specifically, the extent of hypovolemia, caused by applications of HEMI stimuli.

Blood flow index (BFI)

Wu et al. (2018) describe a real-time, non-invasive technology that measures and quantifies regional blood flow in tissues using **“Laser Speckle Contrast Imaging.”**

The authors describe how this technique succeeded in measuring, for up to 30 minutes, the increased blood flow after a nerve block.

This imaging technique is able to quantify objectively and reliably the blood flow changes after a vasodilatory intervention which in our case would be a series of HEMI stimuli.

Value for HEMI projects: We hypothesize that the increase in blood flow would be caused by, and proportional to, the amount of work performed by the muscle. We demonstrated in our previous HEMI-4 report (Murray 2020) that deleterious effects correlate with work. Work leads to increased blood flow. Therefore, this Laser Speckling technique could be used to differentiate between HEMI duty cycles

due to the increase in work caused by the work imposed by the MEMI stimuli, and used as a measure of deleterious effects.

Furthermore, this technique could be used to follow the recovery of the muscles as evidenced by the rate of decrease in blood flow as the muscles recover (due to building up the oxygen and energy stores.)

Several newer cardio-vascular monitors were found in the literature search, as well as expanded applications for existing monitors. With the advent of affordable and readily availability of advanced signal processing, the monitors are being combined, thereby expanding the diagnostic information derived.

Some of these newer technologies have been tested and validated in swine: Maisch et al. (2011), Oliveira et al. (2009)

The monitors described here focus on “volume responsiveness” i.e., they can identify and quantify hypovolemia which is presumed, but not yet proven, to be a major contributing factor to the marked decrease in BP when the HEMI stimuli are discontinued (Figure 24)

Further value could be obtained from the newer monitors by accurately noting the volume of infused fluid necessary to reverse and treat the “volume responsiveness” by administering IV fluids.

The effect of respiration on the cardio-vascular system (BP, heart rate, pre-load, after load, etc.) has been know for many years. With spontaneous breathing, the changes are called “Pulsus Paradoxus.” With positive pressure breathing, the changes are called “Valsalva maneuver.” The relevance to the HEMI project is that these changes can be used to differentiate between a volume deficit (“Volume Responsiveness”) and cardiac failure.

Several different terms have been coined to describe the elements of the so-called “heart lung interactions.” Pinsky (2005) wrote an excellent review article outlining the history and terminology at that stage.

- The “Systolic Pressure Variation” (SPV) is defined as the difference between the highest and lowest systolic blood pressure (SBP) values during one positive pressure breath.
- The “pulse pressure” is the difference between the systolic and diastolic pressures. The “Pulse Pressure Variation” (PPV) is defined as the difference between the largest and smallest pulse pressures during a positive pressure breath. The variation is also evident in the plethysmography (SPO2) monitor. (Figure 27)

Pinsky et al. (1985) argued that it is necessary to measure both the magnitude of PPV and SVV, as well as their its phase relationship to inspiration. If pulse pressure decreases with inspiration, one could presume a preload-responsive pattern, whereas if the pulse pressure increases with inspiration, interdependence is indicated, thereby one may presume heart failure or cor pulmonale.

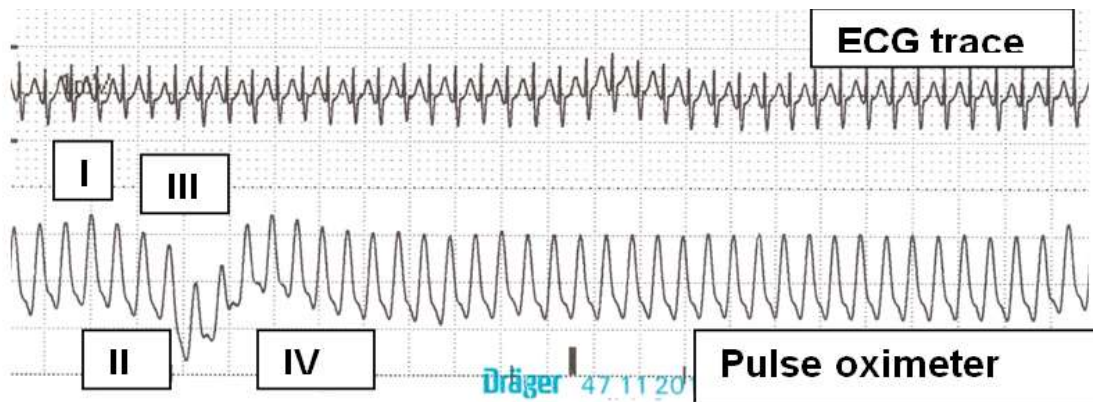
Perel et al. (1987) showed that the Systolic Pressure Variation (SPV) increased with graded blood loss in anesthetized dogs, and the SPV decreased with re-infusion of blood. Maguire et al (2011) published a large clinical study in humans confirming the relationship.

The heart-lung interactions are also present in swine models:

- Maisch et al. [2011] demonstrated in a swine model that electric impedance tomography changes of the chest reflected PPV changes
- Oliveira et al. [2009] also demonstrated that cyclic changes in intra-thoracic pressure (with positive pressure ventilation) caused changes PPV in a swine model.

Pinsky et al. (2012) combined the PPV and SVV to calculate a ratio which defined the dynamic central arterial compliance and varied with vasomotor tone. This combined ratio was found to be able to identify the need for vasopressor therapy in hypotensive patients, thereby differentiating the need therapy from IV fluids.

Figure 27: Effect of a Single Positive Pressure Breath on the Pulse Oximeter



The figure shows an ECG trace in the upper panel and a pulse oximeter trace in the bottom panel.

- *I indicates the positive pressure breath leading to an increase in the amplitude of the pulse plethysmograph.*
- *II indicates the decrease in the amplitude due to the decrease in venous return*
- *III indicated the end of the positive pressure breath causing a fall in amplitude, as the pulmonary venous return to the left ventricle decrease*
- *IV indicates a return to the baseline state*

For more details, see text.

Further developments in invasive and non-invasive monitoring systems that would be able to illuminate the cardio-vascular changes caused by applications of HEMI stimuli, include the following.

Invasive technologies:

- DeCastro et al. [2006] used the arterial pulse contour to assess stroke volume variation (SVV.) They compared the stroke volume and stroke volume respiratory variation measured by axillary

arterial pulse-contour and found satisfactory correlation with measurements by aortic Doppler echocardiography

- Lee et al (2010) showed that the arterial pulse pressure power spectrum, as calculated by Fourier analysis, could be used to predict volume responsiveness in patients in shock.
- It would be useful to consider placing a Millar catheter in the central aorta which should be the least affected by 19 Hz limb movements. The frequency response of a Millar catheter would be sufficient collect data suitable for signal processing, filtering and calculating the original arterial waveform.

Non-invasive technologies:

- Finger plethysmography was shown by Maguire et al (2011) to accurately reflect PPV in a sample of more than 2,000 patients. (Figure 27)
- Non-invasive aortic root tonometry, using ultra-sound, was shown by Dufour et al (2011) to accurately calculate PPV in ICU patients.
- Maisch et al. (2011) used thoracic impedance for estimates of intra-thoracic blood volume and found that the method mirrored PPV changes
Reisner et al. (2011) used a lower body negative pressure protocol to “unmask” hypovolemia. This could be a useful method for the HEMI project in that it does not negate further studies of hypovolemia as would infusion of IV fluids
- Sipkens et al. (2011) *used a noninvasive finger pressure blood pressure monitoring device (Nexfin, Leiden, Netherlands) to demonstrate an excellent beat-to-beat fidelity when compared with intra-arterial blood pressure measurements.*

The non-invasive technologies have been shown to be accurate in humans, but might need modifications and adjustments for use in swine

In addition to collecting a full set of raw A-D derived digital data, it might be useful to consider adding hardware filters during data collection for initial filtering and smoothing. Such an initial hardware derived data set would make it easier for a human to discern clinically relevant events, and therefore, know where to focus efforts for analysis

Contraction Induced Vaso-dilation

In terms of understanding the CVS changes imposed by HEMI stimulation, there are still important physiological questions to answer which include:

- The cause of the BP increases during HEMI induced tetanic contractions
- The amount of perfusion (decrease) in muscles during HEMI induced contractions

The possible causes of the increase in BP include:

- Heart-lung interactions as exhibited by the Valsalva maneuver where the intra-thoracic pressure increases the measured arterial pressure outside the chest. In this scenario, the stroke volume (SV) and the cardiac output (CO) are **INcreased**.
- Increased systemic vascular resistance (SVR) due to the muscular contractions may lead to increased arterial pressure. In this case, the stroke volume (SV) and cardiac output (CO) are **DEcreased**.

