



Human Electro-Muscular Incapacitation (HEMI) Mechanisms Study 4:

An Examination of the Deleterious Physiological Effects of Variations in the Duty Cycle Parameters

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EXECUTIVE SUMMARY

This study is the fourth in a series of studies, the collective objective of which is to elucidate further the mechanisms of action of HEMI devices, their relationship to alternate duty cycles, and the deleterious effects caused by various duty cycles. Understanding how the mechanisms of action and work lead to the deleterious effects is critical to better quantifying and qualifying the actual safety risks, identifying vulnerable populations, as well as attempting to improve the efficacy and safety of this family of devices.

From previous studies, it was clear that HEMI stimuli produced responses that overwhelm physiological compensatory mechanisms and produced biochemical abnormalities well above the measurement capabilities of laboratory equipment. We therefore endeavored to measure “work” as a method to differentiate the deleterious effects of a wide range of duty cycles. We used accelerometers that measured movements in three (3) directions (i.e., the X, Y and Z directions). The movements were used to calculate power and reported as work.

In the pilot studies, we explored the use and development of new methods of measuring work and deleterious effects. In the main study, we examined interrupted HEMI stimuli using repeated duty cycles (“trains”) of 5 seconds of 18 Hz HEMI stimuli, interrupted by “Off” periods from 0 to 3,000 milliseconds (msec) for total durations of 35 seconds. The deleterious effects included the decrement in work generated during subsequent HEMI stimuli. We identified several new phenomena, which will inform and guide future HEMI studies. These results of the main study as well as these new concepts and ideas are presented in detail in the body of this report and summarized in this Executive Report.

- There was a statistically significant correlation between shorter durations of interruptions of HEMI stimuli and the development of deleterious effects, specifically as measured by the PCO₂.
- There was a statistically significant **correlation** between the total amount of work produced by the HEMI stimuli with varying durations of interruptions (0-3,000 msec) and the development of deleterious effects as measured by the decrement of work in subsequent runs.
- There was a statistically significant **difference** between the amount of work produced at 30-35 seconds by the HEMI stimuli with durations of interruptions of 3,000 msec, compared to HEMI stimuli with zero interruptions.
- Anatomical positions of the HEMI probes significantly affect the amount of work generated, and hence the development of deleterious effects. We postulate that the position of the HEMI probes, in a formal study, will have a significant effect on the amount of work generated in addition to the effect of variations in the duty cycle.
- There was evidence, using the measurement of work, of various rates of onset of tetanization with the initial onset of HEMI exposure. In the First Five Seconds of HEMI exposure we found and classified five unique patterns of work, indicating biological differences between animals which may be correlated with work and the development of deleterious effects.
- In the pilot studies, we demonstrated the feasibility of measuring standard human neuromuscular junction tests such as Train-of-Four (TOF) and Post-Tetanic Facilitation (PTF) tests of muscle function and weakness. We also demonstrated the feasibility of measuring the natural frequency of oscillations of a limb as a measure of deleterious effects. This is based on the clinical observation of muscle stiffness developing after HEMI stimulation.

At present there is no accepted understanding of which of the deleterious effects need to be minimized. There have been multiple biochemical abnormalities identified, but no single one of these has been shown to fully and clearly correlate with the deleterious effects. Many of these biochemical measures (e.g., lactate and EtCO₂) increase to such an extent that the present physiological laboratory instrumentation is not able to read such high values. Other biochemical parameters (e.g., potassium) seem to have such rapid changes that intermittent sampling cannot capture the peak values. Moreover, there is at present no single accepted measure of “deleterious effects” that could be used to compare one duty cycle with another. This has been a major barrier to the development of meaningful and relevant safety standards for this family of systems.

- One of our main recommendations, therefore, was to develop a general, generic measure of the deleterious effects, so that studies on variations in the duty cycle have “outcome measures” that can be used to compare one duty cycle to another. Specifically, previously we proposed that the measurement of “WORK” could be a sensitive measure of deleterious effects. There would also not be a limitation on the upper ranges of measurement. We demonstrated that the measurement of work using 3-D accelerometers was feasible and provided accurate and sensitive data. We therefore **recommend that the measurement of work be refined in future studies:** specifically by application of HEMI stimuli to individual muscles and/or muscle groups to separate out whole body deleterious effects and intra-muscular cellular effects.
- We propose the **development of new techniques for automated signal detection**, as well as processing and error trapping of 3-D movements using accelerometers. Signal processing and smoothing would be able to remove the movement artifacts from signals such as the invasive blood pressure monitor, intra-thoracic esophageal monitor, respiratory signals ((etCO₂, etO₂, flow, etc.) Automated analysis would be extremely valuable when literally thousands (18 Hz over multiple minutes per animal) of individual HEMI stimuli have to be detected, mathematically treated, and correlated with the resulting individual muscle movements.
- We propose **investigating the concept of “stunning.”** This is based on studies in electric eels where the prey was stunned after an electric shock by the eel. If stunning was shown to exist in human musculature, it would greatly expand the upper ranges of interruptions to be considered. Stunning has two components. First, relaxation time of muscles, which could be used as a new measure of deleterious effects. Relaxation is an active process and requires calcium, energy stores and oxygen, which might be in short supply in muscles exhausted by HEMI stimuli. Second, delay of muscle activation, measured as electro-mechanical delay after initiation of HEMI stimuli. This delay might be also used as an indicator of deleterious effects.
- We propose **investigating the use of standard human neuro-muscular junction tests** such as Train-of-Four (TOF) and Post-Tetanic Facilitation (PTF) tests of muscle function and weakness as new measures of deleterious effects. Calculation of the natural frequency of oscillation of a limb as a measure of stiffness and deleterious effects would expand the information gained from such studies of the neuro-muscular junction.

INTRODUCTION

Background

The abundance of, and reliance upon, HEMI (Human Electro-Muscular Incapacitation) devices continues 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. 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 that includes human surrogate testing to gain insight into the effects and their thresholds. This remains an important component of the formal acquisition process.

Purpose

This study is the fourth in a series of studies, the collective objective of which is to elucidate further the mechanisms of action of HEMI devices and their relationship to alternate duty cycles. Understanding the deleterious effects of a range of duty cycles on the physiology is critical to better quantifying and qualifying the actual safety risks, identifying vulnerable populations, and attempting to improve the efficacy and safety of this family of devices. The tasking of this specific effort was identifying the deleterious effects of a range of duty cycles to determine the feasibility of a longer duration of interrupted HEMI stimulation, with a goal of 180 seconds while using an equivalent inspired oxygen concentration of 21%. Included in this investigation was the amount of work generated by application of HEMI stimuli and its correlation with (and possibly causes of) the deleterious effects. There are two important lines of inquiry in this study of mechanisms.

The **first** of these is the objective measurement (via accelerometers) of work imposed by HEMI devices. As mentioned, the amount of work is expected to correlate with, and even cause, the deleterious effects, which is an important thrust of this investigation. This investigation includes correlations with possible mechanisms related to observed deleterious effects and mitigation of these effects through variations of the duty cycle. The **second** line of inquiry is the clarification of the interaction of the interruption of the duty cycle with the amount of work generated by the HEMI stimuli. The current study includes elements of both lines of inquiry. The **third** line of inquiry is the identification of new markers of deleterious effects that would not be overwhelmed by the physiological changes (such as what happens to the standard biochemical markers.)

Our Penn State team compared the amount of work generated with physiological responses, using a wide range of different duty cycles in order to quantify and qualify the aggravating or mitigating effects across a number of animals. These duty cycles were based on our previous studies and included a theoretically optimal duty cycle (mitigating and operationally relevant), as well as a wide range of duty cycles (0 to 3,000 msec) that span and bracket the envelope of physiologically and strategically feasible duty cycles. The mitigating aspects of the duty cycle are intended to allow for blood oxygenation via enabling perfusion. The interruptions studied varied from 0 through 3,000 millisecond pauses of the HEMI stimulus, potentially enabling a period of perfusion of the muscle cells.

This study operated under one animal protocol approved by both The Institutional Animal Care and Use Committee (IACUC) at The Pennsylvania State University and the Department of Defense Director of Veterinary Affairs through the Chief, Bureau of Medicine and Surgery, Department of the Navy.

METHODS

Animal Model

Fourteen (14) healthy pigs (*Sus scrofa*) 3-5 months in age and weighing 65.2 ± 7.2 kg (minimum – maximum: 53-77 kilograms, 117-170 pounds) were used for this study. Swine are commonly used as models for a variety of human diseases and conditions and have previously been used in HEMI studies. Swine were selected due to relevant similarities in size, cardiovascular and epithelial systems, and general mammalian physiology. This study was simplified (e.g., less movement of the subjects enabling better and more accurate monitoring) but perhaps limited by, the use of anesthesia to prevent pain and suffering of the animals.

Although we believe that the presence of anesthesia does not unduly affect the physiologic responses to the devices, it may contribute to the apparent inability of the animals to ventilate under muscular stress of extended duration (by maintaining the animal in an unconscious state with the accompanying respiratory depression).¹ In this study, it was elected not to ventilate the animals during the application of the HEMI stimulus as spontaneous breathing was important to the study design. We did notice that the starting saturation, when breathing spontaneously on room air, as measured by the SPO2 monitor and arterial blood gases were below normal values, e.g., a saturation of 88-90%. We decided to negate the depressant effects of the anesthesia by increasing the inspired oxygen concentration to obtain a saturation of at least 94-95%. No animal model is a perfect or an ideal model of the human anatomy and/or physiology. However, the cardiovascular system of the swine model closely resembles that of a human, and is therefore used extensively for testing involving the cardiovascular system. In addition, the size of the swine model is also in the range of the human sizes so that scaling problems (e.g., voltage and amperage) of the HEMI devices are minimized. Each animal in this study received a complete physical examination including blood collection for a complete blood count (CBC) and pre-anesthetic chemistry profile.

Experimental Setup

The operating room layout is shown in **Figure 1** (Experimental Layout).

Anesthesia. Swine were pre-anesthetized using 1mg/kg xylazine + 6-10 mg/kg ketamine (intramuscular–IM). An auricular vein indwelling catheter was inserted and anesthesia induced with equal parts 100 mg/ml Xylazine and 100 mg/ml ketamine mixed in a syringe and given intravenous (IV) to effect. Swine were endotracheally intubated in a supine position, while all the further studies occurred in the dorsal position. The animals were ventilated with oxygen, air and isoflurane (as needed) until the animal was completely instrumented.

Prior to experimental HEMI exposures, the isoflurane was turned off, a propofol infusion was started, and the pig was allowed to breathe spontaneously. Anesthesia was maintained with a continuous propofol infusion delivered by a syringe pump to maintain adequate anesthesia as per standard clinical procedures, typically in the range of 100-120 ug/kg/min. Compared to volatile agents, total intravenous anesthesia

¹ Hughes, Edward L., M.J. Kennett, W.B. Murray, J.R. Werner, D.M. Jenkins, *Electro-Muscular Disruption (EMD) Bioeffects: A Pilot Study on the Effects of Continuous Application of the TASER®X26 Waveform on Swine*, Applied Research Laboratory, The Pennsylvania State University, 30 November 2008. [USMC Contract M67854-05-D-5153/DO03]

(TIVA) is less likely to cause cardiovascular (Kurita 2003²) or pulmonary (Ziser 2003³) inhibition, and causes fewer decreases in arterial and mixed venous oxygen partial pressure (Schwarzkopf 2003⁴) in pigs. Intravenous anesthetics were continued and adjusted when indicated, to ensure adequate depth of anesthesia. Veterinarians were on-hand during the entire study to administer and monitor anesthesia.

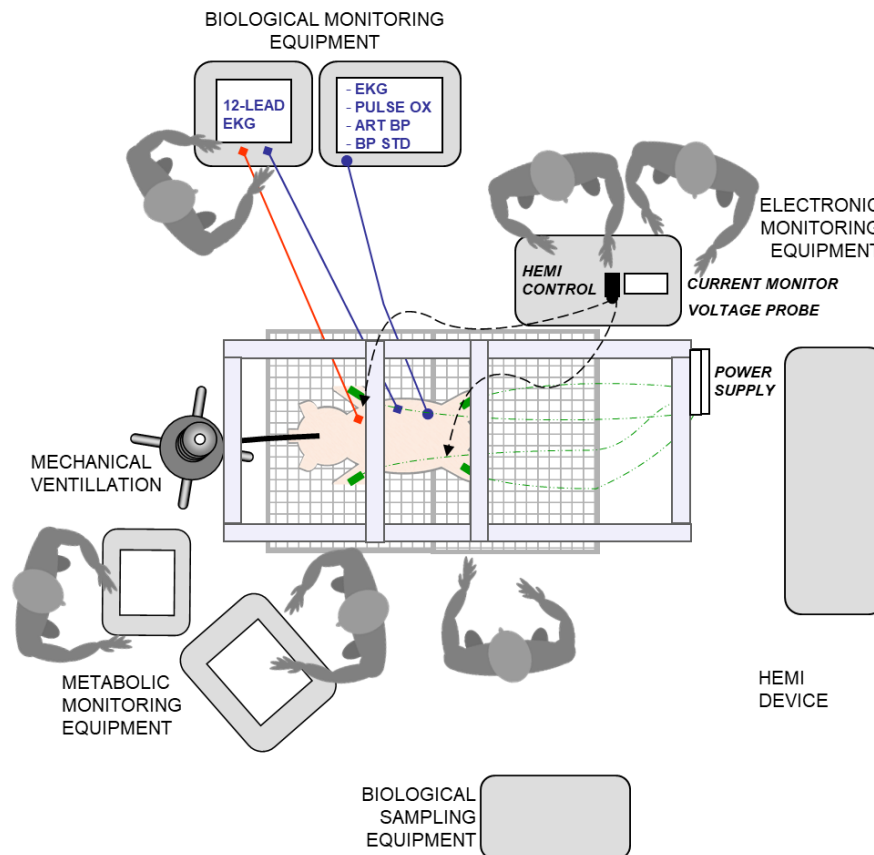


Figure 1: Experimental layout

Ventilation. The animals were allowed to breathe spontaneously after intubation with an endotracheal tube. We noticed that the respiratory rate of the animals changed (slowed) when connecting the endotracheal tube to the circle system of the anesthesia machine. We compared the flow signals with the animal breathing room air (no breathing system), the anesthesia circle system, and an open ended (no valves, no anesthesia reservoir bag) t-piece breathing system.

There was less change in the flow signal from room air (no breathing system) to the t-piece compared to the exhalation flow rate with the circle system, where it seemed there was a decreased exhalatory flow rate. We therefore elected to use the open-ended t-piece for studies when using increased oxygen concentrations, and not using volatile agents.

² T. Kurita, K. Morita, T. Kazama, S. Sato, Comparison of isoflurane and propofol-fentanyl anaesthesia in a swine model of asphyxia, *Br. J. Anaesth.* 91 (2003) 871–877.

³ A. Ziser, R.A. Strickland, M.J. Murray, Propofol does not induce pulmonary dysfunction in stressed endotoxic pigs receiving Intralipid, *Crit. Care. Med.* 31 (2003) 2029–2033.

⁴ K. Schwarzkopf, T. Schreiber, N.P. Preussler, E. Gaser, L. Huter, R. Bauer, H. Schubert, W. Karzai, Lung perfusion, shunt fraction, and oxygenation during one-lung ventilation in pigs: the effects of desflurane, isoflurane, and propofol, *J. Cardiothorac. Vasc. Anesth.* 17 (2003) 73–75

Animal Monitoring. A pulse oximeter with a plethysmogram was attached to the animal and linked to a computerized unit to monitor pulse rate and oxygenation (SPO₂). A capnograph measured end tidal CO₂. Data were displayed continuously. The swine were connected to a 12-lead electro-cardiogram (EKG) monitoring system, which provided automated analysis of the EKG. Intravenous catheters were placed in the lateral auricular veins. For metabolic monitoring studies, prior to EMD application, a CareFusion VMAX metabolic monitor (VMAX Encore 29, Vmax Software Version 28-4, CareFusion, Yorba Linda, CA) was connected to the animal. The metabolic monitor measures flow (“ventilation”), CO₂ production VCO₂), oxygen consumption (VO₂), as well as calculates tidal volume (TV), Inspired to expired (I:E) ratio, and other derived metabolic parameters. During the preparation, the metabolic monitor was calibrated using an auto-calibration method provided by the manufacturer. In order to measure respiratory and metabolic function, the animal was directly connected to the metabolic monitor. The endotracheal tube remained in the animal, which was connected to an open-ended t-piece breathing system (without a reservoir bag) to the anesthetic machine, which enabled the appropriate oxygen tension to be administered. An arterial catheter was placed (Seldinger technique, or cut-down) in a branch of the femoral artery to allow arterial blood sampling.

Blood Sampling. Swine were instrumented with peripheral venous and arterial catheters for repeated blood sampling, intermittent administration of intra-venous anesthetics, and continuous arterial blood pressure monitoring. A Seldinger and/or cut down approach to an artery and/or vein was completed for catheterization. A veterinarian and an anesthesiologist were on-hand during the experiment and performed the procedure. When necessary, an incision was made and soft tissue bluntly dissected to expose the artery and/or vein. Sterile catheters were placed in each vessel and held in place with nylon sutures. At the end of the experiment, the pig was euthanized and the catheters removed.

Electrocardiogram. Animals were instrumented with a ten-lead electrocardiography (EKG) monitor (DRE True Advance Channel EKG System, Louisville, KY, USA) to obtain digitized traces for computer-derived measurements (before, during and after HEMI application).

HEMI Device Monitoring. During the portion of the experiment that examined the effects of variations of the duty cycle, the HEMI device leads or contacts were placed in the same position to enable maximal potential current to traverse (pass through) the heart (left axillary area and right groin). During the portion of the experiment studying the conduction of the electrical stimulus in the body, leads were placed at a variety of positions in accordance with the diagram at **Figure 2**. The leads were consistently placed at least 10mm into the skin to ensure reliable contact and avoid dislodgement during movements caused by the HEMI device. The voltage and current characteristics of the HEMI device were monitored before and after exposure across a 250Ω resistive load (*Ohmite*: 250Ω, 225 Watt Resistor). Voltage was measured using a high voltage divider (Ross Engineering: VD90-4.1-A-LB-A-F). This voltage divider placed 360MΩ across the HEMI device probes. Current was measured using a current probe (Pierson: Model 101) surrounding the HEMI device leads.

Accelerometers. In order to measure relative movement with respect to the varied duty cycles, the 3-D accelerometers were placed with the x-direction as superior to inferior (with respect to the animal’s anatomy), the y-direction from left to right, and the z-direction from anterior to posterior (“up and down” with respect to gravity.) The accelerometers were placed as indicated in the diagram at Figure 2. See below for further description of the fixation to the animals, the data collection and signal processing and analysis of the signals.

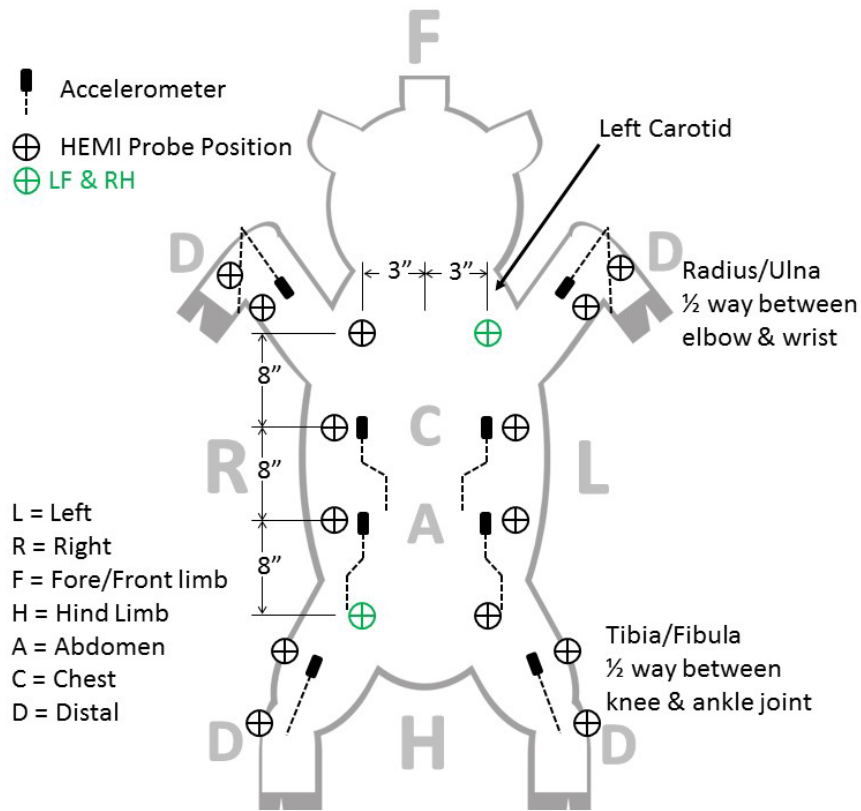


Figure 2. HEMI Probe and Accelerometer Lead Placement

Suspension Frame and Positioning. The overall purpose of the animal and limb suspension frame and the fixtures described herein, was to position and keep the limbs in a relatively consistent position by pre-tensioning and also limiting the excursion of the limbs. Less movement was expected to introduce less interference with the signals that were being collected. We therefore used a known weight with the primary purpose to limit and minimize the extent of the movements. A secondary purpose was to support force calculations by calculating the work expended to move the known weight. The weight was of sufficient mass to enable consistent measurements that could be correlated across animals, but not too much as to restrict movements and thus prevent signal generation. The weight tightly connected to the booted hoof to prevent extraneous movement.

The frame of the test fixture was designed around t-slot framing, this was chosen due to its rigidity (test measurements) and fixture adjustability (animal size). Design considerations included:

- Space (dimensional restrictions) in the operating room, under the ceiling mounted operating room (OR) lights and spanning the pallets/test bed on which the animal resides;
- Getting the fixture/frame in and out of the room and over the test bed with ability to be rigidly fixed for testing (mobility, locking/leveling casters);
- Affixing animals within a size range (adjustable cross members and adjustable length shackles for weights/scales/springs and the size of the boots/clogs); and
- Limb suspension fixtures for desired measurements and repeatability (springs, digital scales, shackles, weighted accelerometer holder and boots/clogs/glue).

Given these design considerations, desired test measurements, and repeatability, a gantry style frame was designed that fit the dimensional restrictions and would be rigid enough to accommodate the strong and marked muscle movements during the test. Leveling casters were selected for mobility with the ability to lower feet of the frame to the floor to rigidly affix the fixture. The t-slot framing was selected due to its adjustability, making it easy to adjust the cross members of the fixture to the size range of the test animals.

The animal would be tested lying on its back so not to restrict its airway that would otherwise would be hindered if the animal were to be suspended by slings under its body/abdomen in the prone position. Given this design restriction, it was decided that the limbs/hooves of the animal would be suspended from the gantry/frame. This limb/hoof suspension fixture would have its own design considerations:

- Acquiring the desired measurements of “net” or “movement/isotonic” work. In contrast to “tension” or isometric work. $Work = mass * displacement$. A mass (5 lbs. which included the weighted accelerometer holder, associated hardware, clog and glue) moved by the net effect of the stimulus applied to the animal measured by accelerometers, differentiated for net displacement;
- Accurately acquiring the desired measurements in a repeatable fashion (springs, tensioning device, adjustable shackles and digital scales); and
- Accommodating a range of limb length and hoof size (adjustable shackles, clogs/glue).

The selected design was a solid fixture method with minimal slippage and/or stretching. The aim was to keep the limbs suspended from the frame. The fixture method consisted of solid boots (see Figure 3). *Solidworks* was used for the modeling portion of the fixture and the boots. A high strength material was needed for the boots, and the material decided upon was not available on any of our in-house printers, so a third party was used (Stratasys Direct Manufacturing). The boots were glued solidly on the lower side to the hoofs, and boots were bolted on the lower side to the weight. The boots that were glued to the hoofs were 3D printed using Select Laser Sintering (SLS) with fiber-glass filled nylon12 powder (very strong and solid). The shape of the boot was determined by a prone hoof position, and made large enough to accommodate hoofs of the determined animal size range. Slots were used for the mounting holes to also accommodate different size/shaped animals and also to align the limb axially with the limb suspension fixture. Two insertion holes were used for injection of the glue - one on the bottom of the boot and one on the toe for filling the boot with epoxy.

We needed a glue for the connection between the hoof and the boot that would rapidly set/cure/solidify in 5-10 minutes. The two-part epoxy that was selected to glue the hoofs in the boots had a few essential requirements. Since we could not sedate the animal overnight to glue the hoofs to the boots for setting/curing overnight, the cure time had to be very short. The other requirement was that we were gluing plastic to hoof and flesh, so the epoxy had to be compatible with a wide variety of materials. The epoxy also had to be relatively viscous, since we were manipulating the animal and bolting the boots to the weights while the epoxy was curing and want to prevent leakage of the epoxy. The epoxy that was used was Chem-Set™ 633LV Low-Viscosity Rodding Epoxy from Chemical Concepts. The epoxy, 633LV, has a cure time of 10-20 minutes and adheres to a variety of materials including stone, metal and plastics.

Figure 3: Three-dimensional (3D) hoof, boot, weight, digital scale and turnbuckle. The figure shows a three-dimensional (3D) hoof was produced using a digital printer (left). The 3D hoof was used to design a boot that was also produced using the digital printer. The animal's hoof was glued onto the boot. The picture on the right shows the mechanism for suspension of each limb (laid out horizontally on a table). At the far left is a model of a hoof inserted into the boot. On the far right is the hoof inserted into the weight connected to a digital scale. The turnbuckle is used to tension the limb to a standardized value. For more details, see text.

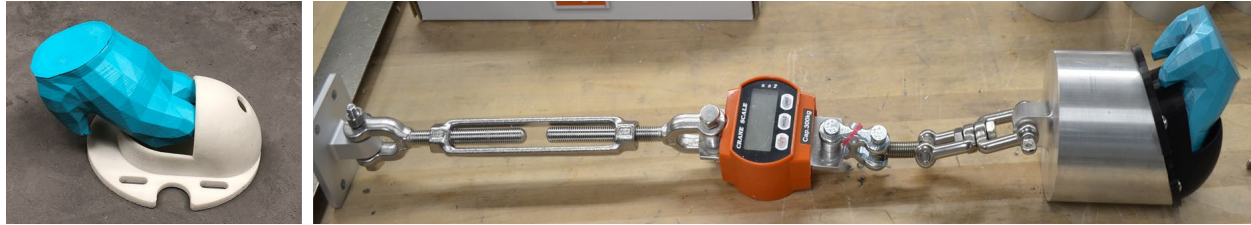


Figure 4: The suspension apparatus in the laboratory setting. The figure shows the actual suspension apparatus fully instrumented and properly oriented in the laboratory setting.



Applications and Measurements

HEMI Stimulus Application. The interrupted HEMI stimulus was applied multiple times for 2.5 or 5-second duration periods from 35 up to 360 seconds (6 min) for each exposure. HEMI exposure was defined as one group (series) of multiple 2.5 or 5-second applications using a TASER Model X26 waveform. The interruption time between exposure periods (duty cycle) was varied to study changes in deleterious effects with various duty cycles. Animals remained under anesthesia for the duration of the experiment. The HEMI device leads (“probes”) were placed to enable maximal potential current to traverse (pass through) the heart (right sub clavicular area and left lower rib cage). The leads were placed at least 10mm into the skin to ensure reliable contact and avoid dislodgement during movements caused by the HEMI device. We had previously set the definition of death as no cardiac function for 1-2 minutes. We therefore had data collection in the peri-mortem phase, including post mortem.

Blood Sampling. We sampled arterial blood before, and 1 minute after, each exposure. The labs drawn “just before the next stimulus” also was used as the “*post-rest period*” lab values for the previous HEMI exposure. Measurements on the arterial blood included pH, PO₂, PCO₂, HCO₃, base excess, Hct, calculated Hgb, Na, K, Cl, and lactate. Blood gas and electrolyte values were obtained by processing samples using the *i-STAT* handheld clinical analyzer (HESKA Corporation, Loveland, CO).

Evidence of muscle breakdown was sought by observing the color of the supernatant (plasma) after centrifuging. Heparinized blood samples were placed on ice and were immediately processed (blood gases and electrolytes). The heparinized blood was spun in a centrifuge to collect plasma and the plasma remains stored at -40°C in the event it is needed for further analysis.

Electrocardiogram. The EKG leads were placed in the following anatomical positions. The left and right foreleg electrodes were placed just below the point of the elbow on the back of the forearm, and the left and right hind-leg electrodes were placed on the loose skin at the stifle in the region of the patella. V1 was placed in the 6th intercostal space on the left side of the thorax along a line parallel to the level of the point of the elbow. V2 was placed in the 6th intercostal space on the left side of the thorax along a line parallel to the level of the point of the shoulder. V3 was placed over the dorsal thoracic spine of T7 at the withers. V4-6 were placed similarly on the Right side of the animal. Significant electrical and movement interference, due to the HEMI stimulus and muscle response made the EKG signal uninterpretable and unrecordable *during* the application of the HEMI stimulus. We used the arterial line tracing to check for non-regular beats such as extra-systoles and missed beats.

Table 1 shows the first three HEMI exposures of each animal in chronological order where:

- 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, 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.

Table 1: 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

Respiration and Tidal Volume. The animals breathed spontaneously during the HEMI stimulus. The animals were placed on a ventilator using 100% oxygen and between runs to promote recovery. Prior to, as a baseline, during and after a period of HEMI stimulation, we used a CareFusion VMAX metabolic monitor (VMAX Encore 29, Vmax Software Version 28-4, CareFusion, Yorba Linda, CA) as a source for a respiratory flow signal. The flow signal from the metabolic monitor does differentiate between inspiratory and expiratory flow directions. The metabolic monitor had an “Actual Flow setting” which measured the real-time flow signal. We used this mode to have the flow signal in-phase with the HEMI signal to obtain the best possible estimation/calculation of the electro-mechanical delay (i.e., the delay from the onset of the 1st HEMI stimulus signal to the onset of muscle movements.) The metabolic monitor also measured continuous breath-by-breath concentrations of inspired and exhaled carbon dioxide and oxygen. The transit delay of the side stream nature of the CO₂ and O₂ analysis resulted in a phase shift between the flow and the gas concentration signals. Furthermore, the analysis chamber for the oxygen measurements has a slower time constant than that of the CO₂ chamber, hence the oxygen signal is not necessarily “in-phase” with the flow and CO₂ signals.

End-tidal CO₂ Measurements. The capnographic signal (EtCO₂) was monitored continuously, and measured on a breath-by-breath basis by a VetPro monitor (Surgivet Advisor Vital Signs Monitor, Version 1, Smiths Medical, Waukesha, WI). An automatically generated output of vital signs values was printed on the paper strip at five (5) minute intervals. The metabolic monitor also provided a continuous, breath-by-breath capnographic signal, which was captured by the metabolic monitor.

End-tidal O₂ Measurements. The oxygen concentration signal (EtO₂) was monitored continuously and measured on a breath-by-breath basis by the metabolic monitor.

Measurement of leg muscles enlargement after HEMI exposure: We had noticed in previous studies that the hindquarters of the swine seemed to be swollen and edematous after the HEMI exposures. We marked and measured the circumference of the upper hind limb before and after HEMI exposures. Considering the limb as a circular object, we calculated the increase in volume.

Paper recording of vital signs: The strip recordings for the vital signs (ECG, BP via arterial line, capnograph, SPO₂, etc.) were obtained at the typical speed of 25 mm/second unless otherwise indicated. The scale for the arterial line was 0 – 160 mmHg. The ECG used a scale of 10 mm per mV. The SPO₂ size was auto-scaled by the equipment.

Duty Cycle Variations. During this project, the physiological responses to variable HEMI pulse rates were of interest. For that reason, a TASER X26 was modified to enable a variable pulse rate.

Modification of HEMI stimulus for HEMI 4. A Taser X26 series TASER was used as the stimulus device. The battery was replaced with a DC power supply to ensure the output was consistent and did not depend on battery charge. For long pulse tests (5 seconds on, 100 milliseconds off/6 minute long/30 second continuous), a programmable logic controller was wired to the safety and trigger of the Taser (X26, TASER International (now Axon), Scottsdale, Arizona) to control it. The programmable logic controller had inputs for on time, off time, and length of test. This hardware setup worked well for longer pulse trains, but was not capable of doing very short or irregular pulse trains. For shorter pulses and more intricate pulse trains, the Taser was disassembled and the high voltage module was controlled directly with an Arduino Micro (Arduino Micro, Arduino, Ivrea, Italy.) The Arduino Micro was programmed with the Arduino IDE (1.8.12, Arduino, Ivrea, Italy.) The waveform on the control line for the high voltage module was mimicked and replicated. It was found that the waveform for a single discharge contained a 500Hz square wave oscillation for 21 pulses (duration of 46 milliseconds). For a continuous pulse train, the 500Hz, 46 millisecond pulses were repeated every 18 Hz, or 56 milliseconds. These waveforms were implemented

as timers in the Arduino. With the Arduino able to replicate the control waveform for the Taser's high voltage module, different pulse trains such as one pulse every second were easily constructed. For each animal, the hardware was set up and tested at the beginning and end of the day to ensure the system was consistent throughout the tests. The Taser was discharged into a resistive load, and the output current waveform was monitored by the data collection system.

Train-of-Four (TOF) and Post-Tetanic Facilitation (PTF). We adapted the equipment to provide a Train-of-Four (TOF) stimulus as well as Post-tetanic Facilitation (PTF) and Post-tetanic Count (PTC) stimuli using the HEMI device. The post-tetanic count also requires a single twitch at a one second interval. This one second stimulus enables analysis of the natural frequency of each limb and each central muscle group (chest and abdominal muscles).

Data Collection Hardware (A-D converters).

- **Accelerometers to "work."** Movements of the limbs, chest wall and abdomen were measured with accelerometers (4030-006-120, TE Connectivity Measurement Specialties, Hampton, Virginia.) The accelerometers measured acceleration in 3 dimensions (3-D). The data acquisition (DAQ) was performed on a PXI controller (PXI-8105, National Instruments, Austin, Texas.) in a PXI chassis (PXI-1031 DC, National Instruments, Austin, Texas.) that controlled three NI-4472 8 channel A-D converters and additional PXIe chassis (PXIe-1073, National Instruments, Austin, Texas.) with a DAQ card (PXIe-6366, National Instruments, Austin, Texas.). Three DAQ tasks (banks) were used for the DAQ system. For software management, see below.
- **HEMI stimulus.** Using the A-D system described above, the HEMI stimulus was converted by the A-D converters to a digital format at two separate frequencies: 1,000 Hz (later 10 kHz) and 100 kHz and stored on the hard drive.
- **VMax signals** (carbon dioxide and oxygen concentrations, as well as respiratory flow rates). Using the A-D system described above, the out signals (carbon dioxide and oxygen concentrations, as well as respiratory flow rates) of the VMax (VMAX Encore 29, Vmax Software Version 28-4, CareFusion, Yorba Linda, CA) were fed into the A-D converters and converted to a digital format at 1,000 Hz (later 10 kHz).
- **VetPro monitor and vital signs.** The signals of the vital signs (BP, HR, SPO2, end tidal and inspired carbon dioxide concentration, as well as rectal temperature) from the VetPro monitor (Surgivet Advisor Vital Signs Monitor, Version 1, Smiths Medical, Waukesha, WI) were available as digital data via a serial port at a frequency of once per 5 minutes. The digital values for the day were collected in a separate CSV file.

Data Collection Software (Management of A-D data).

- **Collected Data Structure.** The data acquisition (DAQ) was performed on a PXI controller (PXI-8105, National Instruments, Austin, Texas.) in a PXI chassis (PXI-1031 DC, National Instruments, Austin, Texas.) that controlled three NI-4472 8 channel A-D converters and additional PXIe chassis (PXIe-1073, National Instruments, Austin, Texas.) with a DAQ card (PXIe-6366, National Instruments, Austin, Texas.). Three DAQ tasks (banks) were used for the DAQ system. The three tasks could be started in software at the same time, but would vary in start time by a few hundred milliseconds. Each bank recorded the HEMI pulse that was used to synchronize the three channels in post processing. The first DAQ task (Low Speed Sampling Rate - LS1) consisted of seven of the eight 3-axis accelerometers (4 limbs, 2 chests, and the Left abdomen), and the HEMI current. The second (Low Speed 2 - LS2) consisted of the remaining 3-axis accelerometer (right abdomen), the VMAX signals and the HEMI current. The final task (High Speed 1 - HS2) recorded the HEMI pulse

at a higher sample rate. Data acquisition utilizes National Instruments LabVIEW software with DAQmx. The general scheme defines the channels in each task, defines the timing, defines the logging, starts the task, reads the task, and stops the task. For each channel the gains and channel names are defined and assigned to a single task; other channel specific settings are also set such as coupling (DC or AC) as needed. Timing is set for an entire task using the card's onboard clock. Logging was set up to stream the task's data directly to disk in TDMS format. TDMS format is a format that combines binary data with meta data that is either intrinsic to the channel format or can be user defined. The three tasks write to separate TDMS files with a common file name differing only by a LS1, LS2, or HS2 string. Each file also writes its data in the group that matches the task name (LS1, LS2, HS2) so that if combined into 1 file the groups do not need to be renamed. All three tasks are started in software at the same time, and logged and read at the same time. A small subsample of data is displayed to the user while acquiring data, but is not necessary. At the end of the test each task is stopped and files closed.

- **Software Time Alignment.** Time aligning the three files (task/banks) is the first step that is performed. The first HEMI pulse was used to perform this, and could be done manually or automatically. When done manually the three files would be opened in Diadem and the three HEMI currents were plotted together. From there the user would find the first HEMI pulse and record it in the test config file. Note: Alignment times could be any arbitrary artifact in the data that would show up in all three currents. This of course is time consuming and only done when the automatic method could not be used. The automatic method tried to do the same but would sometimes be fooled by spurious spikes that typically are present in A-D data sampling and conversion.
- **HEMI Pulse Identification.** To automatically find the HEMI start, the pseudo-median and maximum deviation from the pseudo-median would be found. The pseudo median was found by finding the weighted mean of a median of 100,000 samples. The weights were the size of data used to find the median, such that if the last median was only done on a single point it would not greatly influence the prior medians found. Each HEMI pulse would be identified by unit scaling of the HEMI current by the pseudo-median and the maximum deviation. HEMI pulses were then identified as a data point greater than a threshold chosen by the user usually this was in the range of 0.10 to 0.32 or 10% to 32%. The user would adjust the threshold usually starting at 0.32 and adjusting it until the number of pulses per pulse train were correct and there were no extra or missing pulses. The maximum frequency between pulses could also be defined but was almost always left at 25 Hz. This process would also generate "Quick Look Graphics" that were utilized to confirm that the process worked correctly. **Figure 5** shows the pulse detection graph's primary purpose is to quickly identify missing pulses. The X axis is pulse number and the Y is time. The black (upper) trace is plotting the item from the beginning of the file versus the pulse number. It would be expected that the black trace would have a constant slope except during pulse train dead times. These steps would also be expected to have the same step size as shown below. The "res plot" is the estimated frequency of the pulses based on the time between pulses. Some pulses could not be detected, or did not happen, as identified by frequencies dropping from about 18 Hz to around 9 Hz. Finally, the green trace (upright) indicates the start of a pulse train which was defined as 3 - 18 Hz periods or more.

Figure 5. Pulse Detection Graph. The figure shows a method of error detection and exclusion using a Pulse Detection Graph. For further details, see text.

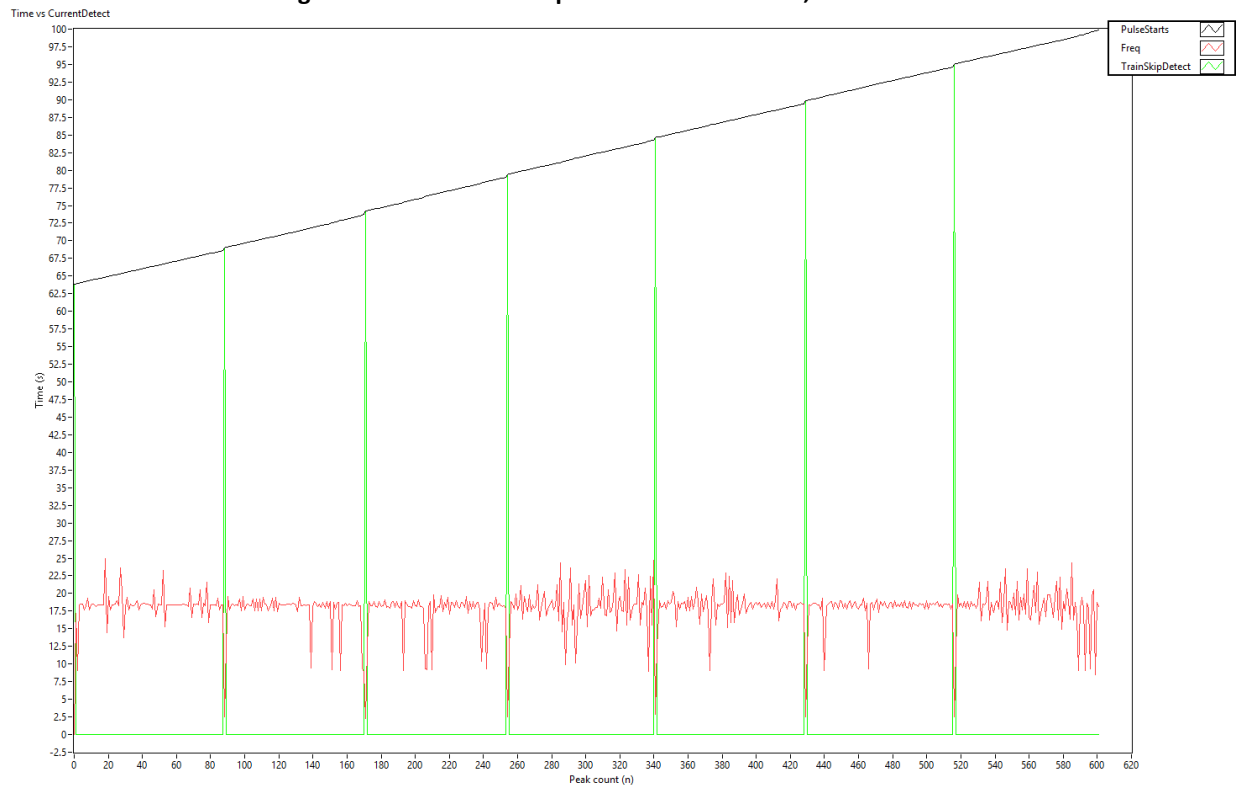


Figure 6 shows expanded details of the same information as in **C5**. It does not include the estimated frequency. **Figure 6** plots the time of a pulse train pulse versus the pulse train pulse number. The axes are the same as in **Figure 5**. Each plot represents a successive pulse train. Ideally the pulse trains should have the same number of pulses. Variations of plot lengths should be only a handful from missed pulses, and ideally should be the same, i.e., differing by only 1.