Naik et al (1999) measured arterial pressure in dogs, as well as the hind limb blood flow (ultra-sound). They showed that

- i. the ***blood flow decreases to almost zero during tetanic stimulation of sciatic nerve of the hind limb***– 20 Hz for 1 sec. The blood pressure demonstrates only a minor increase during this period, as only a single limb is being stimulated. This supports the concept that increased peripheral resistance does contribute to the increase in BP.
- ii. After a tetanic induced contraction, venous dilation occurred in the relevant limb. This would decrease the venous return and contribute to the post-HEMI hypotension.

Monitors using ultra-sound to measure limb blood flow DURING a period of HEMI stimulation could be considered in a future study.

Nociception monitor

Meijer et al. (2020) describe the Nociception Level (NOL) monitor (Medasense Biometrics Ltd, Ramat Gan, Israel) which is able to reliably detect and quantify mild through intense noxious stimuli during painful events including surgery under anesthesia. The monitor combines multiple physiologic and autonomic signals (finger photo-plethysmogram “SPO2”, skin conductance, heart rate, heart rate variability and their time derivatives in a single monitor producing the Nociception Level (NOL) on a scale of zero to 100. While the human version might not be directly applicable to swine, it would be possible to adapt the device for use in animals.

Value for HEMI projects: The device would be useful during the periods of HEMI stimulation, as well as following the rate of decrease in the nociception levels during recovery.

A general principle demonstrated by the Naik et al. (1999) and the Meijer et al. (2020) studies, is to combine monitors from different physiological systems to generate a higher level of insight and understanding of the relevant physiology. The use of in-phase digitized monitors would make such interpretation feasible.

H. RESPIRATORY WAVEFORMS

Respiratory effects of HEMI stimuli can be explored using the existing HEMI data sets. Due to the 3-D nature of the eight accelerometers used, differentiation of inhalation and exhalation should be feasible. Some investigators found inhalation occurred with the onset of HEMI stimuli, while others found exhalation. We developed a new working hypothesis to be explored: the probe positions would have a major influence on the phases of respiration. For instance, stimulation of the phrenic nerve would cause inhalation via the diaphragm, while stimulation primarily of the internal intercostal muscles would cause exhalation.

In-phase signal processing of the existing data set would also be able to elucidate the relationship between the duration of the interruptions and the size of the breath (tidal volume of both inhalation and exhalation) as a measure of a “Good Breath.”

The available equipment for measurement of the VO_2 , VCO_2 leading to the calculation of the respiratory quotient (RQ) and anaerobic point is not able to use and analyze the highly irregular breath sizes due to the HEMI contractions. It would be necessary to separately measure the respiratory flow rates and gas concentrations, and apply real time signal processing to calculate the RQ and anaerobic point. Simultaneously, a “mixing box” could be used to calculate the above parameters.

Several effects of the breathing systems (circle system t-piece, etc.) were found on the respiratory parameters when the animals were under anesthesia. The tidal volumes, the respiratory rates, and the I:E (inspiratory to expiratory) ratios seemed to be affected during the baseline and resting periods. Signal processing involving the ETO_2 , etCO_2 , tidal volumes, etc. would be necessary to further elucidate these interactions.

The interactions between respiration and the blood pressure (Valsalva phenomenon, pulse pressure variation, etc.) as well as the effect of respiration on the T-waves are discussed in separate sections.

I. BIOCHEMICAL PARAMETERS

Finsterer et al. (2012) has published a series of articles on biochemical markers which were found to be useful in supra-maximal exercise in humans. However, most of these biomarkers have limits of measurement, which are typically exceeded due to HEMI stimulation. Hence the search for other biomarkers of deleterious effects that would be able to differentiate between duty cycles.

A literature search identified the concept of micro-vesicles. The publications show that strenuous exercise may lead to the release of tiny micro-vesicles.

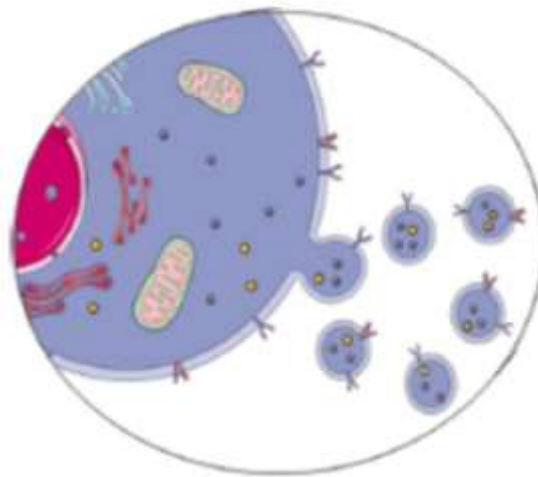
Micro-vesicles are sections of cellular membranes that are formed by cells under stress, including supra-maximal exercise. (Wilhelm et al. 2018) These micro-vesicles may contain DNA or RNA whereby the cells of origin can be determined.

Such micro-vesicles may be valuable to the HEMI effort for measurement of the level of deleterious effects - therefore, being able to detect differences between duty cycles.

There are several synonyms for micro-vesicles. **Extracellular vesicles (EVs)**, is a collective term covering various **subtypes of cell-released, membranous structures, called exosomes, micro-vesicles, microparticles, ectosomes, oncosomes, apoptotic bodies, and many other names.**

Microparticles can be released as extracellular micro-vesicles from red blood cells, white blood cells, platelets, muscle, or endothelial cells. These biological microparticles are thought to be shed from the plasma membrane of the cell as lipid bilayer-bound entities that are typically larger than 100 nm in diameter.

Figure 28: Micro-vesicles breaking off from the cell wall of a stressed cell



The figure shows the development (exocytosis) of micro-vesicles (Wilhem et al. 2018)

Because extracellular micro-vesicles retain the signature membrane protein composition of the parent cell, microparticles and other extracellular micro-vesicles may carry useful information including

biomarkers of disease and deleterious effects of noxious stimuli. They can be detected and characterized by methods such as flow cytometry or dynamic light scattering. Such detection methods might be sufficiently sensitive to differentiate between duty cycles.

As mentioned, most of the biochemical parameters are not sufficiently sensitive to differentiate between duty cycles. For instance, there is quite a large variability in the increase in Hct (hematocrit) after HEMI exposures. It might be useful to analyze the purported **cause of the increase in the Hct** – fluid leakage into the muscle cells during and after supra-maximal tetanic stimulation.

We had noticed that the hindquarters of the swine seemed to clinically enlarge within a few minutes after the HEMI stimulus.

There seemed to be two (or more) time courses for the development of the swelling:

- i. Swelling was evident within a few minutes after the HEMI exposure.
In one instance, the hind limb circumference increased from (752 mm, 29.6) inches by 25 mm (1 inch) after the HEMI stimulus to 778 mm (30.6 inches.)
- ii. The swelling took almost an hour to develop.
In another instance, the pre-HEMI exposure circumference was 832 mm (32.7 inches) It did not increase measurable immediately after the HEMI exposure. However, the circumference had increased by 12.7 mm (0.5 inches) after 53 min.

It might be useful to use a more sensitive measure of swelling than the circumference of a limb. Ultra-sound over specific muscles would give a sensitivity of 1- 2 mm.

As noted in a previous section, the natural frequency of the limbs might increase due to swelling.

FINDINGS AND RECOMMENDATIONS

In this study, we explored the needs of and gaps in the data required for development of the simulation model of HEMI stimuli in a human entity.

From a high-level perspective, the overall study accomplished the following major objectives:

- We analyzed and determined the extent to which the existing HEMI 4 data set would be able to fulfill the needs of the mathematical model
- We performed a literature study to search for new methodologies and monitors to support the creation of the simulation model

The following are the findings of the sub-tasks, and the recommendations based on the findings:

- The existing data set enabled the identification and calculation of examples of the **electro-mechanical delay** (EMD.) These examples are necessary for insertion into the simulation model. This involved characterizing limb movements caused by HEMI stimuli to be able to differentiate from passive movements (e.g., gravity) and oscillations after HEMI induced movements. We recommend that the natural frequency of the oscillations be studied as an indicator of deleterious effects, as natural frequency is postulated to increase as edema forms in muscles performing HEMI imposed supra-maximal work, resulting in such muscles becoming “stiffer.”
- Based on the literature search, we recommend that the existing HEMI data set be used for the calculation of the characteristics of **active contraction** of muscle. The characteristics include: duration of contraction and peak rate of active contraction which will be useful for the modeling project.
We also recommend that the existing HEMI data set be used to show that fatigued muscles have a **slower rate of contraction**, and therefore a **longer duration to travel** the same distance of contraction. Both of these newly identified characteristics could be used as indicators of deleterious effects.
- **Relaxation of muscle** is an active process requiring energy (ATP) to re-absorb ionic Calcium from the cytosol into the endoplasmic reticulum. We recommend that characteristics of relaxation (e.g., duration and peak rate of relaxation) be calculated from the existing data set. The literature search provided some examples of characteristics and measurements that could be used as a starting point. We recommend that alterations in such characteristics of relaxation (in the existing data set) be studied as potential indicators of deleterious effects.
- **The onset of tetanization** is a gradual process, not an event with an immediate onset. The existing HEMI data set provides a large number of instances of the onset of tetanization. We recommend that the data be used to calculate the rate of onset of tetanization for incorporation in the modeling project.
The literature search provided a variety of methods to quantify the rate of onset as well as the degree of tetanization including the concept of “Fusion Index.”
- The effects of the **interruption of HEMI stimuli** are evident in the HEMI data sets. These effects include an increased contraction strength due to post tetanic facilitation (PTF). The literature

also provided methods of quantifying the increase in contraction strength after tetanic stimulations, called Post Tetanic Facilitation (PTC) or Post Tetanic Potentiation (PTP). The quantification method has been published under the term “Boost and Sag.” We recommend that this methodology be applied to the HEMI data sets.