The Primary way of detecting HEMI pulses is done with the High Speed (HS2) task. This becomes evident when looking at the relative amplitudes of the current waveforms in **Figure 7**. This plot is used to identify that the first pulse has been identified correctly. All three tasks' currents are plotted after being aligned. Enough time is shown in the plot to include any erroneous pulse before and/or after the first pulse, if such erroneous pulses had been present.

Figure 8 is used as a double check of the first pulse as being identified correctly. The high speed HEMI current measurement, red, is compared to the Z direction of the 3-D accelerometer for the relevant limb. What is examined here is that when the HEMI pulses start and stop, the major movements (as determined by the accelerometer), start and stop essentially at the same time.

Finally, **Figure 9** shows the method used to confirm synchronization between the current measured at Low Speed (LS1) and High Speed (LS2). Movements should start at the same time, and follow about the same positive and negative movements. The example shown is data from the Z direction of an abdominal signal.

Figure 6 Pulse Trains. The figure shows an analysis and data checking of the Pulse Trains. For further details, see text.

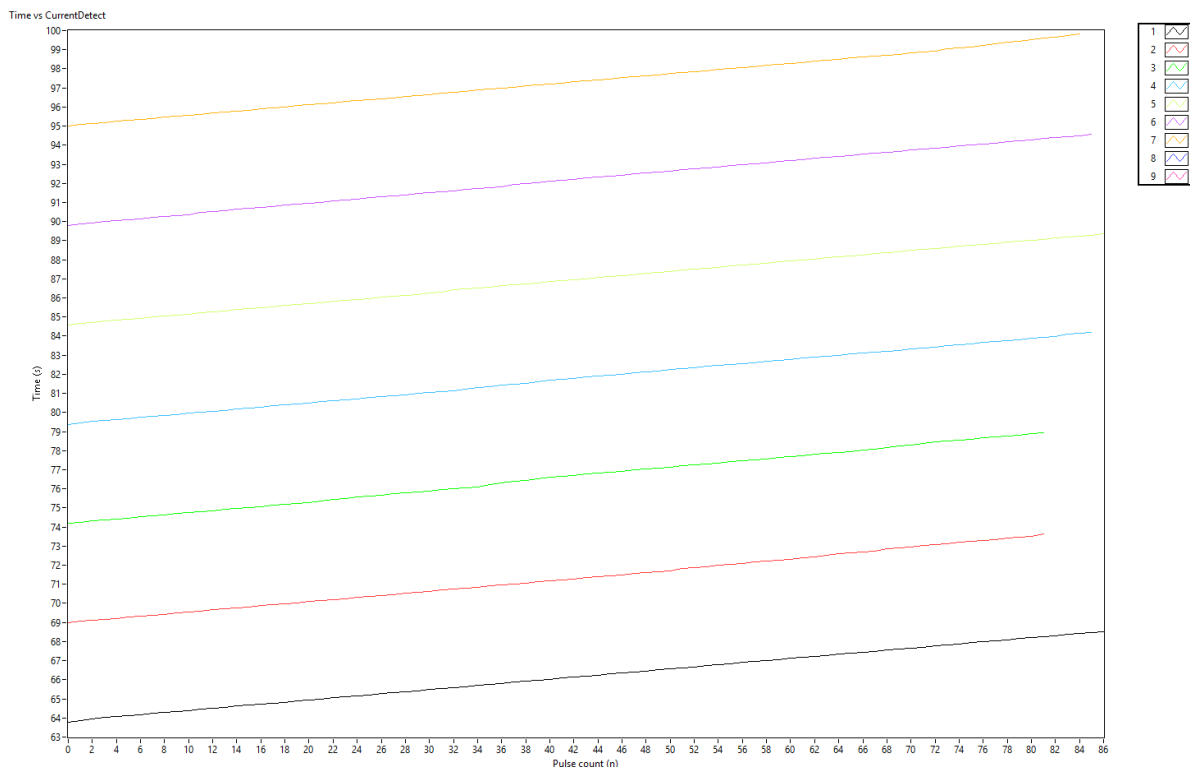


Figure 7: Error checking for correct identification of the First Current's Pulse. The figure shows error checking for correct identification of the First Current's Pulse. For further details, see text.

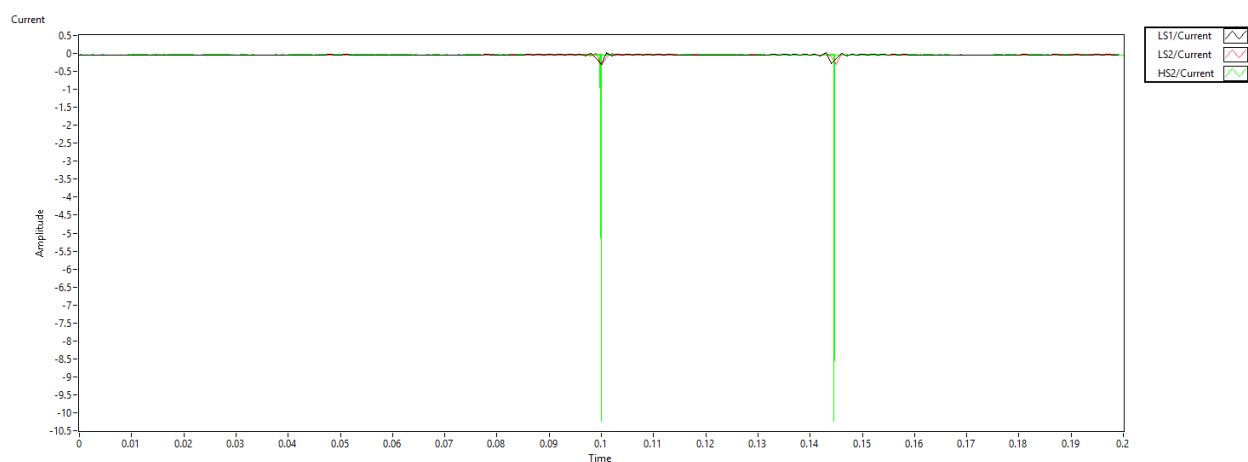


Figure 8 High Speed Sampling (HS2) current and Foot Z Direction. The figure shows the method to ensure that the High Speed Current (HS2) and accelerometer signals are synchronized, by checking that they start and stop at the same times. For further details, see text.

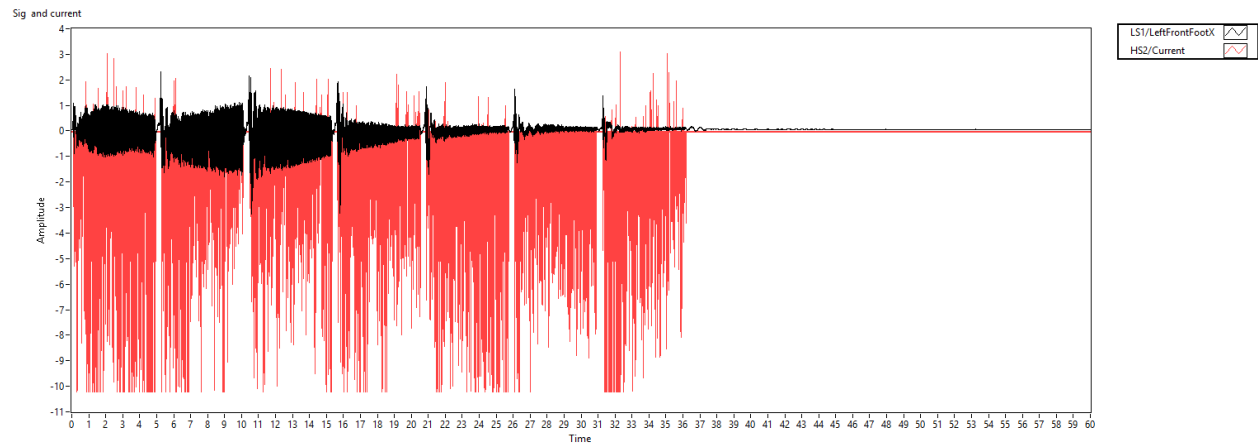
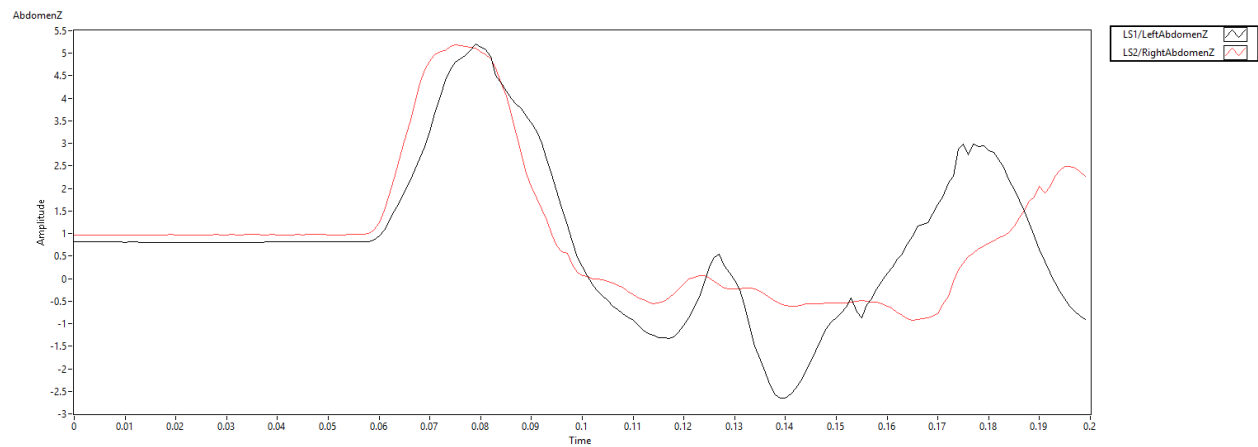


Figure 9 Abdominal Movements. The figure shows the method used to confirm synchronization between the current measured at Low Speed (LS1) and High Speed (LS2). For further details, see text.



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 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.”

Statistical Methods

The data were entered into a Microsoft Excel spreadsheet and data entry checked. Accuracy was further confirmed by ensuring maximum and minimum data points were within physiologically acceptable (expected) ranges. Averages, standard deviations, standard errors, counts, maximums, and minimums were calculated by programming the spreadsheet. The Excel program was also used to calculate linear fits of parametric data. Statistically significant differences between groups were sought using Student's t-test for parametric data. Because of the small data sets, we employed a Fisher Exact Probability Test, as for many of these data sets there was an expected value in a cell below 5, and a Chi square test would not be suitable. In these small data sets, we compared the before and after values in the animals by ranking the combined set, identifying the median value, and then sorting the numbers as above or below the median value. The numbers of animals above the median were separated into 2 groups being compared, e.g., in the pre- and post- values. Similarly, the number of animals with values below the median were grouped into the groupings of interest. This provided a two-by-two table for the Fisher Exact Probability Test. P-values of less than 0.05 were considered statistically significant.

RESULTS

Overview of Results

Fourteen (14) healthy pigs (*Sus scrofa*) 3-5 months in age and weighing 65.2 ± 7.2 kg (53-77 kilograms, 117-170 pounds) were used for this study. The pigs were sexually immature while the male pigs either castrated prior to sexual maturity or not castrated but sexually immature. See Table 2 for the weights of the males and females. All animals survived the first three runs of 35 seconds each.

	Male	Female	Table 2. Weights and Male-Female Ratio. The table shows the weights and genders of the 14 animals in chronological order. The weights of males and females are comparable (in a similar range). The variances of the weights (as shown by the standard deviation) are also comparable (males 8.6% and females 10.2%).		
A1 01	58.0				
A2 01		73.6			
A3 01	53.0				
A4 01		67.7			
A5 01	66.0				
A6 01		63.6			
A7 01		68.0			
A8 01		77.3	AVG	Male	Female
A9 01		71.4	Count	6	8
A10 01	66.0		STD	5.302	6.838
A11 01		56.8	SE	2.371	2.584
A12 01		60.5	Minimum	53.0	56.8
A13 01	65.0		Maximum	66.0	77.3
A14 01	63.6				

Examples of Raw Signals and Conversion to “WORK”

As an introduction to the next section of the results which focuses on the WORK generated by the animals, we show examples of the various formats of presenting the work data in graphical formats. We also introduce graphical examples of **Cumulative Work** which is used as the basis for the calculation of **Total Work** for each limb. The graphical format will elucidate the later examples of tabular format. As described in Methods, the average of the cumulative work of the last 5 seconds was used as the **Total Work** generated over the 35 second period.

Figure 10 (next page, top left) shows an example of the raw data as generated by the accelerometers, which shows “positive” excursions (Z direction – upwards - contraction) as well as “negative” excursions (Z direction – downwards - relaxations).

Figure 11 (next page, top right) shows an example of the data which had been generated by the accelerometers, then converted to work. Both the negative and positive excursions in the previous graphic are shown as work (positive) and used later to calculate TOTAL WORK. Figure 12 shows an example of the work data summed cumulatively, to calculate TOTAL work. Both the negative and positive excursions in the previous graphics are used to calculate TOTAL WORK.

Figure 10. Example of raw data generated by the accelerometers.

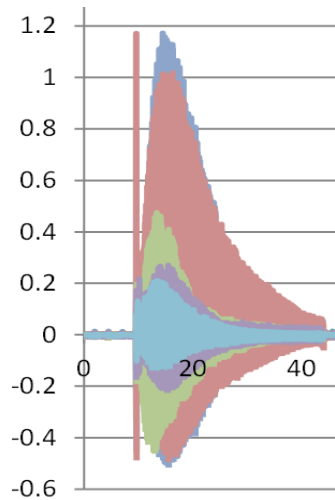


Figure 11. Example of the accelerometer raw data converted to Work. The figure shows an example where the x-axis is in seconds and the y-axis is in units of work (Joules .sec⁻¹). For further details, see text and Figure 10.

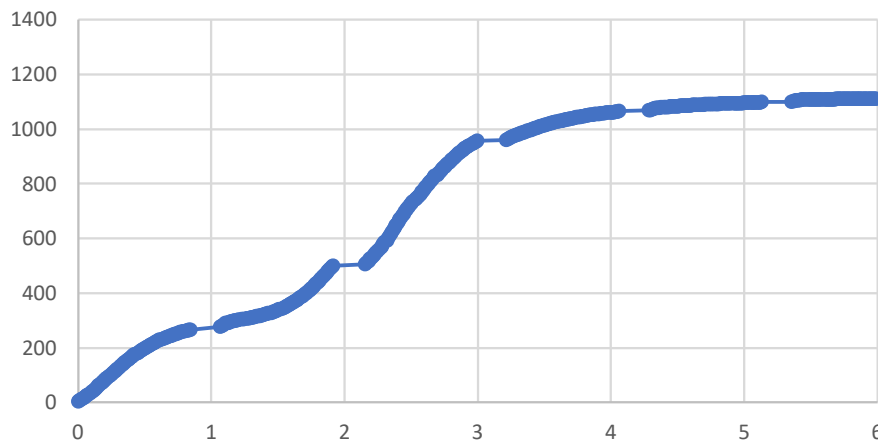
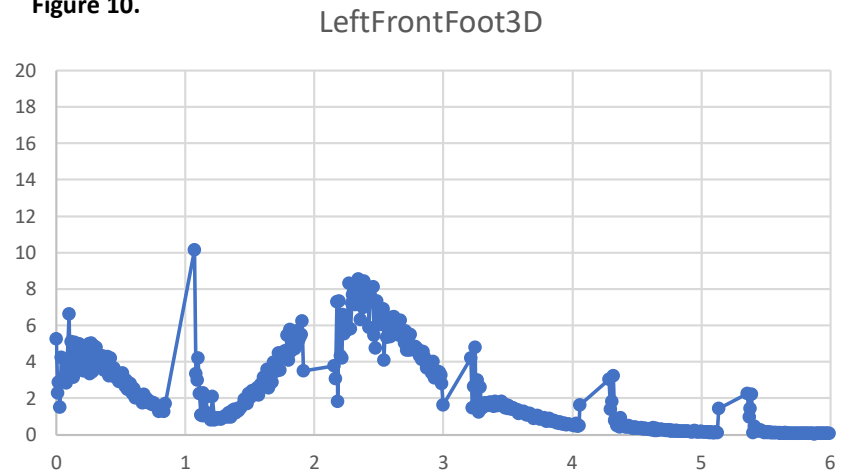


Figure 12. Example of Cumulative Work for Run 1. The figure exemplifies cumulative work data. The work caused by each individual HEMI stimulus is added to the prior sum of work. The x-axis is in seconds, the y-axis is in units of work (Joules .sec⁻¹), Note most of the increment in work occurs in the first 15-20 seconds.

Figure 13. Example of the work data generated in each 5 second period. The work caused by each individual HEMI stimulus in each train is added to the prior sum of work of the specific train of HEMI stimuli, where the x-axis is in seconds and the y-axis is in units of work (Joules .sec⁻¹).

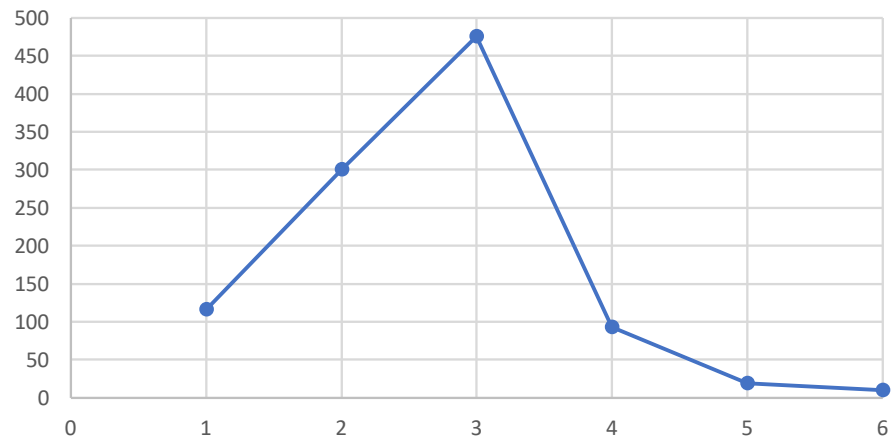
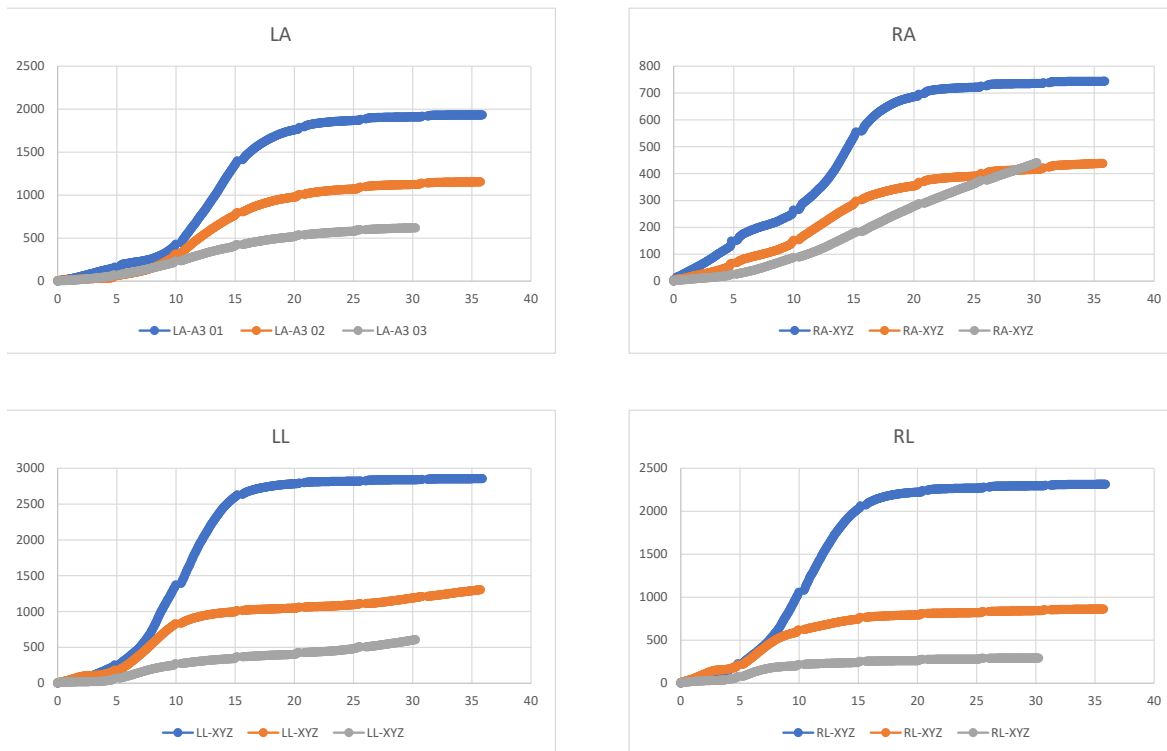


Figure 14 shows an example of the work data summed cumulatively, to calculate TOTAL work. Both the negative and positive excursions in the previous graphics are used to calculate TOTAL WORK.

Figure 14: Example of Cumulative Work for Run 1, Run 2 and Run 3. The figure shows an example of Cumulative Work for Runs 1, 2, and 3. The figure exemplifies cumulative work data. The work caused by each individual HEMI stimulus is added to the prior sum of work. The x-axis is in seconds, the y-axis is in units of work (Joules .sec⁻¹), the upper line (blue) is Run 1, the middle line (orange) is Run 2, and the lower line (gray) is Run 3. Note most of the increment in work occurs in the first 15-20 seconds. For further details, see Figure 10.



A3 01 02 03 5000 200

Overall Total Work

This section shows the results of the total work generated for a given limb over ± 35 seconds. During this period, six HEMI trains of five seconds each were applied. The trains were interrupted by various periods, ranging from 0 to 3,000 msec. For consistency, six periods of five seconds each were used, with various periods of interruption, hence the data collections for the longer periods of interruption were more than 35 seconds. The work generated by each individual HEMI stimulus was summed over the whole period, and the total, cumulative work per limb is presented, i.e., six trains of 5 seconds each. The durations of the interruptions were used to sort the animals from the shortest duration of interruption (0 msec) to the longest interruption (3,000 msec). There were three consecutive runs of 35 seconds for each animal, designated Run 1, Run 2 and Run 3. There was an hour for rest and recovery after each run.

Run 1 - raw	Duration interruption	LA-XYZ	RA-XYZ	LL-XYZ	RL-XYZ
A13 0	0	1008.9	418.9	2327.2	1944.8
A14 0	0	1456.9	1079.8	3795.6	1067.3
A11 100	100	1424.3	597.0	2981.9	3016.3
A12 100	100	889.4	614.6	1176.5	1394.0
A3 200	200	1931.2	743.1	2853.3	2311.6
A4 200	200	2263.0	2130.0	1323.4	2549.6
A6 200	200	1383.6	1306.0	439.6	1259.7
A8 200	200	569.7	716.0	1636.5	1668.6
A1 1000	1000	927.9	232.8	1254.9	1134.6
A2 1000	1000	1309.5	697.9	1588.8	1526.2
A5 1000	1000	918.6	721.1	2159.7	2247.5
A7 1000	1000	1109.1	548.9	1338.7	1320.5
A9 3000	3000	676.0	357.2	1272.1	1611.3
A10 3000	3000	1213.5	1686.1	1502.8	2830.2
	Per Limb	1220.131	846.381	1832.211	1848.733
		463.055	532.546	889.959	641.295
	14	14	14	14	14
		128.428	147.702	246.830	177.863
		569.7	232.8	439.6	1067.3
		2263.0	2130.0	3795.6	3016.3

Table 3. Run 1 – Overall Total Work (Individual Limbs – Raw Data). The table shows the total, cumulative work for each limb over a period of 35 seconds, where:

- Run 1 raw represents the First Run of the day with the raw work data, in contrast to later tables which will show percentages.
- Axy zzzz represents the chronological sequence of the animals, while the zzzz represents the duration of the interruption between the trains of HEMI stimulation
- LA XYZ represents the left forelimb, while the XYZ indicates that the 3-D data (X, Y and Z axes of the accelerometers) has been summed in 3-D space.
- RA, LL, RL represent right forelimb, left hind limb and right hindlimb respectively.
- STDev represents standard deviation.
- SE represents the standard error of the mean.

Table 4. Run 1 – Total Work (All 4 limbs). The table shows the average of the total work generated by all four limbs for Run 1. Note the large standard deviation (46.0 %), which is shown (on the right) as a percentage to demonstrate the large variability and also to enable comparisons with other tables.

Run 1 All 4 limbs - raw data		Run 1 All 4 limbs %	
Average	1436.9	Average	100.0
STDev	765.378	STDev	46.0
Count	56		
SE	103.203		
Minimum	232.8		
Maximum	3795.6		

Table 5. Run 1 – Forelimbs compared to Hind Limbs (Raw Data). The table shows a comparison of the average (of 14 animals) of the total work generated by the smaller forelimbs (LA+RA – left forelimb plus right forelimb) compared to the larger hindlimbs (LL+RL – left hindlimb plus right hindlimb). This data is also presented as a percentage to enable relative comparisons.

- Note that the hind limbs produce significantly more work than the forelimbs ($p < 0.001$, Student's t-test)
- The table on the right shows the distribution of percentages of work generated by the forelimbs compared to the hindlimbs. For ease of comparison, the variation from 100% is presented.

Run 1 – raw	LA + RA	LL + RL	Run 1 - %	LA + RA %	LL + RL %
Average	1033.3	1840.5	Average	71.9	128.1
STDev	525.3623	761.2039			
Count	28	28			
SE	101.106	146.494			
Minimum	232.8	439.6			
Maximum	2263.0	3795.6			

Table 6. Run 1 – Work per Individual Limb. The table shows the average (of 14 animals) of the total work for each limb for Run 1. For ease of comparison, the percentage of the total work produced by each limb is also shown. For further details, see Table 5.

Run 1 - raw	LA	RA	LL	RL	Run 1 - %	LA	RA	LL	RL
Average	1220.131	846.381	1832.211	1848.733	Average	84.916	58.905	127.515	128.664
STDev	463.055	532.546	889.959	641.295					
Count	14	14	14	14					
SE	128.428	147.702	246.830	177.863					
Minimum	569.7	232.8	439.6	1067.3					
Maximum	2263.0	2130.0	3795.6	3016.3					

Overall Total Work – Run 2 Limbs

This is the second (Run 2) of the three consecutive runs of 35 seconds for each animal, with an hour for rest and recovery between each run.

Run 2 - raw	Duration interruption	LA-XYZ	RA-XYZ	LL-XYZ	RL-XYZ
A13 0	0	34.0	20.8	85.0	86.4
A14 0	0	37.9	33.8	82.7	83.0
A11 100	100	1413.3	960.3	1244.0	2016.3
A12 100	100	973.3	1159.4	1196.0	1536.8
A3 200	200	1150.7	433.9	1258.7	859.4
A4 200	200	2201.5	1977.2	1243.3	1444.5
A6 200	200	938.7	885.7	596.1	2809.5
A8 200	200	505.7	554.1	462.7	402.5
A1 1000	1000	557.4	168.0	337.4	249.4
A2 1000	1000	745.3	216.6	204.5	188.5
A5 1000	1000	1323.4	777.0	1661.6	2022.9
A7 1000	1000	1021.7	575.7	1082.3	1175.3
A9 3000	3000	1321.6	391.3	856.0	597.1
A10 3000	3000	301.2	460.2	425.9	1561.5
Per Limb		894.690	615.287	766.875	1073.803
		591.401	521.245	515.924	856.910
		14	14	14	14
		164.025	144.567	143.091	237.664
		34.0	20.8	82.7	83.0
		2201.5	1977.2	1661.6	2809.5

Table 7. Run 2 – Overall Total Work (Individual Limbs – Raw Data). The table shows the total, cumulative work for each limb over a period of 35 seconds, where:

- Run 2 raw represents the Second Run of the day, showing the raw work data, in contrast to later tables which will show percentages.
- For further explanation, see Table 3

Table 8. Run 2 – Total Work (All 4 limbs). The table shows the average work generated by all four limbs for Run 2. Note the large standard deviation (72.8%), which is also shown as a percentage to demonstrate the large variability and to enable comparisons with other tables. The standard deviation (72.8%) is larger than for Run 1 (46.0%). For further explanation, see Table 5.

Run 2 All 4 limbs - raw data		Run 2 All 4 limbs %	
Average	837.7	Average	100.0
STDev	642.066	STDev	72.8
Count	56		
SE	86.576		
Minimum	20.8		
Maximum	2809.5		

Table 9. Run 2 – Forelimbs compared to Hind Limbs (Raw Data). The table shows a comparison of the total work generated by the smaller forelimb compared to the larger hind limbs. This data is also presented as a percentage to enable relative comparisons. There is less of a difference between the forelimbs and the hind limbs, as compared to Run 1. Note that, while the hind limbs produce almost 20% more work than the fore limbs, this is not statistically significant. For further details, see Table 5.

Run 2 - raw	LA + RA	LL + RL	Run 2 - %	LA + RA %	LL + RL %
Average	755.0	920.3	Average	90.1	109.9
STDev	565.205	711.4299			
Count	28	28			
SE	108.774	136.915			
Minimum	20.8	82.7			
Maximum	2201.5	2809.5			

Table 10. Run 2 – Work per Individual Limb. The table shows the average (of 14 animals) of the total work for each limb for Run 2. For ease of comparison, the percentage of the total work produced by each limb is also shown. For further details, see Table 5.

Run 2 - raw	LA	RA	LL	RL	Run 2 - %	LA	RA	LL	RL
Average	894.690	615.287	766.875	1073.803	Average	106.808	73.453	91.549	128.664
STDev	591.401	521.245	515.924	856.910					
Count	14	14	14	14					
SE	164.025	144.567	143.091	237.664					
Minimum	34.0	20.8	82.7	83.0					
Maximum	2201.5	1977.2	1661.6	2809.5					

Overall Total Work – Run 3 Limbs

Table 11. Run 3 – Overall Total Work (Individual Limbs – Raw Data). The table shows the total, cumulative work for each limb over a period of 35 seconds, where Run 3 raw represents the Second Run of the day with the raw data, in contrast to later tables which will show percentages. For further explanation, see Table 3.

Run 3 – raw	Duration interruption	LA-XYZ	RA-XYZ	LL-XYZ	RL-XYZ
A13 0	0	25.3	22.9	110.1	76.8
A14 0	0	31.9	26.5	92.3	68.5
A11 100	100	970.3	1101.2	1777.3	2052.4
A12 100	100	696.9	1085.2	313.3	914.4
A3 200	200	611.2	408.4	554.6	291.9
A4 200	200	2004.5	1725.0	2057.8	2472.3
A6 200	200	435.3	406.3	206.1	876.3
A8 200	200	369.1	501.1	230.7	231.3
A1 1000	1000	291.0	68.6	137.3	101.1
A2 1000	1000	478.3	298.4	125.1	124.9
A5 1000	1000	1703.4	808.7	1748.2	1695.9
A7 1000	1000	571.5	543.5	581.8	1045.8
A9 3000	3000	1575.8	397.8	663.7	441.7
A10 3000	3000	362.9	506.7	1767.2	1804.5
Per Limb		723.390	564.313	740.406	871.281
		618.730	476.372	745.937	827.280
		14	14	14	14
		171.605	132.122	206.886	229.446
		25.3	22.9	92.3	68.5
		2004.5	1725.0	2057.8	2472.3

Table 12. Run 3 - Total Work (All 4 Limbs). The table shows the average work generated by all four limbs for Run 3. Note the large standard deviation, which is also shown as a percentage (89.1%) to demonstrate the large variability and to enable comparisons with other tables. The standard deviation is larger than for both Run 1 (46.0%) and Run 2 (72.8%). For further explanation, see Table 5.

Run 3 All 4 limbs - raw data	
Average	724.8
STDev	670.439
Count	56
SE	90.402
Minimum	22.9
Maximum	2472.3

Run 3 All 4 limbs %	
Average	100.0
STDev	89.1

Table 13. Run 3 - Forelimbs compared to Hind Limbs (Raw Data). The table shows a comparison of the total work generated by the smaller forelimb compared to the larger hind limbs. This data is also presented as a percentage to enable relative comparisons. Note that, while the hind limbs produce almost 20% more work than the forelimbs, this is not statistically significant. For further details, see Table 5.

Run 3 - raw	LA + RA	LL + RL
Average	643.9	805.8
STDev	547.857	775.803
Count	28	28
SE	105.435	149.303
Minimum	22.9	68.5
Maximum	2004.5	2472.3

Run 3 - %	LA + RA %	LL + RL %
Average	88.8	111.2

Table 14: Run 3 -Work per Individual Limb. The table shows the average (of 14 animals) of the total work for each limb for Run 1. For ease of comparison, the percentage of the total work produced by each limb is also shown. For further details, see Table 5.

Run 3 - raw	LA	RA	LL	RL
Average	723.390	564.313	740.406	871.281
STDev	618.730	476.372	745.937	827.280
Count	14	14	14	14
SE	171.605	132.122	206.886	229.446
Minimum	25.3	22.9	92.3	68.5
Maximum	2004.5	1725.0	2057.8	2472.3

Run 3 - %	LA	RA	LL	RL
Average	84.916	58.905	127.515	128.664

Table 15. Significant Decrements of Work from Run 1 to Runs 2 and 3. The table shows the significance of the decrement from Run 1 to Run 2, Run 2 to Run 3 and from Run 1 to Run 3. The highlighted values represent statistical significant decrements in the amount of work generated by the specific limb. “Short” represents the runs with interruptions of 100, 200, and 400 msec. “Long” represents the runs with interruptions of 1,000 and 3,000 msec. There were no significant differences for the amount of work generated between the “Short” and “Long” interruptions for individual limbs in any of the runs in Table 3, Table 7 or Table 110.

	LA-XYZ		RA-XYZ		LL-XYZ		RL-XYZ	
	Short	Long	Short	Long	Short	Long	Short	Long
P:Run 1-2	0.21	0.57	0.56	0.21	0.02	0.03	0.18	0.06
P:Run 2-3	0.55	0.88	0.77	0.96	0.79	0.81	0.58	0.83
P:Run 1-3	0.05	0.56	0.35	0.23	0.02	0.04	0.04	0.05

A Comparison of Runs 1, 2, and 3

In this section, we present in a single table, for ease of comparison, the data from all three runs. The upper portion of the table shows the work generated by the front and hind limbs for each Run. The lower left portion of the table shows the decreased amount (as a %) of work generated with Run 2 and Run 3 compared to the amount of work produced by Run 1. The work of 73.069 % generated by the LA+RA in Run 2 represents a decrement of 27% compared to Run 1. Note that the decreased percentage of work generated is greater after the first run (73.069%) compared to the second run (63.31s%).

In Run 1, the hind limbs produced much more work than the forelimbs. In Runs 2 and 3, the decrement of work was greater for the hind limbs (100 – 50.006) than the forelimbs 100-73.06). Note, the “decrement of work” is considered an indicator of “deleterious effects.”

The lower right section shows the significance of the decrement from Run 1 to Run 2, Run 2 to Run 3, and Run 1 to Run 3. The highlighted cells represent statistically significant differences (decreases) of the amount of work generated by the runs as indicated.

Table 16. Comparison of Work generated by forelimbs and hind limbs - Runs 1, 2, and 3.

	Run 1					
n=14	AVG	STDev	Counts	SE	Min	Max
LA+RA	1033.3	525.362	28	14.204	232.8	2263.0
LL+RL	1840.5	761.204	28	6.067	439.6	3795.6
	Run 2					
n=14	AVG	STDev	Counts	SE	Min	Max
LA+RA	755.0	565.205	28	14.204	20.8	2201.5
LL+RL	920.3	711.430	28	6.067	82.7	2809.5
	Run 3					
n=14	AVG	STDev	Counts	SE	Min	Max
LA+RA	643.9	547.857	28	14.204	22.9	2004.5
LL+RL	805.8	775.803	28	6.067	68.5	2472.3
% work	LA-RA	LL-RL		n=14	LA+RA	LL+RL
Run 2	73.069	50.006		P:Run 1-2	0.06	< 0.001
Run 3	62.313	43.785		P:Run 2-3	0.46	0.68
Run 2- 3	85.280	87.559		P:Run 1-3	0.009	0.001

The Value of an Interrupted HEMI Duty Cycle

One of the hypotheses of this study was that the deleterious effects would be driven by, and therefore proportional to, the amount of work generated by the muscles. We also postulated that the decrement in the amount of work generated in the second and third runs would be useful as a measure of deleterious effects. A secondary goal was to study the value of an interruption of the HEMI duty cycle.

The table shows the work generated by duty cycles with zero interruptions (0-0) compared to duty cycles ("All") with some duty interruptions (100, 200, 1,00, and 3,000 msec). The notation "0-0" represents the runs with no interruptions, and "All" represents all the other runs which did have interruptions. The lower left section shows the significance of the decrement from Run 1 to Run 2, Run 2 to Run 3, and Run 1 to Run 3. The lower right-hand section shows the p-values of the differences between the "0-0" and "All" data. The highlighted cells are considered significant.

Table 17. Comparison of Work generated by duty cycles with zero interruptions compared to duty cycles with interruptions.

All 4 limbs		AVG	STDev	Counts	SE	Min	Max
Run 1	0-0	1759.9	1054.002	8	398.375	418.9	3795.6
	"All"	1403.4	715.591	48	104.380	232.8	3016.3
Run 2	0-0	58.0	28.565	8	10.796	20.8	86.4
	"All"	967.6	601.308	48	87.710	168.0	2809.5
Run 3	0-0	56.8	34.498	8	13.039	22.9	110.1
	"All"	836.2	661.206	48	96.447	68.6	2472.3

All 4 limbs	0-0	"All"
P:Run 1-2	0.003	0.002
P:Run 2-3	0.94	0.33
P:Run 1-3	0.003	<0.001

All 4 limbs	0-0 vs "All"
P: Run 1	0.37
P: Run 2	< 0.001
P: Run 3	< 0.001

Table 18. Comparison of Work generated by duty cycles with zero interruptions compared to duty cycles with interruptions of 3,000 msec. “0-0” represents the runs with no interruptions, and “3000” represents all the other runs with interruptions of 3,000msec. The lower left section shows the significance of the decrement from run 1 to Run 2, Run 2 to Run 3, and Run 1 to Run 3. The lower right-hand section shows the p-values of the differences between the “0-0” and “3000” data. The highlighted cells are considered significant.

All 4 limbs		AVG	STDev	Counts	SE	Min	Max
Run 1	0-0	1637.4	1054.002	8	398.375	418.9	3795.6
	3000	1393.6	741.131	8	280.121	357.2	2830.2
Run 2	0-0	58.0	28.565	8	10.796	20.8	86.4
	3000	739.4	468.745	8	177.169	301.2	1561.5
Run 3	0-0	56.8	34.498	8	13.039	22.9	110.1
	3000	940.0	651.950	8	246.414	362.9	1804.5

0-0 vs. 3000	0-0	3000
P:Run 1-2	0.003	0.07
P:Run 2-3	0.94	0.50
P:Run 1-3	0.003	0.22

All 4 limbs	0-0 vs 3000
P: Run 1	0.61
P: Run 2	0.005
P: Run 3	0.006

Table 19. Comparison of Work generated by duty cycles with Short interruptions compared to duty cycles with Long interruptions. “Short” represents the runs with interruptions of 100, 200, and 400 msec, and “Long” represents the runs with interruptions of 1,000 and 3,000 msec. The runs with zero interruptions are excluded from this analysis.

All 4 limbs		AVG	STDev	Counts	SE	Min	Max
Run 1	Short	1571.2	851.479	32	152.930	418.9	3795.6
	Long	1257.7	604.195	24	125.983	232.8	2830.2
Run 2	Short	896.5	718.603	32	129.065	20.8	2809.5
	Long	759.2	527.805	24	110.055	168.0	2022.9
Run 3	Short	710.9	716.621	32	128.709	22.9	2472.3
	Long	743.5	618.107	24	128.884	68.6	1804.5

All 4 limbs	Short	Long
P:Run 1-2	0.002	0.002
P:Run 2-3	0.34	0.92
P:Run 1-3	< 0.001	0.003

All 4 limbs	Short vs Long
P: Run 1	0.11
P: Run 2	0.41
P: Run 3	0.86

Table 20: Comparison of Work generated by the forelimbs (LA+RA) with duty cycles with Short interruptions compared to duty cycles with Long interruptions. “Short” represents the runs with interruptions of 100, 200, and 400 msec, and “Long” represents the runs with interruptions of 1,000 and 3,000 msec. The runs with zero interruptions are excluded from this analysis.

LA+RA		AVG	STDev	Counts	SE	Min	Max
Run 1	Short	1214.0	622.710	12	187.754	569.7	2263.0
	Long	866.5	415.756	12	125.355	232.8	1686.1
Run 2	Short	1096.1	546.82	12	164.873	433.9	2201.5
	Long	655.0	396.18	12	119.452	168.0	1323.4
Run 3	Short	859.5	541.692	12	163.326	369.1	2004.5
	Long	633.9	503.884	12	151.927	68.6	1703.4
LA+RA	Short	Long					
P:Run 1-2	0.63	0.21					
P:Run 2-3	0.29	0.91					
P:Run 1-3	0.16	0.22					

LA+RA	Short vs Long
P: Run 1	0.12
P: Run 2	0.04
P: Run 3	0.29

Table 21: Comparison of Work generated by the hind limbs (LL+RL) with duty cycles with Short interruptions compared to duty cycles with Long interruptions. “Short” represents the runs with interruptions of 100, 200, and 400 msec, and “Long” represents the runs with interruptions of 1,000 and 3,000 msec. The runs with zero interruptions are excluded from this analysis.

LL+RL		AVG	STDev	Counts	SE	Min	Max
Run 1	Short	1884.2	836.202	12	252.124	439.6	3016.3
	Long	1648.9	506.492	12	152.713	1134.6	2830.2
Run 2	Short	1255.8	678.254	12	204.501	402.5	2809.5
	Long	863.5	633.87	12	191.120	188.5	2022.9
Run 3	Short	998.2	853.087	12	257.215	206.1	2472.3
	Long	853.1	720.233	12	217.158	101.1	1804.5
Run 1	Short	Long					
P:Run 1-2	0.06	0.004					
P:Run 2-3	0.43	0.97					
P:Run 1-3	0.02	0.006					

LL+RL	Short vs Long
P: Run 1	0.41
P: Run 2	0.15
P: Run 3	0.65

Overall Total Work % Limbs

To enable an understanding of the percentage data, we show Figure 15 as an example of the raw work data, and Figure 16 shows the same data in a percentage (%) format. Specifically, Figure 16 shows that Run 1 is indexed as 100% while Run 2 and Run 3 are shown as percentage work compared to Run 1.