We recommend that the duration of the interruption should be analyzed to determine the extent to which the duration enables (or even “forces”) a respiratory movement, a so-called “Good Breath.”

- The changes in the **cardio-vascular system (CVS)** caused by HEMI stimuli are critical to understanding the increased blood pressure (BP) at the onset of the HEMI stimuli, as well as the marked decrease in BP after discontinuation thereof.
An important series of articles found in the literature discussed in-depth the “Heart-Lung Interactions” based on the concepts described under the “Valsalva Maneuver.” We recommend that newer monitoring techniques such as cardiac ultra-sound, be explored for use in a future human surrogate study, to enable a more in-depth understanding of the differences due to hypovolemia, in contrast to “stunning” of the heart.
Based on improved signal processing, we recommend that several newer monitoring concepts be explored for implementation in a potential future study, including the “Blood Flow Index (BFI)”, “Peripheral Intra-Venous Analysis (PIVA) and “Systolic-Dichrotic Notch Pressure Differential.”
- **Respiratory effects** of HEMI stimuli can be explored using the existing HEMI data sets. Due to the 3-D nature of the eight accelerometers used, differentiation of inhalation and exhalation should be feasible. Some investigators found inhalation occurred with the onset of HEMI stimuli, while others found exhalation. We recommend that the existing data set be used to explore whether the probe positions have a major influence on initiating a specific phase of respiration.
- The literature describes experimental **biochemical parameters** that would be useful to indicate and quantify deleterious effects. For instance, **micro-vesicles** are sections of cellular membranes that are formed by cells under stress, including supra-maximal exercise. These micro-vesicles may contain DNA or RNA whereby the cells of origin can be determined. We recommend that such newer biochemical measurements, including micro-vesicles, be explored as potential measures of deleterious effects after HEMI stimulation.
- The existing HEMI data sets contain a large number of **duty cycles** with varying durations of interruptions, as well as varying durations of total applications. We recommend that these data sets be incorporated for the development of a robust simulation model. Such a model would be useful to examine a large number of combinations and permutations of HEMI frequencies, waveforms, interruptions, total durations, etc. which would not be possible with human surrogates.

The findings and recommendations outlined above are described in more detail in the full report.

We recommend that the HEMI data sets be used to provide quantitative data related to multiple aspects of physics for the modeling project, while further detailed analysis of the physiologic aspects will be able to enhance and highlight understanding of the physiology, which would then be used to feed back into the simulation and modeling.

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APPENDIX

APPENDIX 1: BACKGROUND AND EXPLANATIONS OF HEMI 4

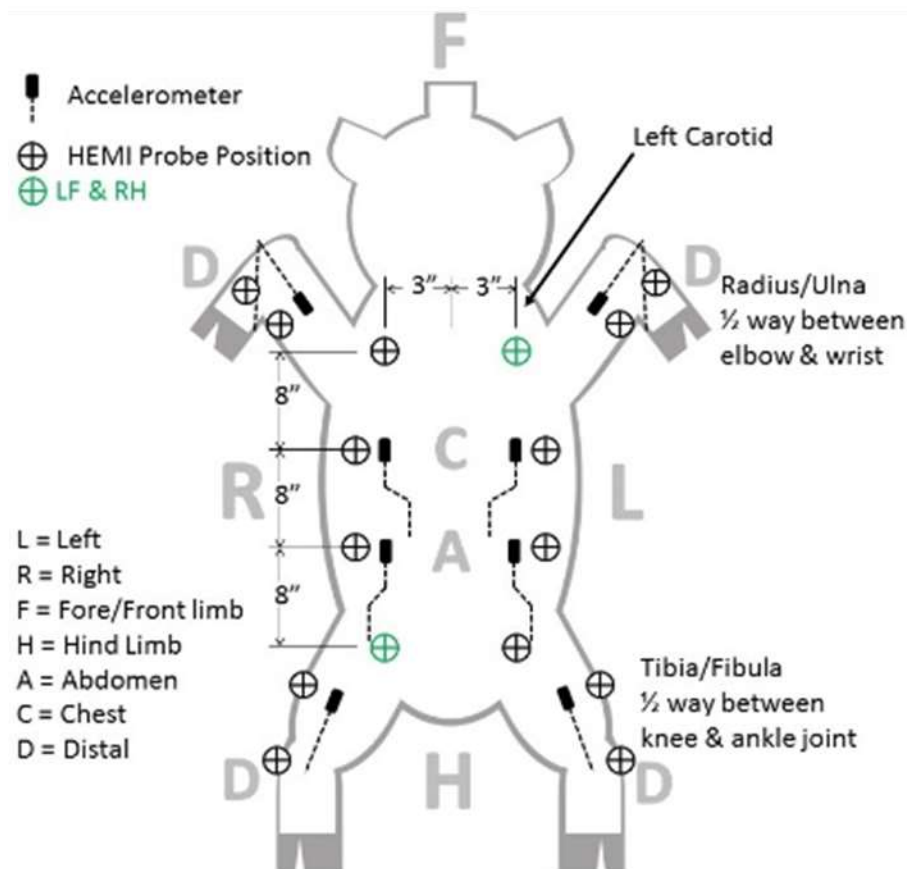
This report builds on the HEMI 4 study. (Murray et al. 2020.) The appendix will start with a brief description of the project and the resulting data set for ease of reference.

The purpose of the HEMI 4 project was to compare various duty cycles and determine if the calculation of Power and Work could differentiate and quantify the deleterious effects of various duty cycles.

The full report is available at: <https://apps.dtic.mil/sti/pdfs/AD1116553.pdf>

As a secondary investigation, data was collected with the HEMI probes placed in different locations.

Figure 29: Accelerometer and HEMI probe placement positions.



The figure shows the varied positions of the HEMI probes. The accelerometers were glued into fixed positions.

The naming conventions for the HEMI 4 exposures are shown in Table 1.

Table 1: Naming convention for the HEMI 4 exposures

- Subject number is the chronological sequence of the animal experiments
- Run number is the sequence of the first three runs of the day
- Full name has the following structure (Aaa bb cccc-dddd eee ff" is the exposure designator):
 - aa is the sequential number of the subject: 1 through 14
 - bb is the number of the exposure for that day;
 - cccc is the duration of the "train" of HEMI stimuli (5,000 msec)
 - dddd is the duration of the interruptions (0, 10, 100, 200, 1,000, or 3,000 msec)
 - eee is the duration of the HEMI exposure (35, 40, 180 or 360 msec)
 - ff is the concentration of the inspired oxygen (FIO₂) (typically 40%)
- Train is the 5 second duration of the HEMI stimulus
- Interruption is the duration of the interruption in msec between the trains
- Total duration is the duration of the series of trains and interruptions
- Probe position is the site of the HEMI probe: LA is left forelimb, RL is right hind limb. These were considered the "standard positions" for the HEMI probes. RA is right forelimb, LL is left hind limb, LC is left chest, RC is right chest, Labd is left abdomen, Rabd is right abdomen
- Train-of-Four (TOF) 4 impulses 500 msec intervals
- Train 5 second trains of 19 Hz with intervals from 100 to 3000 msec for 35 seconds
- Post-tetanic-count (PTC)
 - i. 10 impulses 1 second apart (baseline control for the next stimuli)
 - ii. 5 seconds tetanic stimuli (19 Hz versus 50 Hz)
 - iii. Wait 5 seconds
 - iv. 10 impulses 1 second apart

The HEMI 4 report examined and described the First 3 runs of each animal

The HEMI stimuli were administered using several total number of impulses ("trains") with a variety of inter-train intervals ("interruptions") (Table 2)

Signal processing suggested for twitches as measured by the accelerometers include:

- i. Maximum and Minimum values of the very first "sharp" excursions
- ii. Maximum, minimum and time points of "raw" peaks and valleys (as well as the time points of the zero crossings)
- iii. Curve smoothing of the 3rd and subsequent peaks and valleys
 - Maximum (positive) and minimum (negative) values (and time point) of each of the "smoothed" peaks and nadirs (as well as the time points of the zero crossings)
- iv. - Curve fitting of
 - raw values
 - smoothed values
 (seems to be an exponential type, but it can be anything else – see what "fits" the best)

Table 2: HEMI Run 1, Run 2 and Run 3 per each animal

Subject Number	Run number	Full name	Train (msec)	Interruption (msec)	Total Duration	Probe positions	
A1	01	A1 01 5000-1000 35 40	5000	1000	35	LA	RL
A1	02	A1 02 5000-1000 35 40	5000	1000	35	LA	RL
A1	03	A1 03 5000-200 30 40	5000	200	30	LA	RL
A2	01	A2 01 5000-1000 35 40	5000	1000	35	LA	RL
A2	02	A2 02 5000-1000 35 40	5000	1000	35	LA	RL
A2	03	A2 03 5000-200 30 40	5000	200	30	LA	RL
A3	01	A3 01 5000-200 35 40	5000	200	35	LA	RL
A3	02	A3 02 5000-200 35 40	5000	200	35	LA	RL
A3	03	A3 03 5000-1000 35 40	5000	1000	35	LA	RL
A4	01	A4 01 5000-200 35 40	5000	200	35	LA	RL
A4	02	A4 02 5000-200 35 40	5000	200	35	LA	RL
A4	03	A4 03 5000-1000 35 40	5000	1000	35	LA	RL
A5	01	A5 01 5000-1000 35 40	5000	1000	40	LA	RL
A5	02	A5 02 5000-1000 35 40	5000	1000	40	LA	RL
A5	03	A5 03 5000-3000 35 40	5000	3000	40	LA	RL
A6	01	A6 01 5000-200 35 40	5000	200	40	LA	RL
A6	02	A6 02 5000-200 35 40	5000	200	100	LA	RL
A6	03	A6 03 5000-3000 35 40	5000	3000	40	LA	RL
A7	01	A7 01 5000-1000 35 40	5000	1000	35	LA	RL
A7	02	A7 02 5000-1000 35 40	5000	1000	35	LA	RL
A7	03	A7 03 5000-3000 35 40	5000	3000	35	LA	RL
A8	01	A8 01 5000-200 35 40	5000	200	35	LA	RL
A8	02	A8 02 5000-200 35 40	5000	200	35	LA	RL
A8	03	A8 03 5000-3000 35 40	5000	3000	35	LA	RL
A9	01	A9 01 5000-3000 45 40	5000	3000	45	LA	RL
A9	02	A9 02 5000-3000 45 40	5000	3000	45	LA	RL
A9	03	A9 03 5000-3000 45 40	5000	3000	45	LA	RL
A10	01	A10 01 5000-3000 45 40	5000	3000	45	LA	RL
A10	02	A10 02 5000-3000 45 40	5000	3000	45	LA	RL
A10	03	A10 03 5000-3000 45 40	5000	3000	45	LA	RL
A10	04	A10 04 5000-200 35 40	5000	200	35	LA	RL
A11	01	A11 01 5000-100 35 40	5000	100	35	LA	RL
A11	02	A11 02 5000-100 35 40	5000	100	35	LA	RL
A11	03	A11 03 5000-100 35 40	5000	100	35	LA	RL
A12	01	A12 01 5000-100 35 40	5000	100	35	LA	RL
A12	02	A12 02 5000-100 35 40	5000	100	35	LA	RL
A12	03	A12 03 5000-100 35 40	5000	100	35	LA	RL
A13	01	A13 01 5000-0 35 40	5000	0	35	LA	RL
A13	02	A13 02 5000-0 35 40	5000	0	35	LA	RL
A13	03	A13 03 5000-0 35 40	5000	0	35	LA	RL
A14	01	A14 01 5000-0 35 40	5000	0	35	LA	RL
A14	02	A14 02 5000-0 35 40	5000	0	35	LA	RL
A14	03	A14 03 5000-0 35 40	5000	0	35	LA	RL

For an explanation of the abbreviations and naming conventions, see Table 1

Table 3: Variations of durations and number of HEMI stimuli – data sets available

Subject number	Run number	Full name	Number stimuli	Total Duration	Probe positions	
A8	18a	A8 18a Single Twitch - Pre	ST-1	10	LA	RL
A8	18b	A8 18b Single Twitch - Pre	ST-1	10	LA	RL
A8	18c	A8 18c Single Twitch-Post	ST-1	10	LA	RL
A8	18d	A8 18d Single Twitch - Post	ST-1	10	LA	RL
A8	18e	A8 18e Single Twitch - Pre	ST-1	10	LA	RL
A8	18f	A8 18f Single Twitch - Pre	ST-1	10	LA	RL
A8	18g	A8 18g Single Twitch-Post	ST-1	10	LA	RL
A8	18h	A8 18h Single Twitch - Post	ST-1	10	LA	RL
A8	18i	A8 18i Single Twitch - Pre	ST-1	10	LA	RL
A8	18j	A8 18j Single Twitch - Pre	ST-1	10	LA	RL
A8	18k	A8 18g Single Twitch-Post	ST-1	10	LA	RL
A8	18l	A8 18h Single Twitch - Post	ST-1	10	LA	RL
A8	18m	A8 18i Single Twitch - Pre	ST-1	10	LA	RL
A8	18n	A8 18j Single Twitch - Pre	ST-1	10	LA	RL
A8	18o	A8 18o Single Twitch-Post	ST-1	10	LA	RL
A8	18p	A8 18p Single Twitch - Post	ST-1	10	LA	RL
A9	18a	A9 18a Single Twitch	ST-1	10	LA	RL
A9	18b	A9 18 b Single Twitch	ST-1	10	LA	RL
A10	18a	A10 18a Single Twitch - Pre	ST-1	10	LA	RL
A10	18b	A10 18b Single Twitch - Post	ST-1	10	LA	RL
A10	18c	A10 18c Single Twitch - Pre	ST-1	10	LA	RL
A11	18a	A11 18a Single Twitch	ST-1	10	LA	RL
A11	18b	A11 18b Single Twitch	ST-1	10	LA	RL
A11	18b1	A11 18b1 Single Twitch	ST-1	10	LA	RA
A11	18c	A11 18c Single Twitch	ST-1	10	LL	RL
A11	18d	A11 18d Single Twitch - Post	ST-1	10	LA	RL
A11	18e	A11 18e Single Twitch - Pre	ST-1	10	LA	RL
A11	18f	A11 18f Single Twitch - Pre	ST-1	10	LA	RA
A11	18g	A11 18g Single Twitch - Pre	ST-1	10	LL	RL
A11	18h	A11 18h Single Twitch - Post	ST-1	10	LA	RL
A12	18a	A12 18a Single Twitch - Pre	ST-1	10	LA	RL
A12	18b	A12 18b Single Twitch - Pre	ST-1	10	LA	LL
A12	18c	A12 18c Single Twitch - Pre	ST-1	10	RA	RL
A12	18d	A12 18d Single Twitch - Post	ST-1	10	LA	RL
A12	18f	A12 18f Single Twitch - Pre	ST-1	10	LA	LL
A12	18g	A12 18g Single Twitch - Pre	ST-1	10	RA	RL

ST-1 – Single Twitch, Pre- and Post – before and after a 35 sec run. (see Table 1 for abbreviations)

Table 4: Variations of HEMI probe positions – data sets available

Subject number	Run Number	Full name	Train (msec)	Interruption (msec)	Total Duration	Probe positions	
A2	12	A2 12 2500-1000 20 4	2500	1000	20	LL	RL
A2	13	A2 13 2500-1000 20 4	2500	1000	20	LA	RA
A3	4a	A3 4a 5000-1000 35 4	5000	1000	35	LA	LL
A3	4b	A3 4b 5000-1000 35 4	5000	1000	35	RA	RL
A3	5	A3 5 5000-1000 35 40	5000	1000	35	LA	RA
A3	6	A3 6 5000-1000 35 40	5000	1000	35	LL	RL
A4	04a	A4a 03 5000-1000 35	5000	1000	35	LA	LL
A4	04b	A4b 03 5000-1000 35	5000	1000	35	RA	RL
A4	05	A5 03 5000-1000 35 4	5000	1000	35	LA	RA
A4	06	A6 03 5000-1000 35 4	5000	1000	35	LL	RL
A5	5a	A5 05a 5000-1000 35	5000	1000	40	LA	RA
A5	5b	A5 05b 5000-1000 35	5000	1000	40	Labd	Rabd
A5	5c	A5 005c 5000-1000 35	5000	1000	40	LC	RC
A6	5a	A6 5a 5000-200 35 40	5000	200	35	LC	RC
A6	5b	A6 5b 5000-200 35 40	5000	200	35	Labd	Rabd
A6	06	A6 6 5000-200 35 40	5000	200	35	LA	RA
A6	07	A6 7 5000-200 35 40	5000	200	35	LL	RL
A7	5a	A7 5a 5000-1000 35 4	5000	1000	35	LA	LC
A7	5b	A7 5b 5000-1000 35 4	5000	1000	35	RA	RC
A7	5c	A7 5c 5000-1000 35 4	5000	1000	35	RC	Rabd
A7	5d	A7 5d 5000-1000 35 4	5000	1000	35	LC	Labd
A7	5e	A7 5e 5000-1000 35 4	5000	1000	35	Labd	LL
A7	5f	A7 5f 5000-1000 35 4	5000	1000	35	Rabd	RL

The highlighted are (Subject A7) describes single animal “walk-down” which consists of repeated repositioning of the HEMI probes in a single animal in the positions and sequence as described in the “Probe Positions” columns. The sequence is as listed in the “Run Number” column.