Figure 15. All 4 Limbs Combined Total Work for Runs 1, 2, and 3 – Raw Data. The figure shows total work generated by all 4 limbs for Runs 1, 2, and 3. The decrements in work with the 2nd and 3rd runs of HEMI stimuli are demonstrated. The x-axis shows the Number of the Animal as well as the duration of the interruptions (from 0 through 100, 200, 1,000 and 3,000 milliseconds). The y-axis shows the Total work achieved by the 1st (blue), 2nd (orange) and 3rd (gray) runs of the day. Note the first two HEMI runs with zero interruptions: both the 2nd and 3rd runs of HEMI stimuli produce minimal work compared to the 1st run. This is much lower than any of the other animals where an interruption was employed. Note: compared to the animals with zero interruptions, all the animals with interruptions lead to significantly higher amounts of work during the 2nd and 3rd runs.

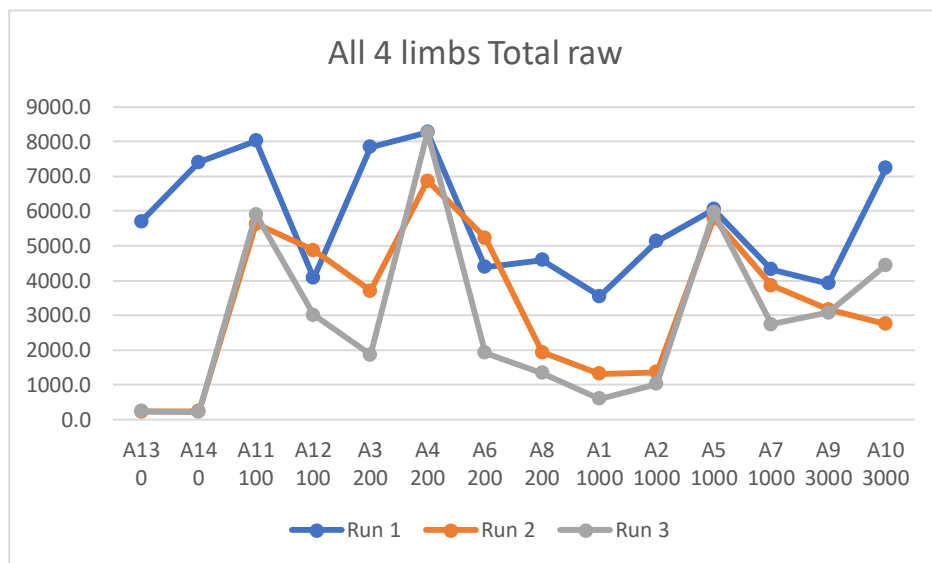


Figure 16. All 4 Limbs Combined: Total Cumulative Work for Runs 2 and 3 as a percentage of Run 1. The figure shows the decrement in work with the 2nd and 3rd runs of HEMI stimuli with the 1st run indexed at 100% for each animal. The indexing takes into account the weight and sex (M / F) of each animal. The x-axis shows the Number of the Animal as well as the duration of the interruptions (from 0 through 100, 200, 1,000 and 3,000 milliseconds). The y-axis shows the percentage of work achieved by the 2nd (orange) and 3rd (gray) compared to the 1st run of the day. The 1st HEMI run of the day (blue) has been indexed as 100%. Note the first two HEMI runs with zero interruptions: both the 2nd and 3rd runs of HEMI stimuli produce minimal work (around 2-3% of the work produced during the 1st run). This is significantly lower than any of the other animals where an interruption was employed. Note also that all the animals with interruptions lead to higher amounts of work during the 2nd and 3rd runs.

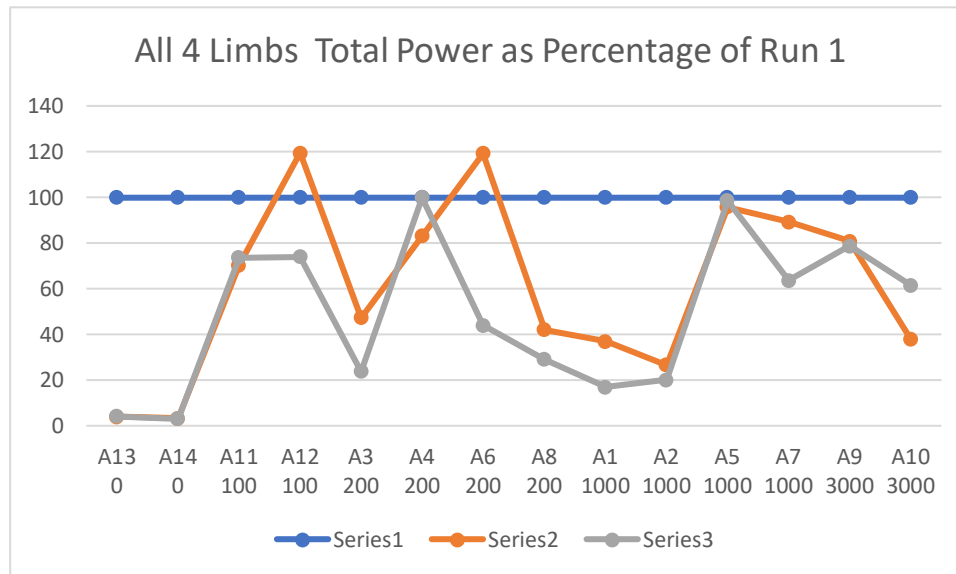


Figure 17. Forelimbs (LA plus RA). Total Cumulative Work for Runs 2 and 3 as a percentage of Run 1. The figure shows the results of the upper limbs. For further details, see Figure 16.

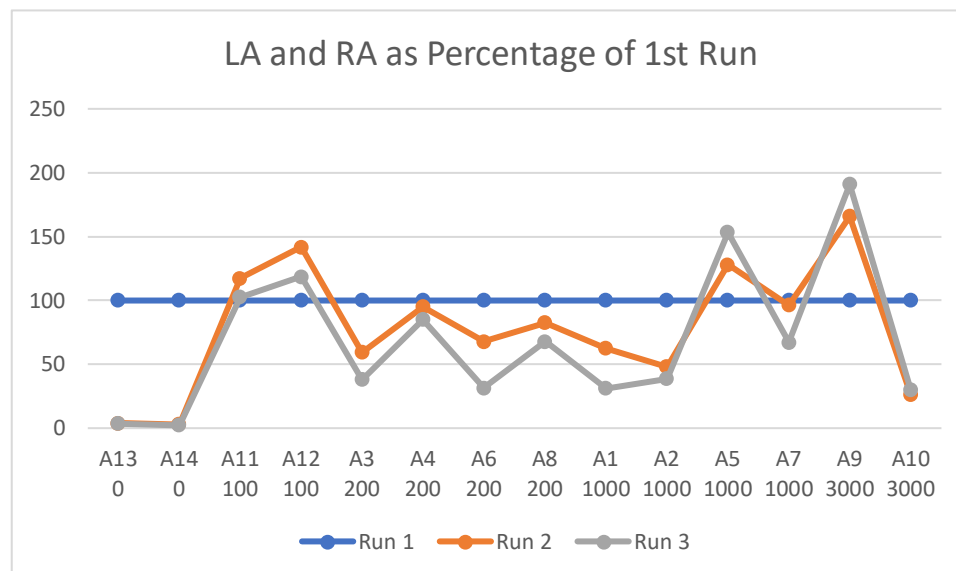
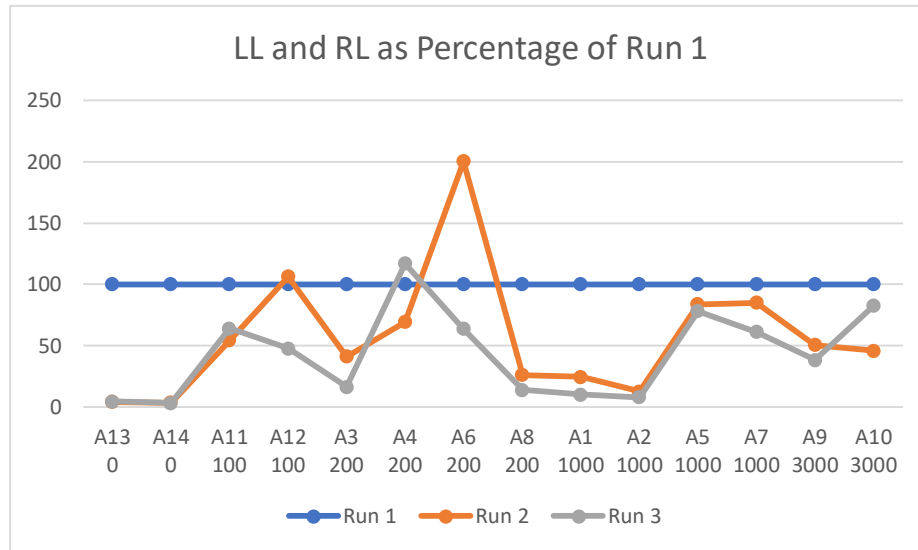


Figure 18. Hindlimbs (LL plus RL). Total Cumulative Work for Runs 2 and 3 as a percentage of Run 1. The figure shows the results of the lower limbs. For further details, see Figure 16.



The next series of figures show the decrement of Run 2 and Run 3 for each individual limb

Figure 19. Individual Limbs. Total Cumulative Work for Runs 2 and 3 as a percentage of Run 1. The figure shows the results for each individual limb. For further details, see Figure 16.

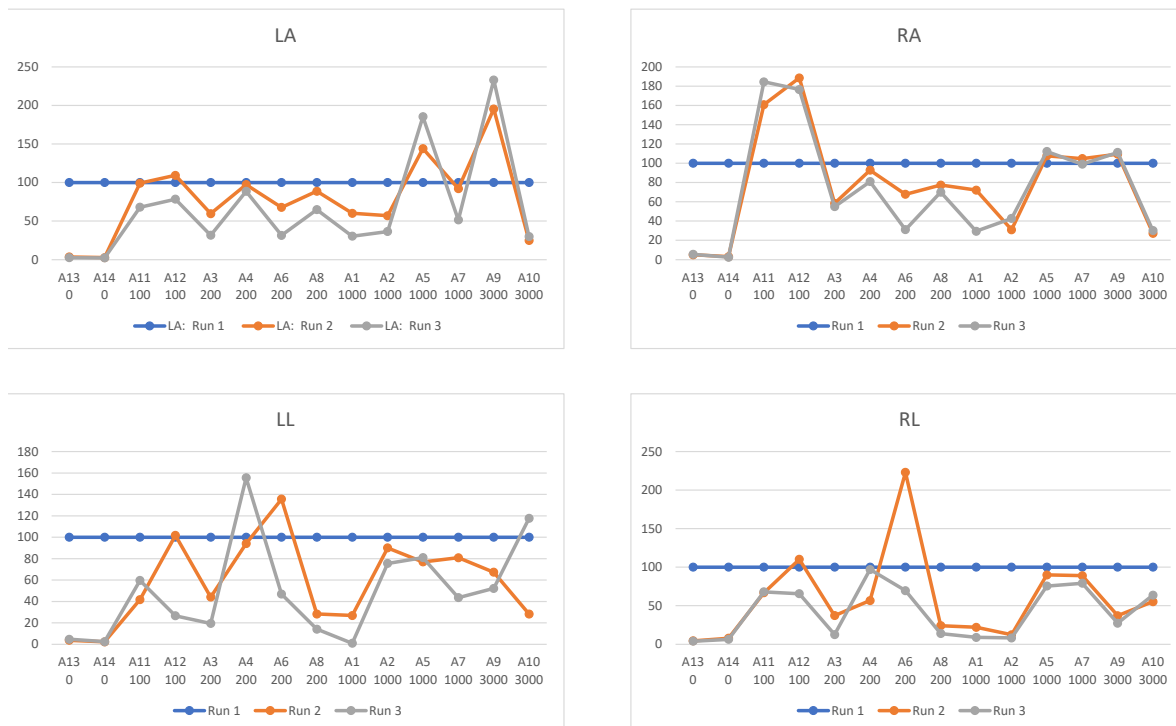
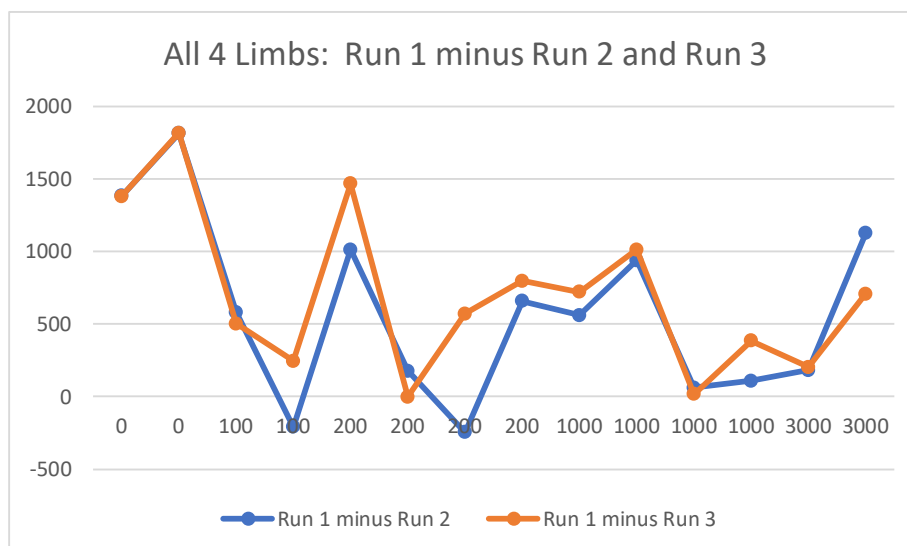


Figure 20. All 4 Limbs – Total Cumulative Work. Run 1 minus Run 2 and minus Run 3 – Raw data. The figure shows the results of the difference of total amount of work from Run 1 to Run 2 and Run 3. Interpretation: a larger difference means more inhibition of Runs 2 and 3, implying more deleterious effects. For instance, the two runs with zero interruptions had the highest levels of inhibition. For further details, see Figure 16.



Linear Fit Calculations: Total Work and Interruptions

Correlations were sought of Work with the durations of the interruptions. Calculations were performed with the raw data, as well as with the data indexed as a percentage of the overall averages.

Figure 21. Example of Linear Fit Total Work and Interruptions. The figure shows the results of the correlation of the total work (all 4 limbs) with the duration of the interruptions for run 3. See Figure 16 for the raw data set. The R^2 is 0.0199, which means that only 1.99% of the variation in the data is explicable by the durations of the interruptions. This is not statistically significant, nor clinically relevant.

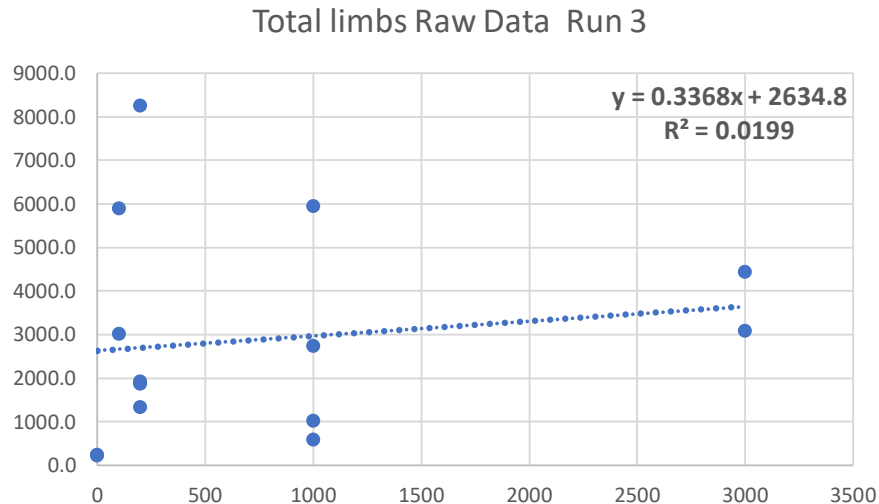
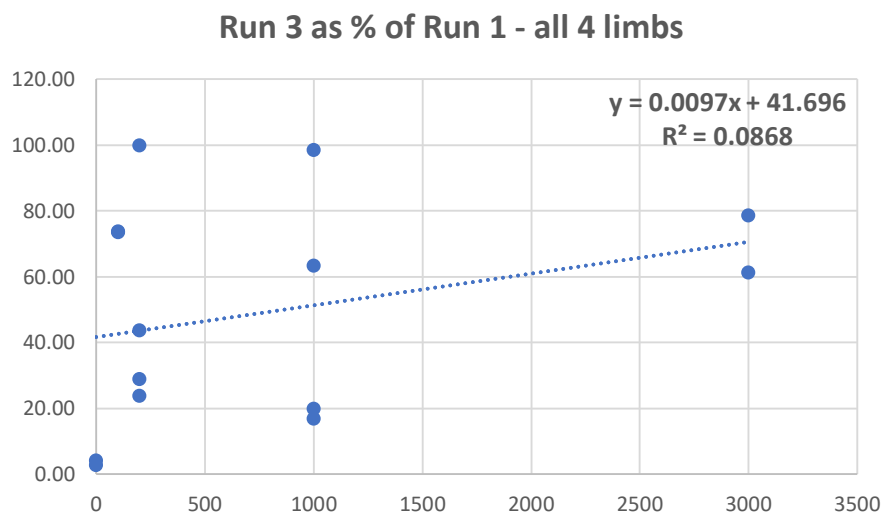


Figure 22. Example of Linear Fit as a % Total Work and Interruptions. The figure shows the results of the correlation of the percentage of total work (all 4 limbs) with the duration of the interruptions. The data for Run 3 is expressed as a percentage of Run 1. The R^2 is 0.0868, which means that only 8.68% of the variation in the data is explicable by the durations of the interruptions. While this is a higher correlation, than with the raw data in Figure 21, this is not statistically significant, nor clinically relevant.



Timing of Maximum Work: Single and Double Peaks

Clinically, in prior studies, we had noticed that in some animals, after an initial few seconds of vigorous contractions, there was a period (several seconds) of less active contractions, followed by increased contractions again for multiple seconds. The clinical impression of this pattern was confirmed by the objective measurements of work in the present study. This phenomenon was **termed a “double peak”** (see Figure 23).

Figure 23. Examples of the Generation of Single and Double Peaks of Work. The figure shows 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 (A14 01 5000-0). 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

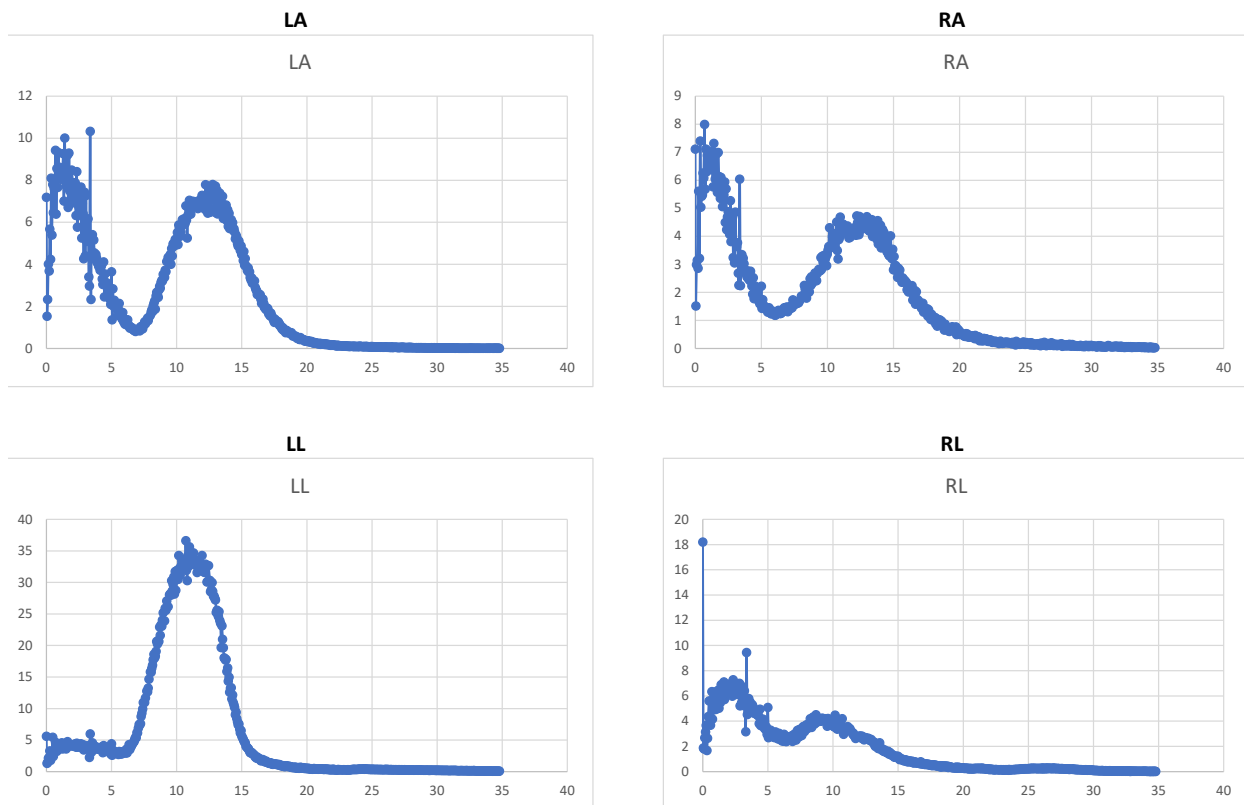


Figure 24. Example of Second Peaks with larger magnitudes than first peaks. The figure shows a different pattern of the maximum peaks of work. The HEMI stimulus was 35 seconds of HEMI stimulation, with interruptions of 1,000 msec (A5 01 5000-1000). The second peak in each of these two limbs was of a higher magnitude than the first peak. Note that in the left-hand figure (LL), there is a marked increase in work after the pause. This is an example of post-tetanic facilitation. For more details, see Discussion.