For an explanation of the abbreviations and naming conventions, see Table 1

Appendix 1: Background and explanations of HEMI 4 (continued)

The accelerometer signals were used to calculate power and work.

The next section provides examples of the difference between work and power as an introductory step to the following section on the actual calculations.

A car drives at a speed of 60 mph - this is the **rate** of travel

After 3 hours, the car has travelled 180 miles - this is the **total distance** of travel.

Similarly:

A given light bulb uses 60 Watts – this is the **rate** of energy consumption and is equivalent to 60 Joules per second.

After 3 seconds, the light bulb has used 180 Joules – this is the **total amount** of energy used.

Phrased differently:

The **POWER** of the light bulb is 60 **Joules per second**

The **TOTAL WORK** performed by the light bulb after 3 seconds is 180 **Joules**

Work and Power calculations. It was desired to determine the amount of energy generated by muscle movements caused by each HEMI pulse. It is assumed that the work was performed on either the sensor for the chest and abdomen accelerometers, or the combination of the sensor and mass for the legs' and arms' accelerometers. The following method can also be applied for any arbitrary time interval.

In physics, work can be calculated as:

$$W = Fd \quad (\text{asdf1})$$

where W is work in Joules, F is force in Newtons, and d is displacement in meters.

Force can be calculated as:

$$F = ma \quad (\text{asdf2})$$

where F is force in Newtons, m is mass in kilograms, and a is acceleration in meters per second squared or Newton per kilogram.

Combining equation (asdf1) and (asdf2), we get:

$$W = mad \quad (\text{asdf3})$$

Finding the rate of both sides of equation (asdf3) we find:

$$\frac{W}{t} = ma \frac{d}{t} \quad (\text{asdf4})$$

Where t is in seconds.

Reducing equation (asdf4) we find

$$P = mav \quad (\text{asdf5})$$

since power is the rate of energy used, or rate of work that is done.

The equation (asdf5) is convenient, since only the velocity is unknown to determine the power, and eventually the work. The velocity can be determined by integrating the acceleration.

Work and Power calculations in practice. The amount of Energy for each time segment (Figure 30) was calculated. A time segment was defined as the time between HEMI pulses, or about 1/18Hz. Calculation of velocity was done over a range of the desired time interval plus 4 samples before and 4 samples after the desired range. These points were needed for initial and final conditions of the velocity calculations, but were truncated for the results when finding the average power for a time period.

Mass utilized either 5 pounds plus 50 grams or 50 grams depending if an accelerometer was mounted on a limb or the body. 5 pounds was the nominal mass of the mass attached to the limbs and 50 grams is the nominal mass of the accelerometer.

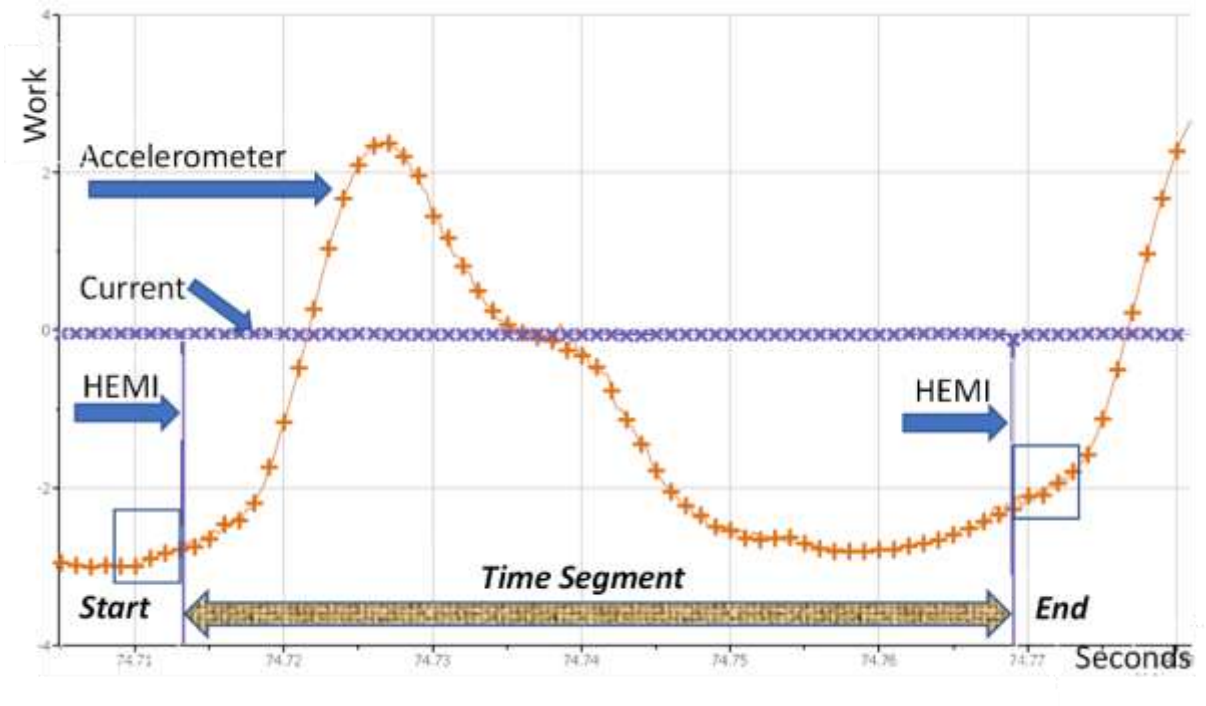
The measured acceleration is comprised of two parts: the DC “gravitational” component, and the AC “movement” component. The DC component of the acceleration was removed by subtracting the average acceleration during each segment. The remaining AC acceleration was multiplied by the mass resulting in the Force. The AC acceleration was integrated using Simpson’s rule to calculate the velocity. Simpson’s rule approximates the input as a quadratic curve, opposed to trapezoidal, approximating as a piece-wise linear curve.

Average power between HEMI pulses is calculated by calculating the work that occurred during each sample period. The energy per data point was calculated by pointwise multiplying the absolute value of the force, the absolute value of the velocity, and the sample period together. The 4 initial and 4 final condition samples were removed and the average of the sample powers was calculated.

A file was then generated that lists the HEMI pulse train number, pulse number within the train, start time, stop time, and power per acceleration per pulse, and the sum of power per 3D accelerometer. A summary file was also generated of work performed on a 3D axis accelerometer for the first 10 pulses, the 10th to last pulse, and for all pulses in a given train.

While the data is presented as power in the Results, the relationships and graphical outputs are essentially the same. Work is a much more common term and better understood, so for clarity, this report will use the term “work” rather than the term “power.”

Figure 30: Time Segment Definition: The time period between two HEMI Stimuli



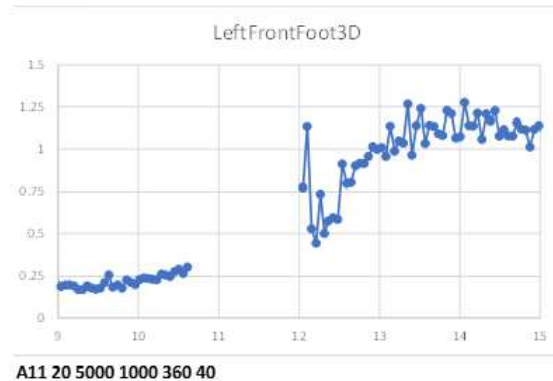
The figure shows an explanation of a Time Segment.

- *“Start” represents the beginning of a given HEMI stimulus which defines the beginning of the Time Segment.*
- *“End” represents the next HEMI stimulus which marks the end of the Time Segment.*
- *“Time Segment” represents the period between two HEMI stimuli.*
- *“HEMI” represents a HEMI stimulus.*
- *“Current” (“x”) represents the quiescent period between HEMI stimuli.*
- *“Accelerometer” (“+”) represents the data collected from the accelerometer where positive movement represents extension of the limb due to muscle contraction caused by the HEMI stimulus.*
- *Negative movement represents relaxation of the limb.*
- *The square boxes identify the 4 samples before and after the time segment used as initial and final conditions for calculating the velocity.*

APPENDIX 2: POST-TETANIC FACILITATION (PTF) AS AN INDICATION OF DELETERIOUS EFFECTS

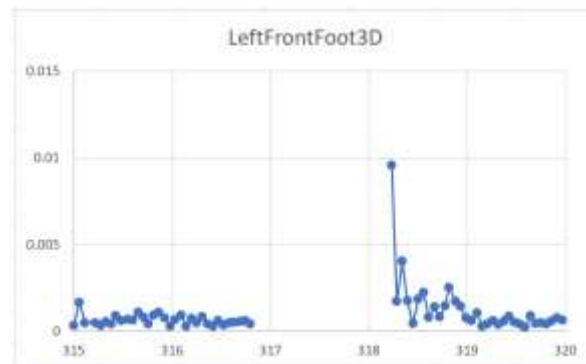
We postulated that the phenomenon of post-tetanic facilitation (PTF) could be used as an indicator of deleterious effects. Here we show an example of the decrease of the PTF as muscle fatigue sets in.

Figure 31: Post-tetanic Facilitation (PTF) – prior to marked fatigue



The figure shows Post-tetanic Facilitation (PTF) – prior to marked fatigue. The figure shows the end of a train of HEMI stimuli at 10.7 seconds, with amount of work as 0.26 units. The next train starts at the 12 second mark, with the amount of work being markedly higher (0.75 units) than at the end of the previous train. This demonstrates the phenomenon of post-tetanic facilitation (PTF)

Figure 32: Post-tetanic Facilitation (PTF) – After marked fatigue has developed



The figure shows Post-tetanic Facilitation (PTF) – After marked fatigue has developed. The figure shows the same animal and the same run as in the previous figure. The time period shown in this figure is shortly after 300 seconds (5 minutes) of HEMI stimulation. Note the markedly decreased amount of work generated by each individual HEMI pulse. Also note the markedly attenuated post-tetanic facilitation after the interruption (at 318.2 seconds). Note that the scale in this figure is one-hundredth of that of the previous figure, emphasizing the fatigue that sets in with HEMI stimulation.

APPENDIX 3: EFFECTS OF VARIATIONS IN THE POSITION OF HEMI PROBES

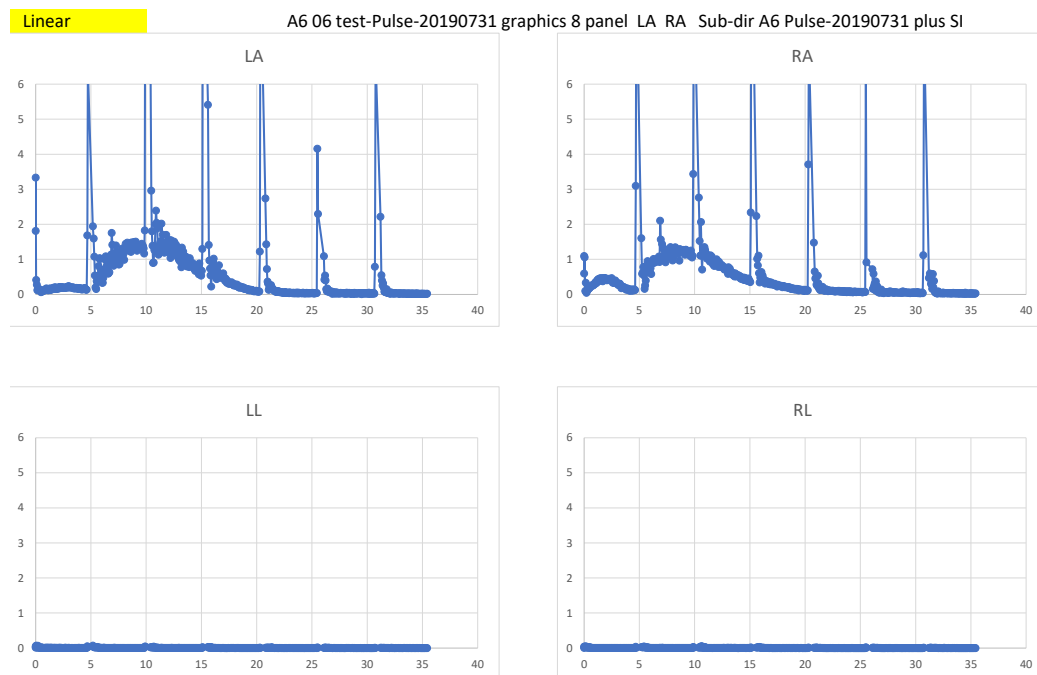
The position of the probes on primarily the forelimbs or primarily the hind limbs have a major influence on the total work imposed on the subject, and hence on the development of deleterious effects.

We measured the effect of placing the probes bilaterally high on the torso (“body”) compared to bilaterally low on the torso. Specifically, in the terms of this project, we placed the probes in different configurations for separate, consecutive runs:

- LA and RA = left forelimb and right forelimb
- LL and RL = Left hind limb and right hind limb

In both instances, we collected the standard data set from all 8 accelerometers and vital signs for 35 seconds. We converted the accelerometer data to work in a 3-D space. In this section, we first present the data in a linear format – this shows that the limbs (distal to the HEMI probe insertion points) have “almost zero” movement and by implication, will be generating zero to minimal work and minimal deleterious effects (Figure 41). We also present the data in a logarithmic format (Figure 42), which indicates that there is definitely some effect of the HEMI stimulation on the musculature of the limbs distal to the HEMI probes. Last, we present the data as the total work as calculated for each of the trains (5 second duration) for each of the limbs, over each train of 5 seconds (Figure 43).

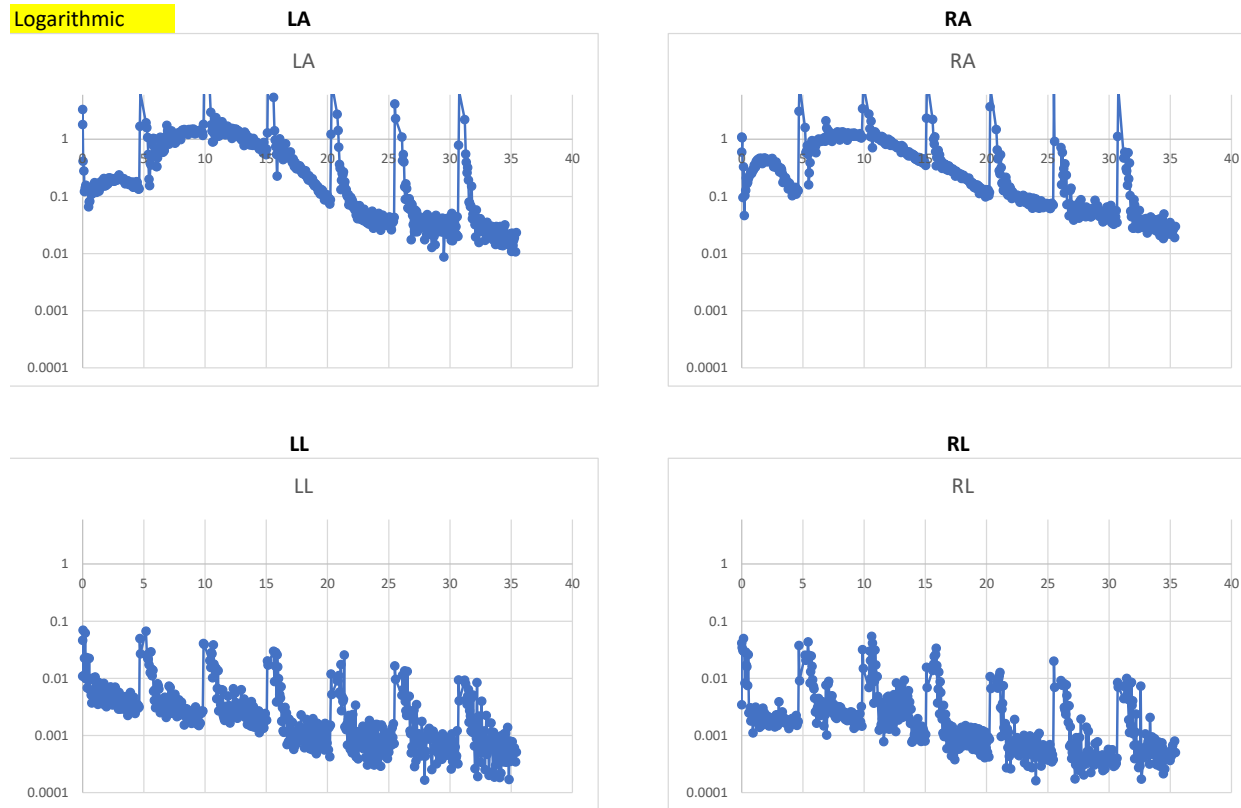
Figure 33: Linear data – probes placed on the forelimbs (LA and RA).