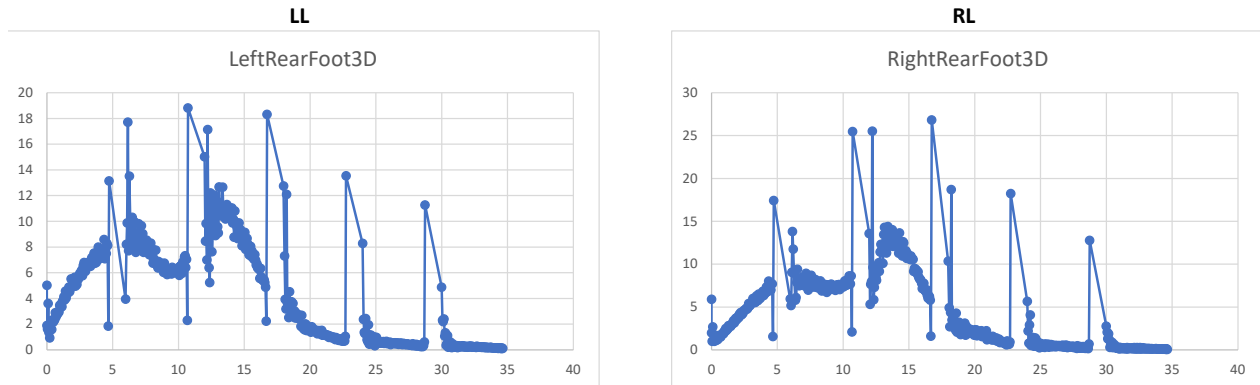


Table 22. Incidence of Single and Double peaks. The LA and RL (highlighted in gray) are the limbs closest to the HEMI probes. LA: A statistically significant majority (10 of 14) of the second peaks in the LA (closest to the HEMI probe) were larger than the 1st peaks compared to the RA which was further away from the probe ($p=0.02$, Fisher Exact Probability Test, two tailed). RA: Six of the 9 second peaks in the RA (further away from the HEMI probe) were larger than the 1st peaks. LL: Two of the 3 second peaks in the LL (further away from the HEMI probe) were larger than the 1st peaks. RL: Three of the 7 second peaks in the RL (closest to the HEMI probe) were larger than the 1st peaks. The forelimbs had a statistically significantly larger number of instances of double peaks compared to the hind limbs ($p<0.01$, Fisher Exact Probability Test, two tailed.) (see Discussion for further explanation and hypotheses).

	LA	RA	LL	RL	Forelimbs	Hind Limbs
Single	0	5	11	7	5	18
Double	14	9	3	7	23	10

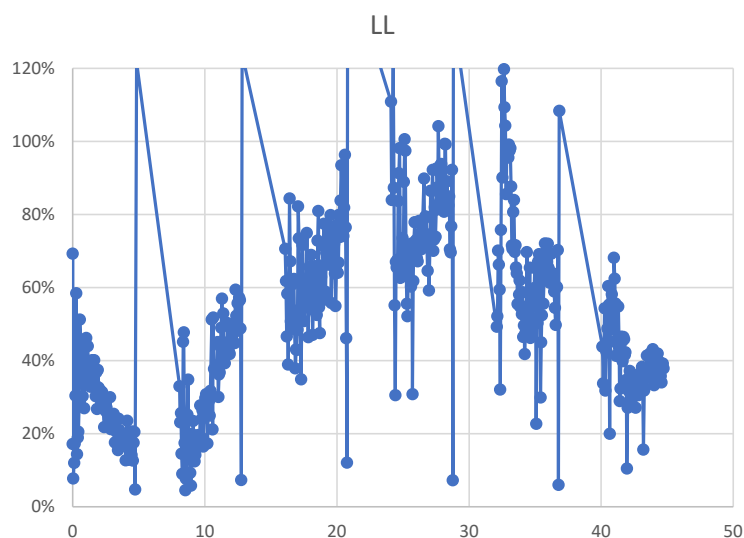
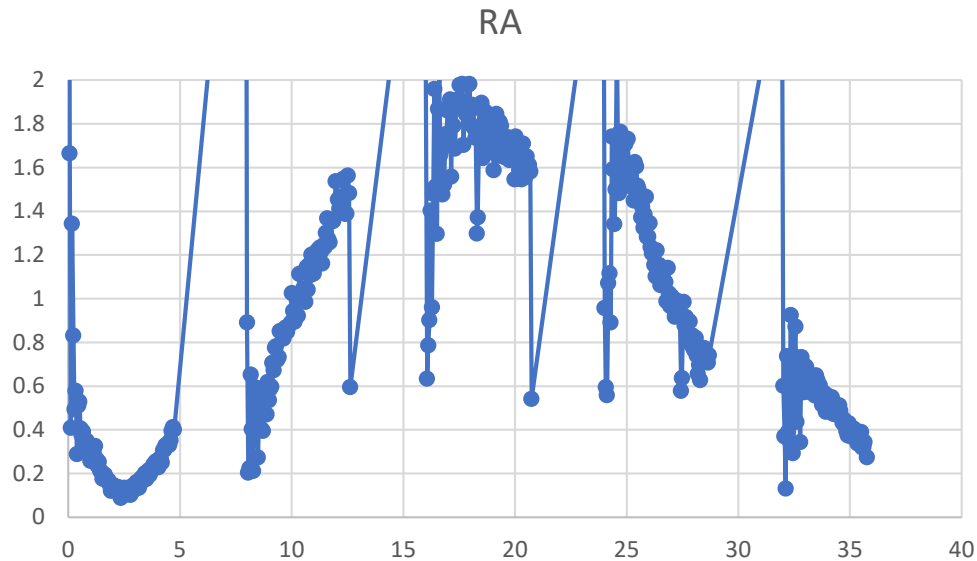


Figure 25. The Second Peak is generated later with longer interruptions. The figure shows the results of HEMI stimulation with an interruption of 3,000 msec. (A10 01 5000-3000.) There is a very early first peak (2-3 sec), with the second peak occurring at ± 30 seconds, which was almost twice the time at which the secondary peaks appeared in HEMI stimuli with shorter durations.

Figure 26. Longer Durations of interruptions result in maintenance of generated work for a longer period. The figure shows that, with interruptions of 3,000 msec, there still is a significant amount of work being generated at the end of the run of 35 seconds. Compare the almost zero amount of work in Figure 24 with interruptions of 1,000msec. The figure also shows that post-tetanic facilitation is still present towards the end of the run at 25 and 52 seconds. For further explanation, see text.

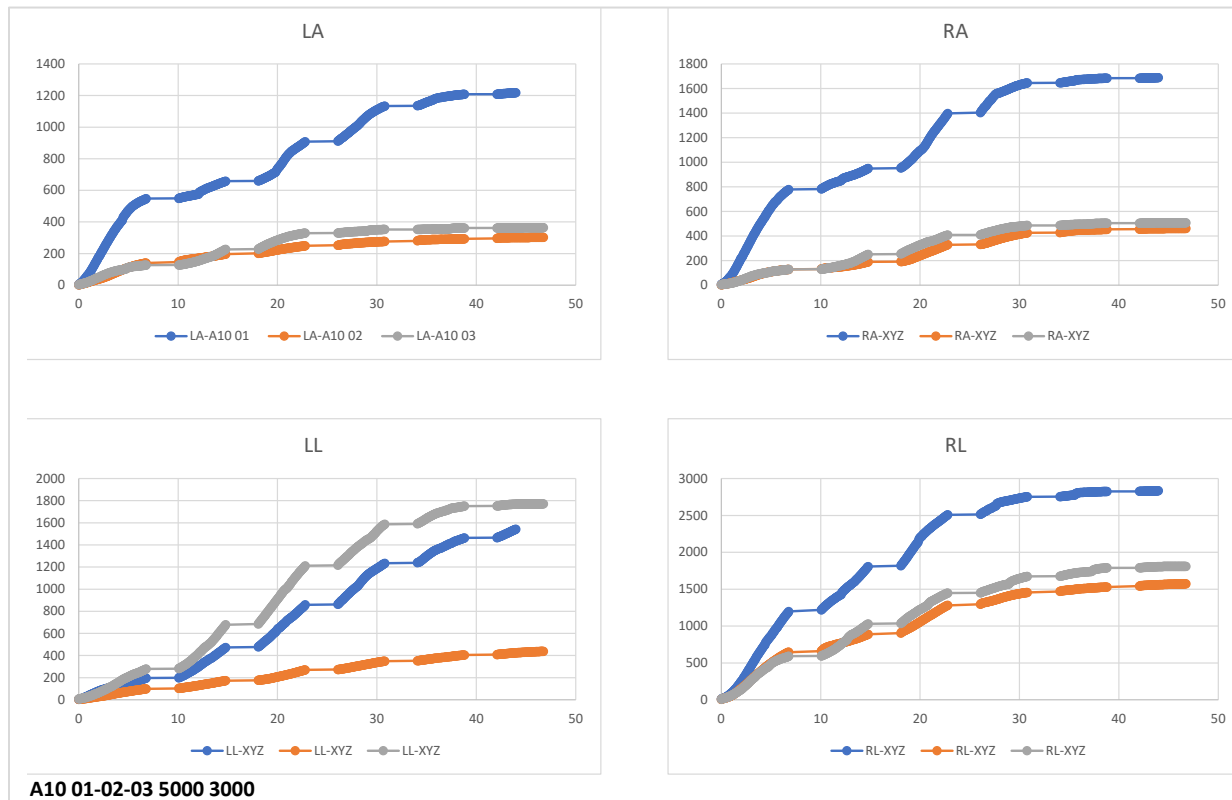


Note that in Figure 26, after each of the 3,000 msec interruptions, the next train of HEMI stimuli rapidly builds up to a higher level than at the end of the previous train. This is the phenomenon of Post-Tetanic Facilitation (PTF.) The post-tetanic facilitation with longer durations of the interruption (3,000 msec) is still present towards the end of the run (see Discussion section for further explanations). Also note that with interruptions of 3,000 msec, there still is a significant amount of work being generated at the end of the run of 35 seconds. This is in contrast with shorter durations of the interruptions, where the amount of work being produced towards the end of the run (at 35 seconds) is approaching zero; and the magnitude of post-tetanic facilitation with the shorter durations of the interruptions is much less, and not present towards the end of the run (see Figure 23).

Sequential Runs of Cumulative Work as a Measure of Deleterious Effects

The results show the feasibility of using the concept that a repeated run of HEMI stimuli can demonstrate that the limbs typically perform less work in a subsequent run compared to the first run (also see Figure 14). Here, we demonstrate variations in the patterns.

Figure 27. Sequential Runs as a Measure of Deleterious Effects. The figure shows instances where Run 3 generated more total work than Run 1. The bottom left panel (LL – left hind limb) shows an instance (bottom left panel) where Run 3 (gray) generated more work than Run 1 (blue) throughout the Runs of 35 seconds. The interruptions (“breaks in the data stream”) in this case were 3,000 msec.



While Figure 14 shows the expected result, where Run 2 generates less work than Run 1, and Run 3 generates less work than Run 2, Figure 27 shows instances where Run 3 generated more total work than Run 1. This again demonstrates the variability between animals. Overall, as a group, the average of total work generated by Run 1 was greater than Run 2, and Run 2 was greater than Run 3 (See Table 16).

Work generated in the First 5 Seconds

We calculated the work generated in the first 5 seconds, before the first interruption. Up to this point, all 14 animals had been exposed to the same protocol. The observed variance, as objectively measured, could therefore mainly be attributed to variations in the anatomy and physiology of the animals.

The variance of the work generated in the first 5 seconds for each limb (Table 23) was calculated as was the work generated by the forelimbs (LL+RL) compared to the work generated by the hind limbs (LL+RL Table 24). Analyses looked for differences, correlations with size (weights) of the animals with the amount

of work generated, and differences in the amount of work generated in the first 5 seconds between the male and female animals. We present the results of these measurements and calculations in this section.

Table 23. Work generated in the First 5 seconds for each limb for each animal. The left forelimb (LA), which is in close proximity to the HEMI probe, does not generate a significantly different amount of work (265.0 ± 116.68 units) compared to the right forelimb (RA) which is further away from the HEMI probe (245.8 ± 142.91 units). The right hind limb (RL), which is in close proximity to the HEMI probe, does not generate a significantly different amount of work (387.7 ± 143.98 units) compared to the left hind limb (LL) which is further away from the HEMI probe (415.0 ± 173.63 units).

First 5 seconds	LA	RA	LL	RL
A1 01	120.5	62.8	413.4	268.9
A2 01	247.2	364.7	485.9	406.6
A3 01	163.9	149.2	255.1	226.6
A4 01	231.5	227.0	453.9	283.1
A5 01	149.8	106.6	470.4	389.1
A6 01	253.2	175.1	215.7	321.0
A7 01	277.1	243.6	376.6	584.8
A8 01	237.8	418.9	617.3	727.5
A9 01	145.4	119.4	254.9	422.3
A10 01	428.1	568.0	142.1	775.6
A11 01	316.4	210.3	194.2	361.2
A12 01	248.8	218.2	184.0	290.1
A13 01	341.7	169.5	192.7	254.0
A14 01	548.7	407.8	346.4	499.3
AVG	265.0	245.8	328.7	415.0
Count	14	14	14	14
STD	116.682	142.912	143.983	173.636
SE	31.184	38.195	38.481	46.406
Minimum	120.5	62.8	142.1	226.6
Maximum	548.7	568.0	617.3	775.6
% of average:	84.5	78.4	104.8	132.3
% variation - STD	44.0%	58.1%	43.8%	41.8%

Work: First 5 Seconds	LA+RA	LL+RL
AVG	255.4	371.9
Count	28	28
STD	128.4	162.6
SE	11.4	12.8
Minimum	62.8	142.1
Maximum	568.0	775.6

Table 24. Comparison of Work generated in the First 5 seconds by the forelimbs compared to the hind limbs. The Table shows the average work generated by the forelimbs (LA+RA), compared to the hind limbs LL+RL, of all 14 animals in the First 5 seconds of HEMI stimulation. The forelimbs generated significantly less work (255.4 ± 128.4 units) compared to the hind limbs (371.9 ± 162.6 units.) This difference was statistically significant ($p < 0.01$, Students t-test.) The decreased work by the forelimbs was expected, as the forelimbs have markedly less musculature, compared to the hind limbs.

Patterns of Work during the First 5 Seconds

During the First 5 Seconds of the HEMI stimulation, the response was not a continuous, uniform increase in work, as would be expected from 5 seconds of continuous, uninterrupted HEMI stimulation, prior to the first interruption. There were several variations of the patterns of increases and decreases in the amount of work generated with each individual HEMI stimulus, after the initial large movements (“jerks”) which typically occur with the onset of the HEMI stimulation (and consequent tetanization.) We identified and classified five unique patterns of the sequences of the peaks and nadirs (“valleys”) of work generated.

- PNP (Peak, Nadir, Peak). Shows an early peak of the amount of work, followed by a nadir (“valley”), which is followed by yet another peak of work – all occurring within the first 5 seconds. See Figure 28D19 (PNP-1 A8 01 RL).
- PN (Peak, Nadir). Shows initially a peak of the amount of work, followed by a nadir (“valley”), which occurs again within the first 5 seconds of HEMI stimulation. See Figure 29 (PN-2 A8 01 LA+RA).
- P (Peak). Shows only a gradual rise to a peak of the amount of work generated in the first 5 seconds. See Figure 30 (P-3 A3 01 LL+RL).
- N (Nadir). Shows only a gradual decrease to a nadir of the amount of work generated. See Figures D22 and D23 (N-4 - A10 01 LL and N-5 - A11 01 RA).
- 0 (Zero). No real change ($\Delta < 10\%$) of the amount of work after the initial large movements (“jerks”) of work with the onset of the HEMI stimulation. See Figure 33 (0-6 - A3 01 RA).

Figure 28. Example of a Peak, Nadir, and Peak of the amount of work (x-axis in seconds and y-axis in units of work). A Peak, Nadir, and Peak (PNP) of work (i.e., an early peak of the amount of work), followed by a nadir (“valley”), which is followed by yet another peak of work – all occurring within the first 5 seconds.

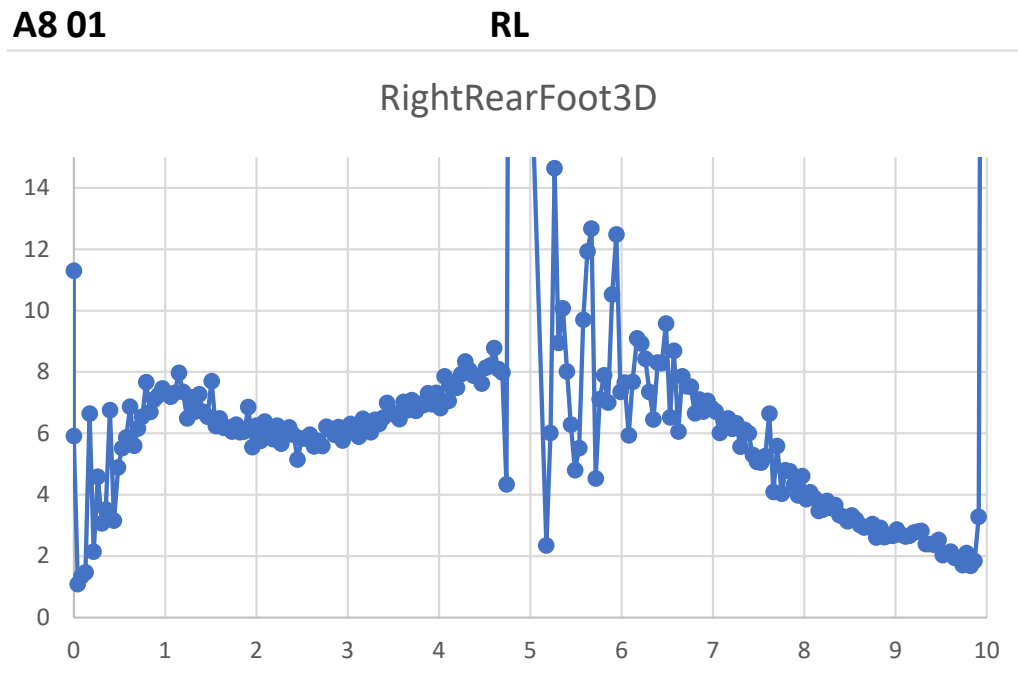


Figure 29. Example of a Peak and Nadir (PN) pattern of the amount of work (x-axis in seconds and y-axis in units of work). There is a Peak and Nadir (PN) pattern in the top two panels (LA-LeftFrontFoot3D and RA-RightFrontFoot3D). Note the contrast, in the same animal, while the top two panels had PN patterns, the lower two panels show a “P” pattern (left) and a PNP pattern (right lower panel).

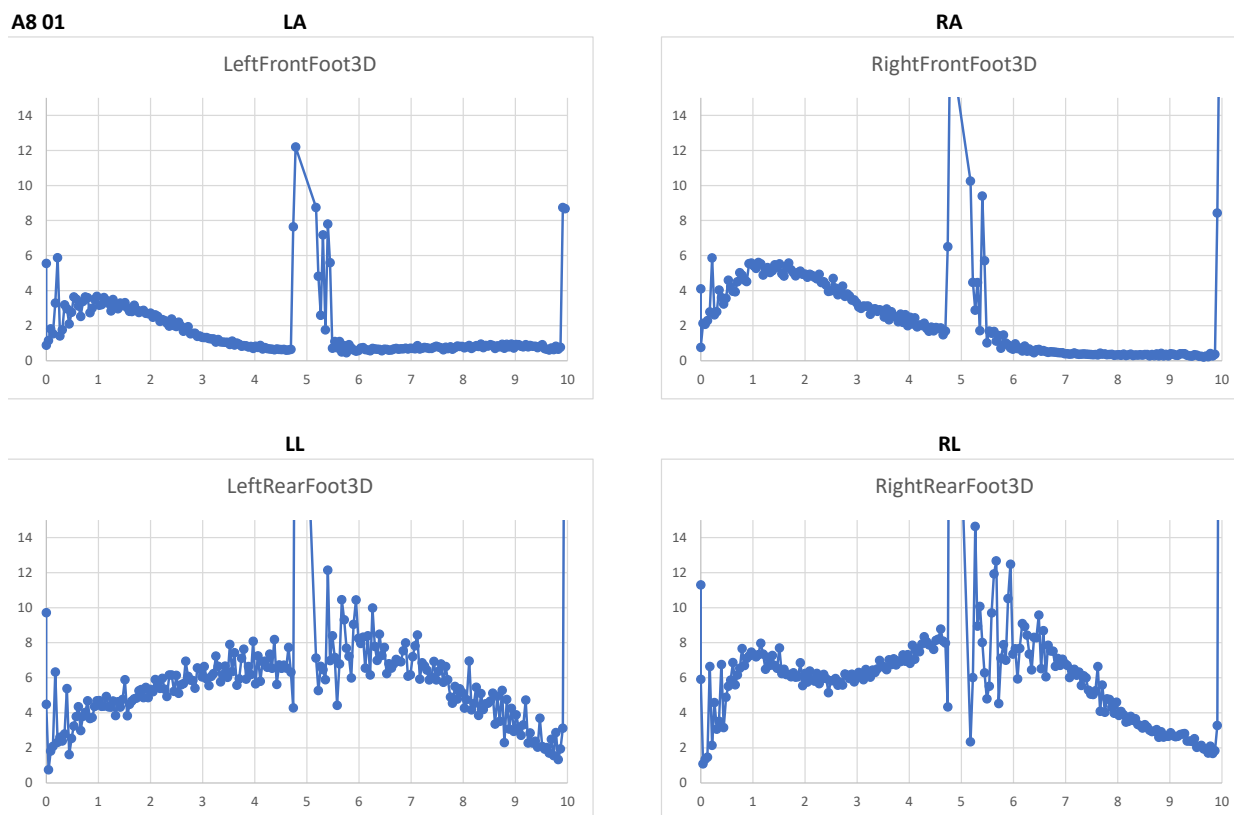


Figure 30. Example of a Peak (P) pattern of the amount of work (x-axis in seconds and y-axis in units of work). There is a gradual rise of the amount of work to a peak, generated in the first 5 seconds.