The figure shows the probes are placed symmetrically close to the upper limbs (LA and RA). The data is presented in a linear format. The work generated by the upper limbs are clearly visualized by the objective measures. There is relatively zero to minimal work being performed by the hind limbs (legs).

The next illustration, Figure 34 shows the same data as presented in the previous Figure 33, presented here on a logarithmic scale, specifically to illustrate the fact that there is definitely an effect of the HEMI stimulation on the lower limbs, which is not clearly visualized on the graphic using a linear scale.

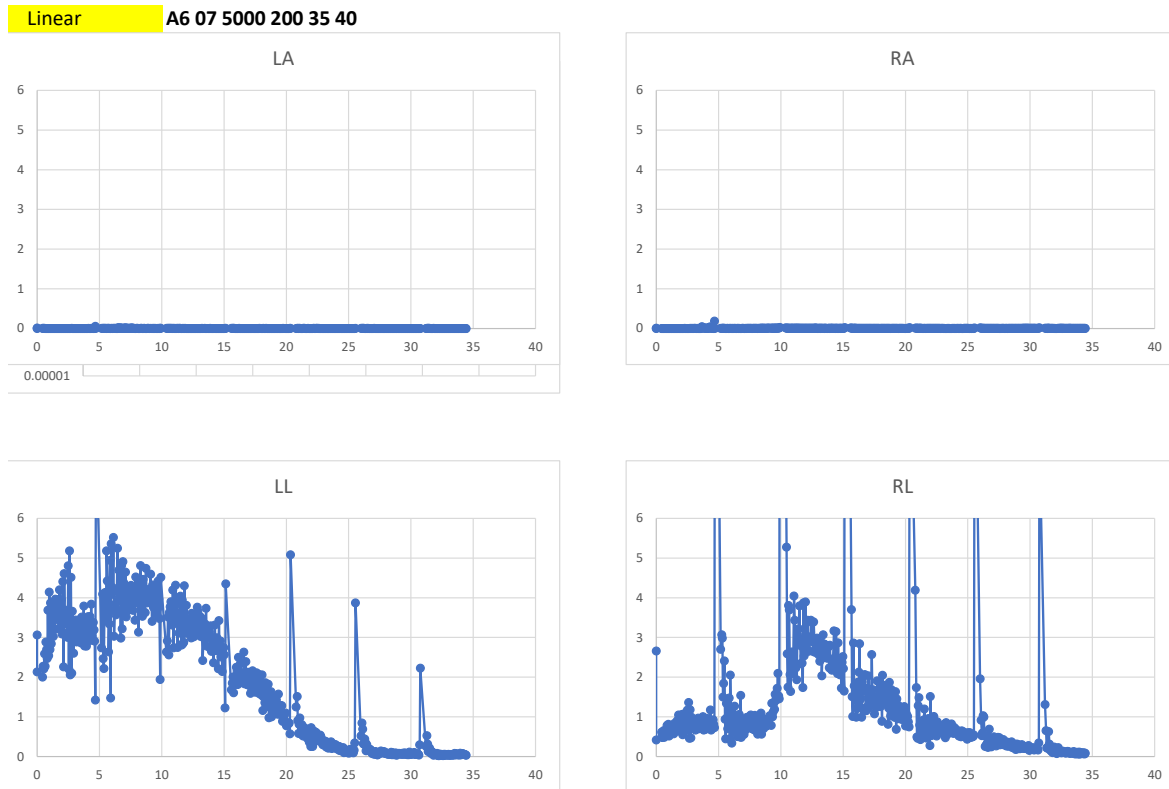
Figure 34: Logarithmic data - probes placed on the forelimbs (LA and RA).



The figure shows the same data as in Figure 33, but on a logarithmic scale as in the prior figure. The work generated by both the upper and the lower limbs are clearly visualized by the objective measures. The work in the upper limbs can be seen to be mainly between 1 and 2 units, while the work generated by the lower limbs, (further away from the HEMI probes), vary from 0.1 to 0.001 units. For further details, see prior figure.

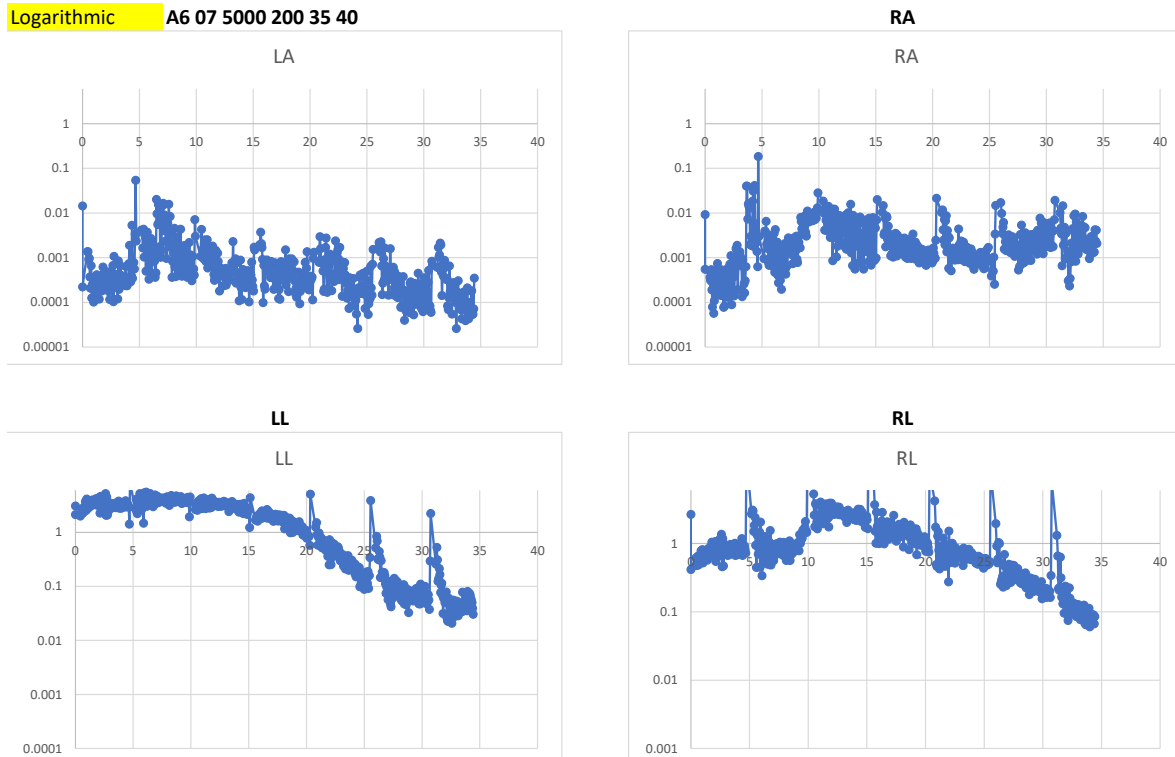
We now present the converse position of insertion of the HEMI probes, i.e., in close proximity to the hind limbs.

Figure 35: Linear data – probes placed on the Hind Limbs (LL and RL)



The figure shows the probes are placed close to the hind limbs (LL and RL). The data is presented in a linear format. The work generated by the lower limbs are clearly visualized by the objective measures. There is relatively zero to minimal work being performed by the upper limbs (LA and RA).

The next illustration, Figure 45, shows the same data as presented in the previous figure, presented here on a logarithmic scale, specifically to illustrate the fact that there is definitely an effect on the upper limbs, which is not clearly visualized on the linear scale.

Figure 36: Logarithmic data – probes placed near the hind limbs (LL and RL).

The figure shows the same data as in the prior Figure 35 except on a logarithmic scale. The work generated by both the upper and the lower limbs are clearly visualized by the objective measures on a logarithmic scale. The work in the lower limbs can be seen to be mainly between 1 and 2 units, while the work generated by the upper limbs (further away from the HEMI probes), vary from 0.1 to 0.001 units. For further details, see prior figure.