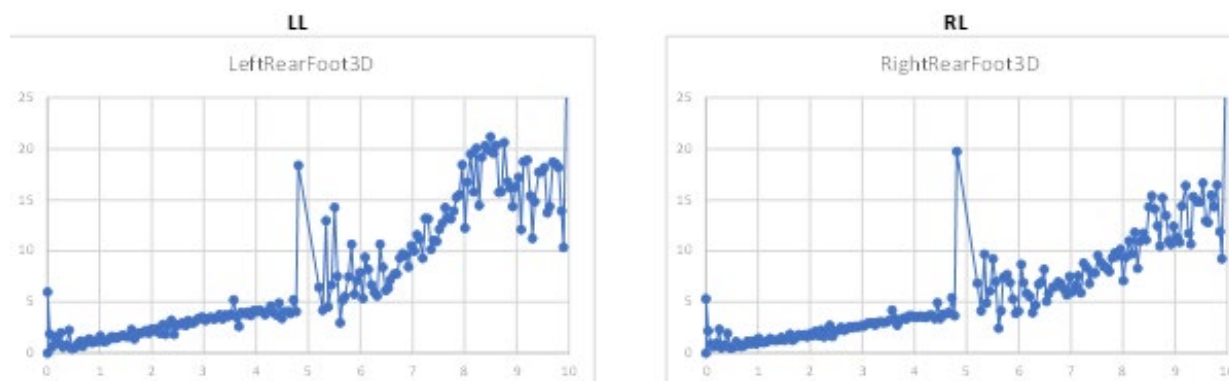


Figure 31 (below). Example of a Decrease to a nadir (N) pattern of the amount of work generated (x-axis in seconds and y-axis in units of work). This shows a gradual decrease, to a nadir “N”, of the amount of work generated in the first 5 seconds. There are typically large excursions (“jerks”) with the onset of the HEMI stimuli and the start of tetanization.

A10 01

LL

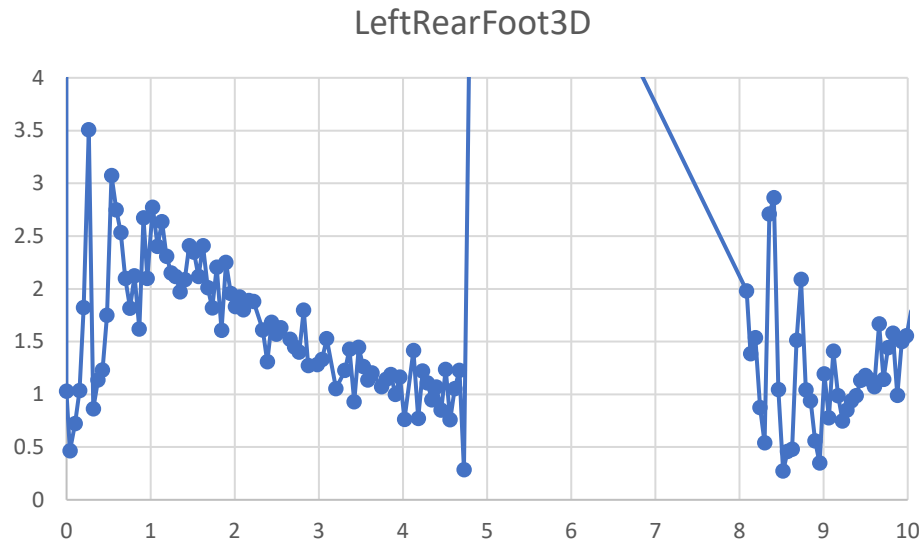
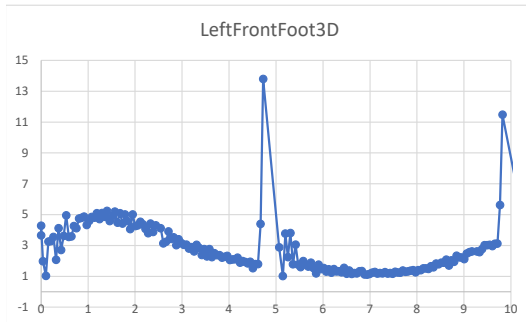


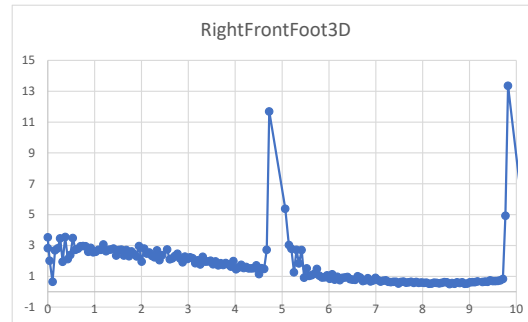
Figure 32 (below). Example of a Decrease to a nadir of the amount of work generated (x-axis in seconds and y-axis in units of work). In the upper right panel, the figure shows a gradual decrease of the amount of work generated in the first 5 seconds to a nadir “N”, after the first large excursions with the onset of the HEMI stimuli. The other panels demonstrate the wide variations of patterns in a single animal in the various limbs. For instance, the lower left panel demonstrates a “P” (Peak) pattern, where there is minimal increase early on, with the peak only developing towards the end of the 5 second period.

A11 01

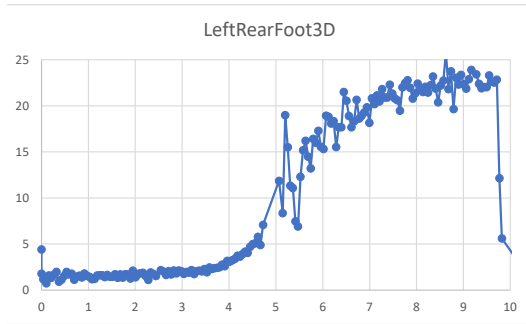
LA



RA



LL



RL

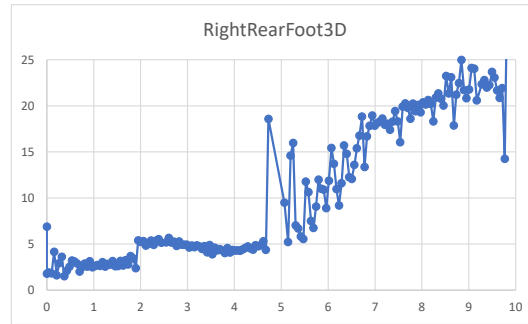
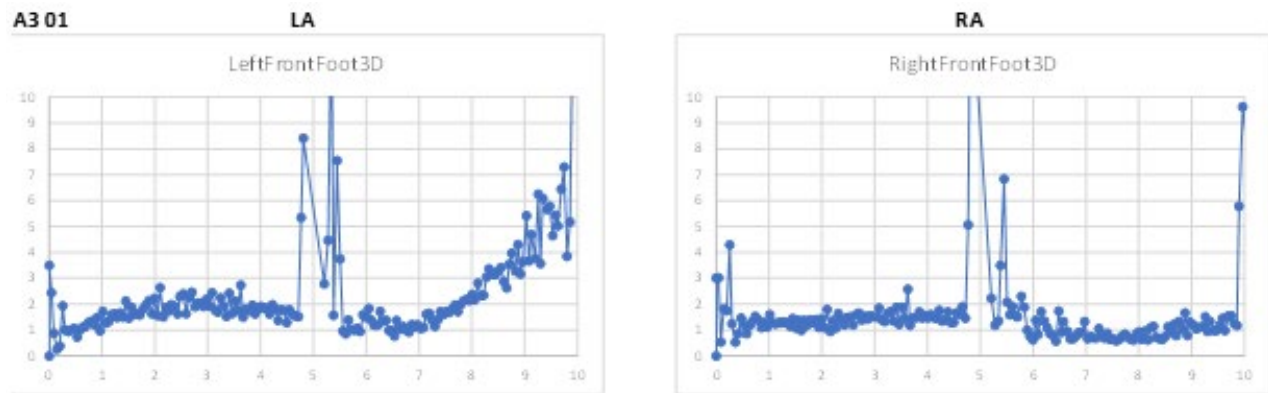
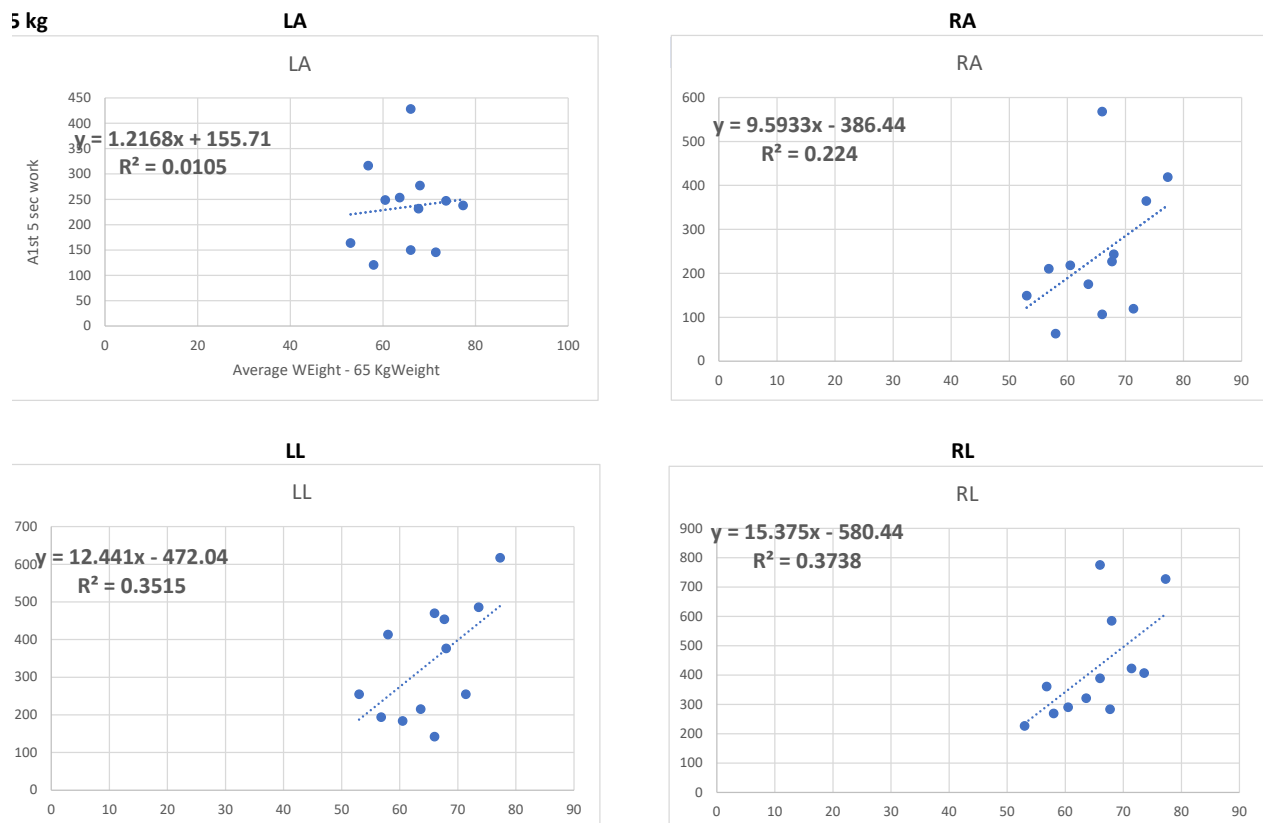


Figure 33. Example of No real change (delta < 10%) of the amount of work (x-axis in seconds and y-axis in units of work). In the panel on the right, the figure shows “No real change” (“0”) of the amount of work generated after the initial large movements (“jerks”) with the onset of the HEMI stimulation. The panel on the left demonstrates one of the variations of patterns (PN) in a single animal in a different limb.



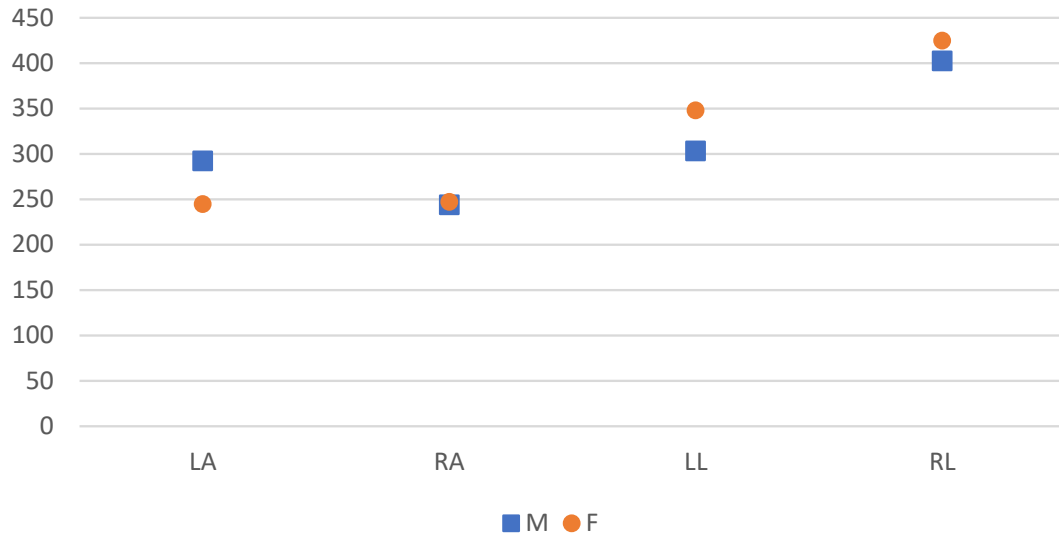
Correlations of Weight and gender with WORK generated during the First 5 seconds

Figure 34: Correlation of weight with work generated during the First 5 seconds. The figure indicates that the variation in the weights of the animals did not greatly influence the amount of work generated (e.g., for the RA, the $R^2 = 0.224$. This means that only 22.4 % of the variation is explained by the weight - 87.6 % is not).



Gender versus the amount of work generated during the first 5 seconds

Figure 35: Correlation of Gender versus the amount of work generated during the first 5 seconds. The figure shows the average amount of work generated by each limb for male and female animals. There were no significant differences between the amount of work generated by male and female animals.



Train-of-Four (TOF) Nerve Stimulation as a potential measure of deleterious effects

We programmed the HEMI device to administer four single twitches which were 500 msec apart.

Figure 36: Train of Four (TOF) twitch – Pre-HEMI stimulation. The figure shows a TOF twitch series prior to any HEMI stimulation. This is the “control” TOF with all 4 twitches expected to be essentially the same size.

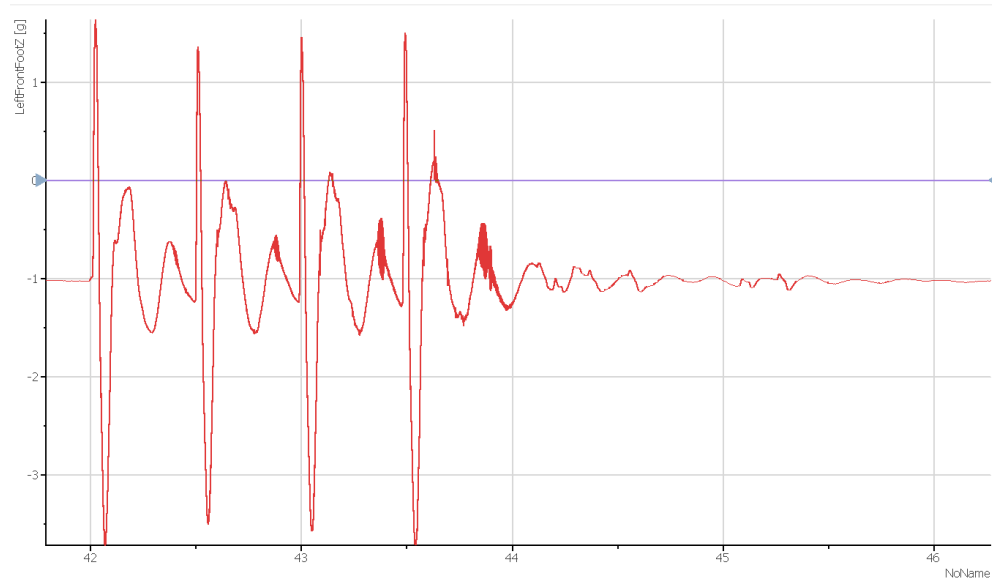
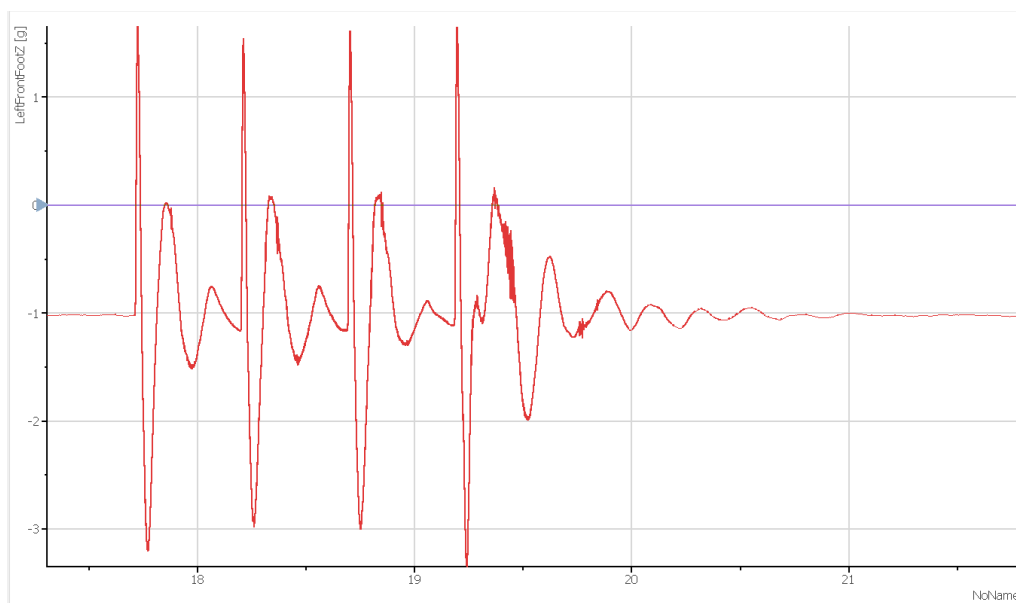


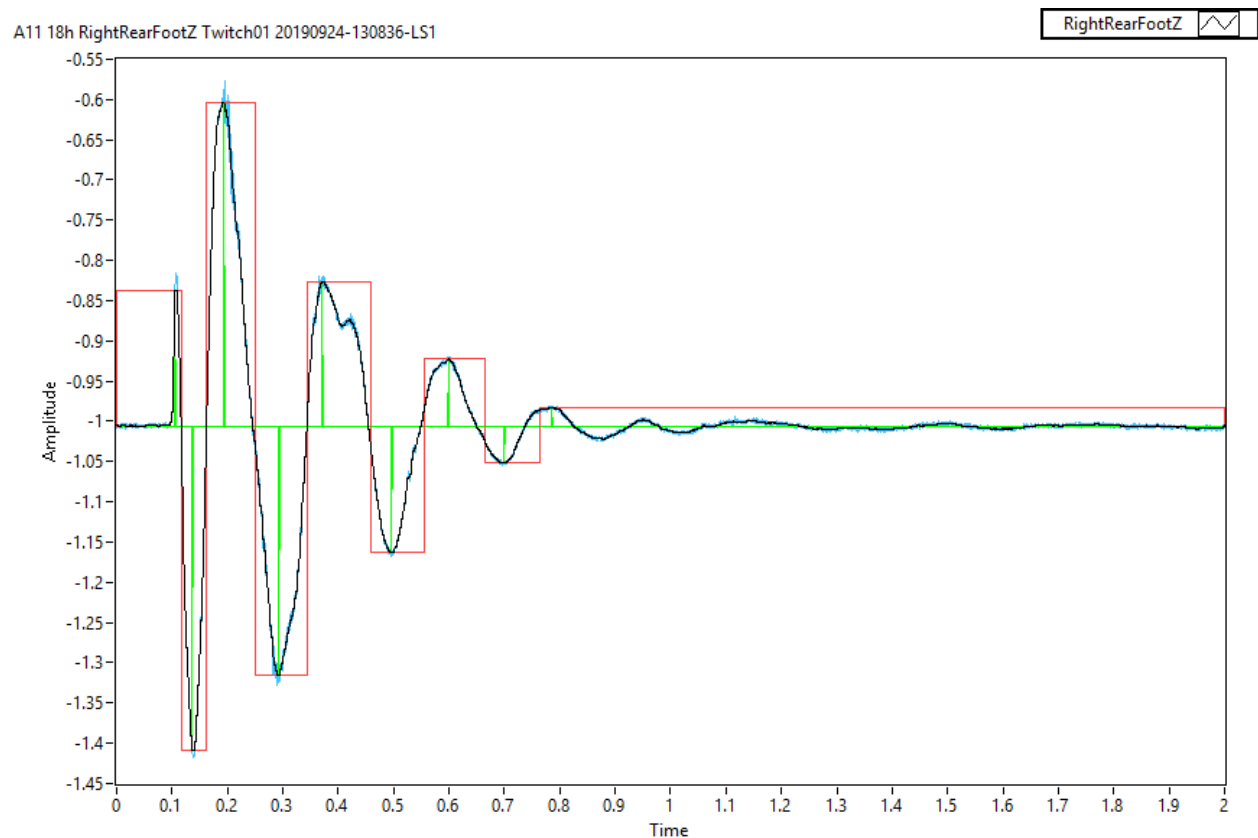
Figure 37: Train of Four (TOF) twitch – Post-HEMI stimulation. The figure shows a TOF twitch series after 35 seconds of HEMI stimulation. The Post-HEMI TOF shows all 4 the twitches as being essentially the same size, which is a similar pattern to the TOF before the HEMI stimulation. A decrement of the 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. For further discussion, see text in Discussion section.