APPENDIX 4: CALCULATION OF THE ELECTRO-MECHANICAL DELAY/LATENCY

Questions:

- i. Which part of the HEMI stimulus will be used as "zero" - see oscillation around HEMI impulse
 - start, peak, end, duration, how many A-D samples for quality control?
- ii. when/where does the contraction start – Define/Describe beginning of the contraction
 - as a percentage (%) of the total movement
 - greater than x% of the baseline A-D conversion variation
 - max dP/dt of curve
- iii. define limits of electro-mechanical delay – statistical (2 STD), range/duration (fatigued)

Electro-mechanical Delay/Latency : Details of Questions and Tasks

Questions to answer:

- i. Given the distance from the probe to the accelerometer, is the electromechanical delay significantly (or clinically) different?
- ii. How far "up and down" the spinal cord does each HEMI stimulus activate relevant spinal nerves? (Unilaterally)
- iii. What is the "recruitment volume" for the various probe positions? (bilaterally)

Data sets to study:

- iv. Probes standard position (LA and RL) (left "arm" and right "leg")
 - compare probe LA to accelerometers LA and RA
 - compare probe RL to accelerometers RL and LLExpected results:
 - delay to RA and LL should be similar – both are mirror images of probe positions
 - LA versus RA and RL versus LL should be shorter – closer to probes
 - accelerometers on the chest (LC and RC) and abdomen (Labd and Rabd) should demonstrate intermediate delays from the two HEMI probes
- v. Probes placed symmetrically LA and RA - similar reasoning
- vi. Probes placed symmetrically LL and RL - similar reasoning
- vii. Probes placed unilaterally left or right (arm-chest, chest to abd, abd to leg)
 - variations in delays to accelerometers should depend on distance

Active stimulated movements are expected to demonstrate greater movement in the Z direction than in the other (YX and Y) directions.

Analyzing the ratios between the X, Y and Z directions might be able to identify and differentiate between the following:

- movement due to a HEMI stimulus
- passive movement (e.g., oscillation)

See Appendix 5 for suggested methods to record, tabulate and analyze the X, Y and Z ratios.

APPENDIX 5: RATIOS OF THE 3-D MOVEMENTS

It is postulated that HEMI stimulation would have different effects on the different types of muscles. This could be examined by calculating the **ratios of the 3-D movements**.

The value of using the **ratios of the 3-D movements** includes the ability to standardize (or “index”) each group of muscles for variations in muscle size in a given animal (e.g., forelimbs versus hind limbs), as well as to standardize across animals for size, etc.

Here we provide suggestions to present the data in table and figure formats, as well as suggestions for statistical comparisons.

The tables will be populated to show

- the absolute distance moved per individual animal (eight accelerometers, X, Y and Z directions)
- the averages and standard deviations of absolute distance moved for all animals (eight accelerometers, X, Y and Z directions)
- the ratios of distance moved per individual animal (eight accelerometers, X, Y and Z directions) (Table Appendix 5)
- the averages and standard deviations of ratios of distance moved for all animals (eight accelerometers, X, Y and Z directions) (Table similar to Table Appendix 5.) Each cell can be compared statistically with the other cells using non-parametric statistics with the Hypothesis: the muscles that move the greatest distance (do the most work), will show the most fatigue

Table 5: Ratio of each 3-D movement to one another and to the TOTAL distance moved

RATIO	x	y	z	Total
x		y/x	z/x	MT
y	x/y		z/y	MT
z	x/z	y/z		MT
Total	x/total	y/total	z/total	Total

The table shows the suggested method to calculate and report the ratio of each 3-D movement compared to the other 3-D movements, as well as to the TOTAL distance moved

Tables will be generated to show the onset, progression and recovery of fatigue for:

- first runs, second runs, third runs, etc.
- beginning of the run, versus the end of the run
- variations in the duty cycles

Figures could be generated from the table to graphically demonstrate that muscles that performed the most work (traveled the longest distances) will show a statistically significant decrease in the ratio to total 3-D distance moved, compared to muscles that traveled shorter distances.

APPENDIX 6: CONVERTING AN ANALOGUE IMAGE TO DIGITAL DATA

Converting a paper strip image to digital data is one of the MATLAB functions:

[Image to data - File Exchange - MATLAB Central \(mathworks.com\)](https://www.mathworks.com/matlabcentral/fileexchange/1282-image-to-data)

Here are the instructions:

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Image to data

Version 1.0.0.0 (4.37 KB) by [Sumeet Yamdagni](#)

Converts images to data points.

im2dat.m is used to convert images to data which can be plotted using the standard MATLAB functions. This is very handy if you have plots on hardcopy and you want to convert them into data that MATLAB can use. The scanned image can be analysed by this function and the output will allow you to perform any calculation/manipulations that MATLAB can perform, e.g. curve fitting. (Not just plotting)

The zip file contains two other functions:

imdatsep.m: This is used to separate multiple plots from one image, after using im2dat.m to analyse the image. The function compares two sets of image data and find all the uncommon elements.(It can also be used to restrict the range of data points)

imdatsort.m: This sorts the output of im2dat.m so that a line plot can be made.

Yamdagni, Sumeet (2020).

Image to data (<https://www.mathworks.com/matlabcentral/fileexchange/1282-image-to-data>), MATLAB Central File Exchange. Retrieved December 3, 2020.

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Other references for converting analogue data to digital:

Badilini, Fabio; Erdem, Tanju; Zareba, Wojciech ; Moss, Arthur J

ECGScan: a method for conversion of paper electrocardiographic printouts to digital electrocardiographic files

Journal of electrocardiology, 2005, Volume 38, Issue 4; 310-318

AMPS (Analyzing Medical Parameters for Solutions) offers a scan service to convert paper ECG traces to digital FDA-compliant format XML files.

<http://www.amps-llc.com/Services/paper-ecg-to-digital> Accessed 2022-03-17

APPENDIX 7: USING ALL THE DATA POINTS TO CREATE AN “ENVELOPE”

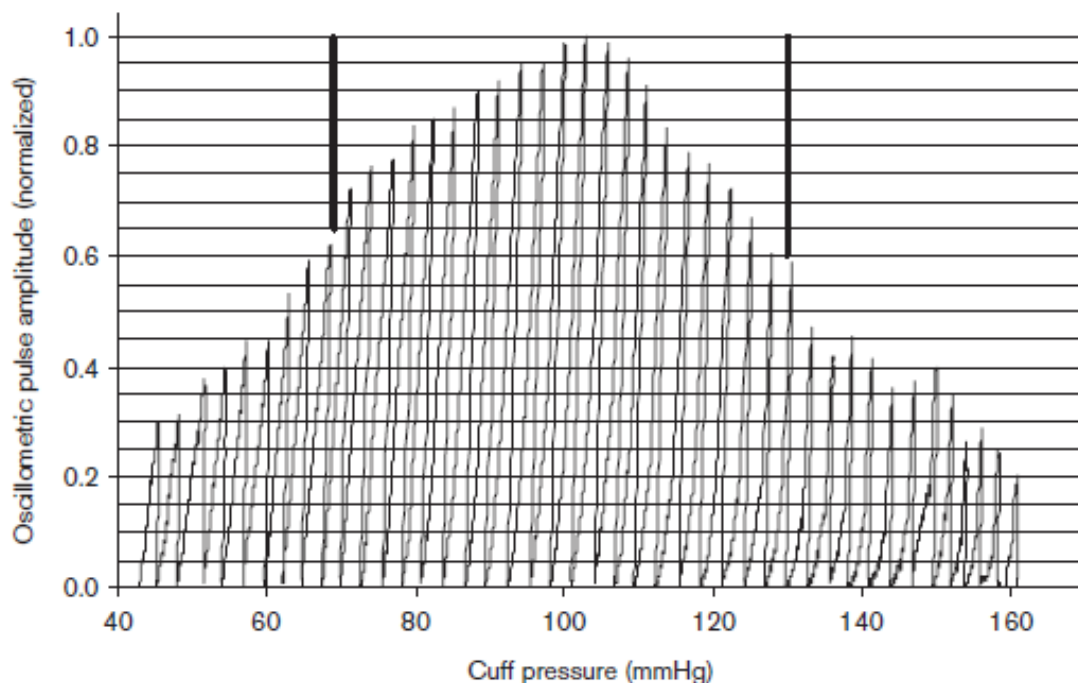
Amoore et al (2007) described measuring the effect of the shapes of the non-invasive oscillometric pulse **amplitude envelopes** to determine the systolic and diastolic blood pressure values.

The principle is to use all the values (the envelope) to calculate various parameters.

Alternative techniques include:

- Calculation a formal multi-factorial equation to describe the envelope
- Calculate a given percentage of a section of the total data set, for instance 30% of the rising values.

Figure 37: Using all the data points (“envelope”) for calculations of parameters



The figure shows the cuff pressure – actually going from high to low – on the x-axis. The y-axis shows the oscillations in the blood pressure cuff – arbitrary units. The upright thicker lines represent systolic BP (right upright line) and diastolic BP (left upright line)

Amoore, J. N. , Vacher, E. , Murray, I. C. , Mieke, S. , King, S. T. , Smith, F. E. & Murray, A
Effect of the shapes of the oscillometric pulse amplitude envelopes and their characteristic ratios on the differences between auscultatory and oscillometric blood pressure measurements.
Blood Pressure Monitoring 2007; 12 (5), 297-305. doi: 10.1097/MBP.0b013e32826fb773.