Single Twitch (ST-1) as a potential measure of deleterious effects

We show, as a pilot, a method of automated calculation of the natural frequency of the oscillation of a limb after a single twitch. We had noticed clinically, that the limbs tended to be quite stiff with post-mortem examination. We postulated that there would be an exponential decay of the magnitude of the oscillations that could potentially be used to calculate the stiffness of a given limb, before and after HEMI stimulation, and that the stiffness could be used as a measure of deleterious effects.

Figure 38. 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 can be identified by an automated program. For further details, see Discussion.



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 (LeftFrontFoot3D).

Figure 39. 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). For further explanation, see Discussion.

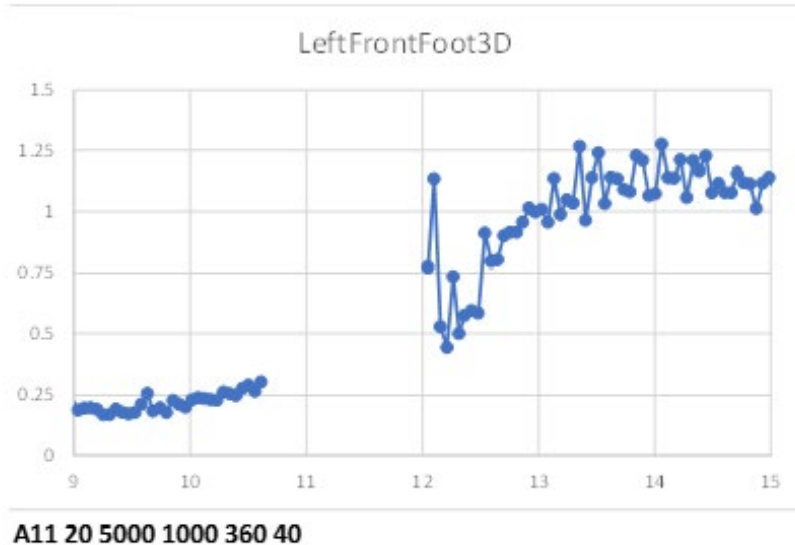
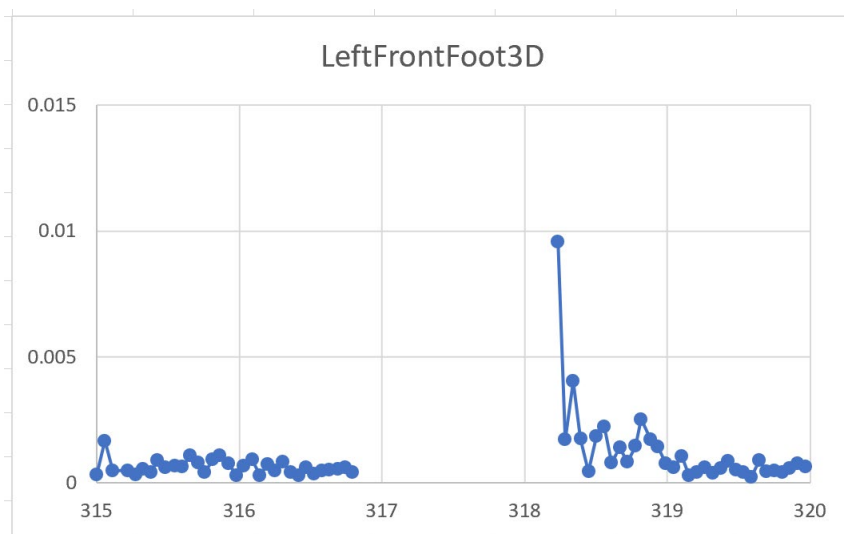


Figure 40. Post-tetanic Facilitation (PTF) – After marked fatigue has developed. The figure shows the same animal and the same run (A11 20 5000-1000 360 40) as in the previous figure. The time period shown in this figure is shortly after 300 seconds (5 minutes) of HEMI stimulation. Note the markedly 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. For further details, see Discussion.



Position of HEMI Probes

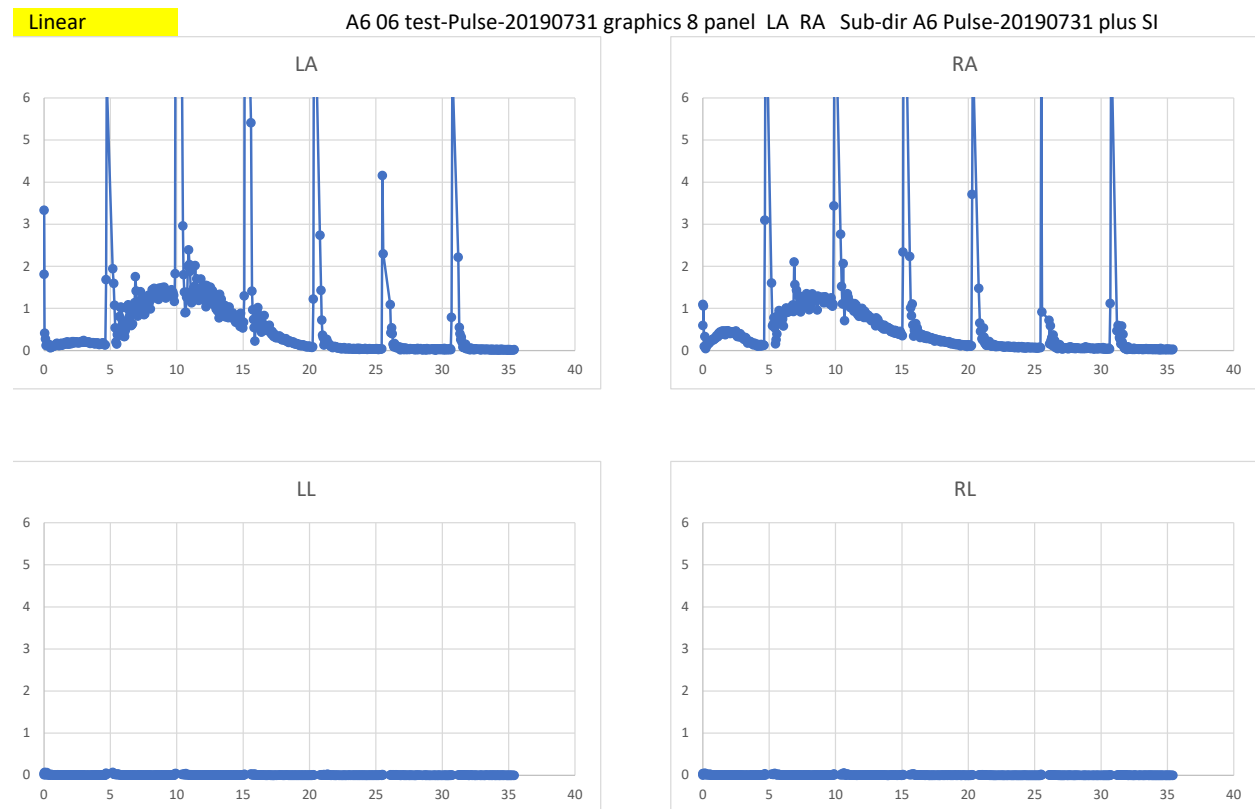
The position of the probes on primarily the forelimbs or primarily the hind limbs have a major influence on the total work imposed on the subject, and hence on the development of deleterious effects.

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 41. 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 lower limbs (legs).



The next illustration, Figure 42 shows the same data as presented in the previous figure (Figure 41), 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 42. Logarithmic data - probes placed on the forelimbs (LA and RA). The figure shows the same data 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.

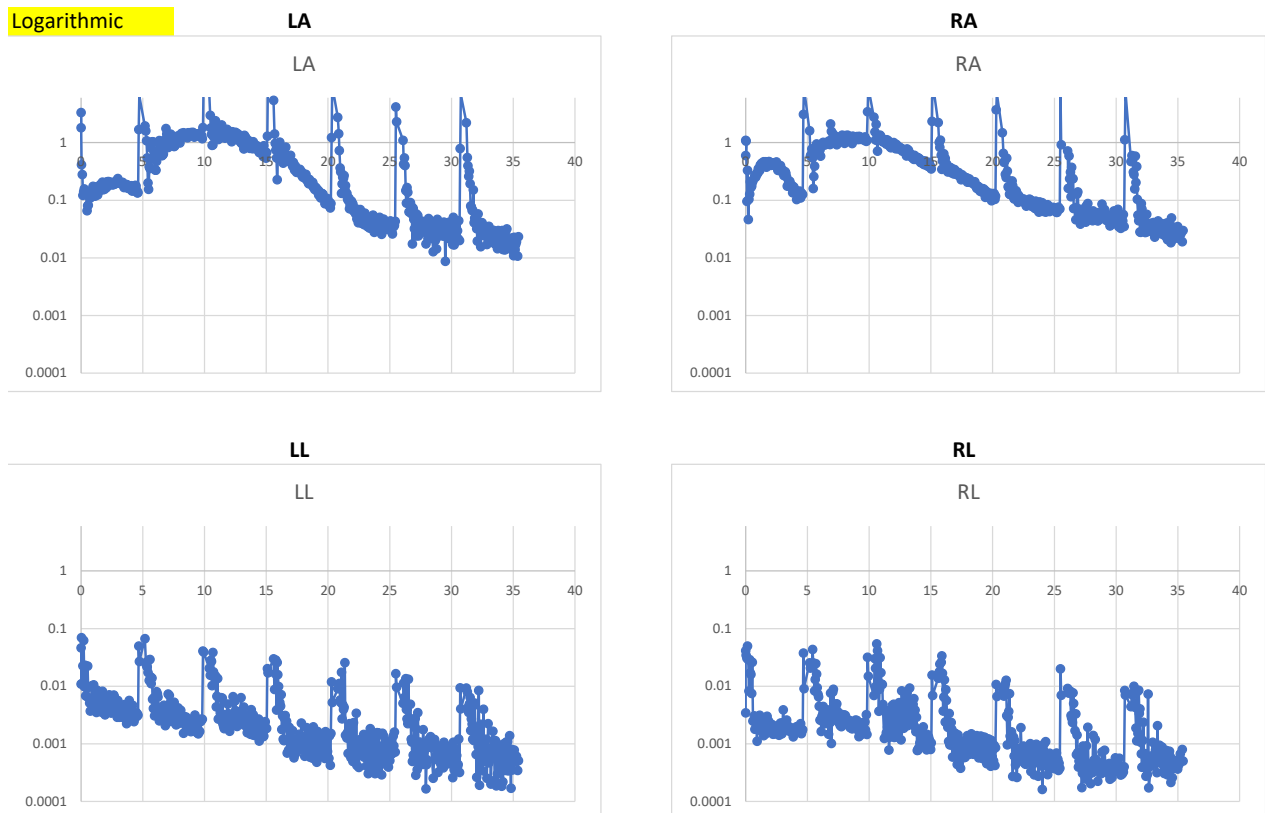
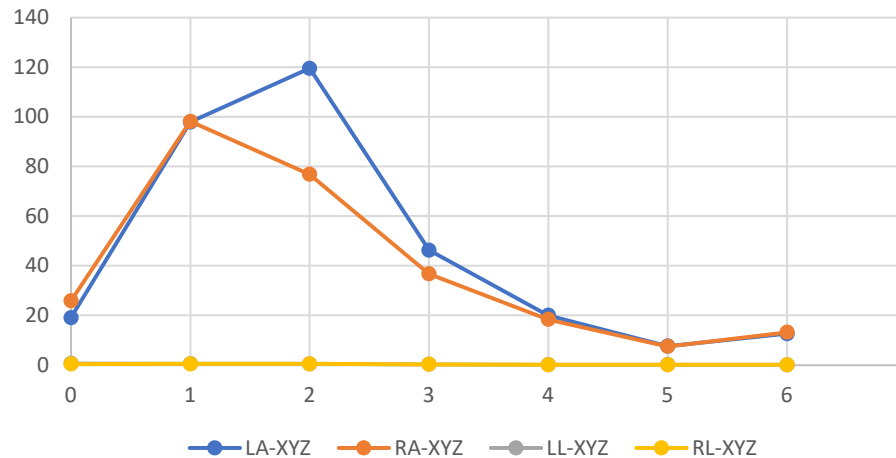
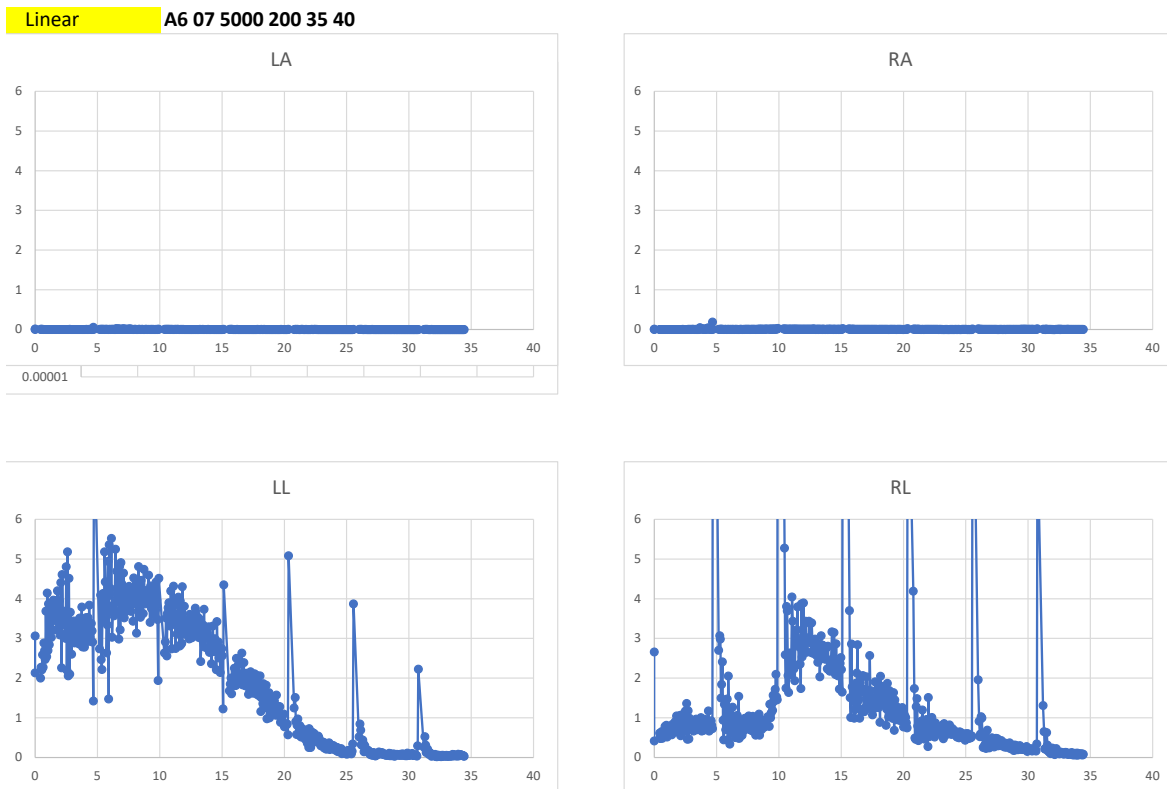


Figure 43. Work per 5 second interval - probes placed proximal to the forelimbs (LA and RA). The figure shows the total 3-D work as measured by each accelerometer over each train of 5 seconds. The probes are placed close to the upper limbs (LA and RA). Note that the work generated by the limbs proximal to the HEMI probes (LA and RA) is much greater than the work measured at the limbs distal to the probe positions. Note also the minimal work measured by the accelerometers placed “distally” (near the hind limbs).



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

Figure 44. 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 45. Logarithmic data – probes placed near the hind limbs (LL and RL). The figure shows the same data as in the prior figure except on a logarithmic scale. The work generated by both the upper and the lower limbs are clearly visualized by the objective measures. 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.

Logarithmic A6 07 5000 200 35 40

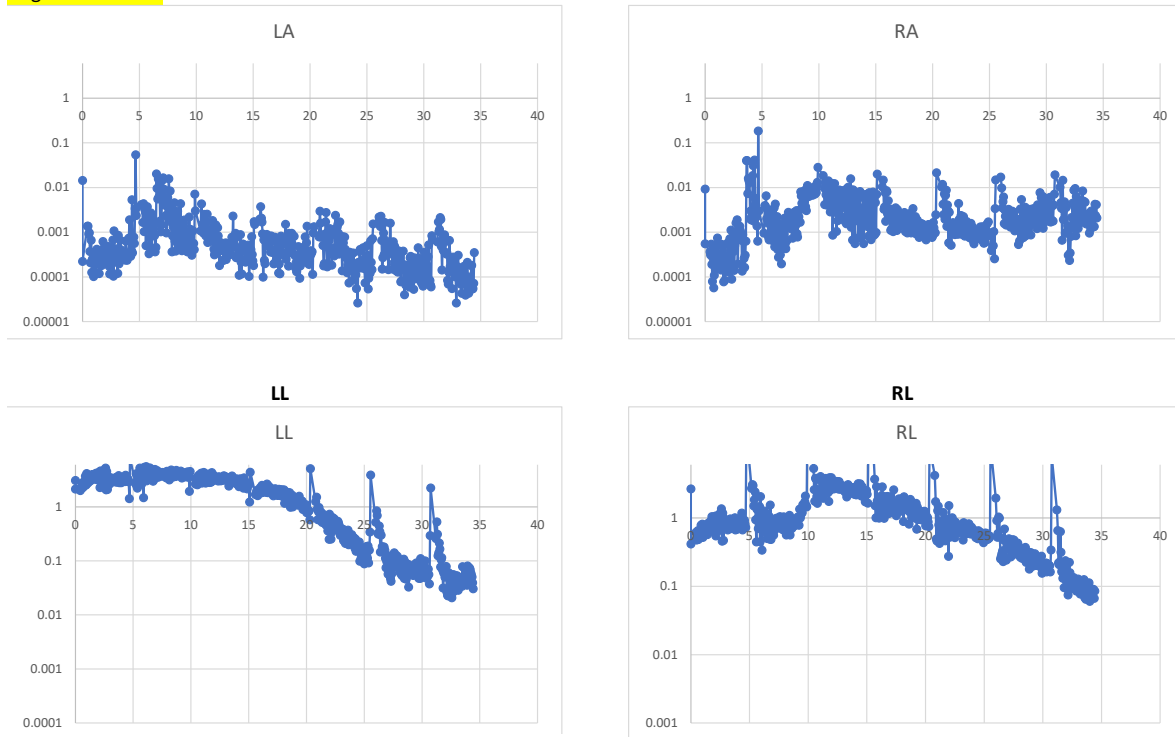
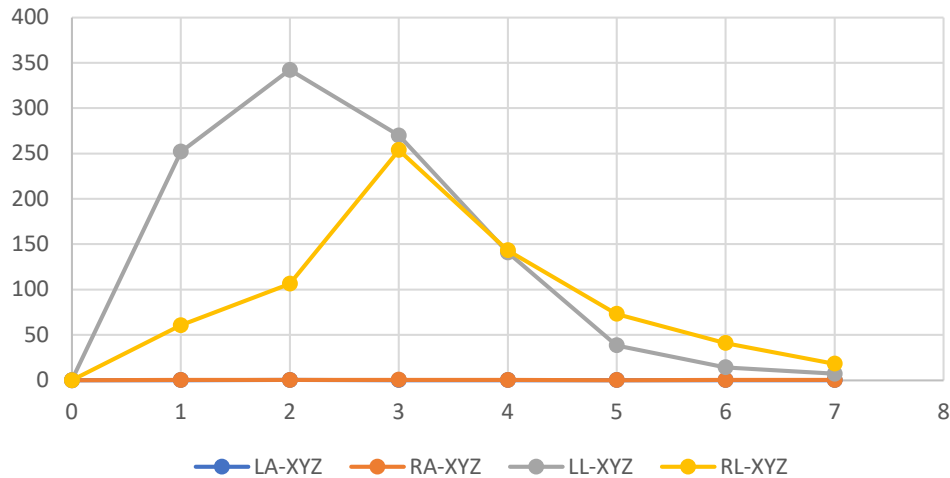
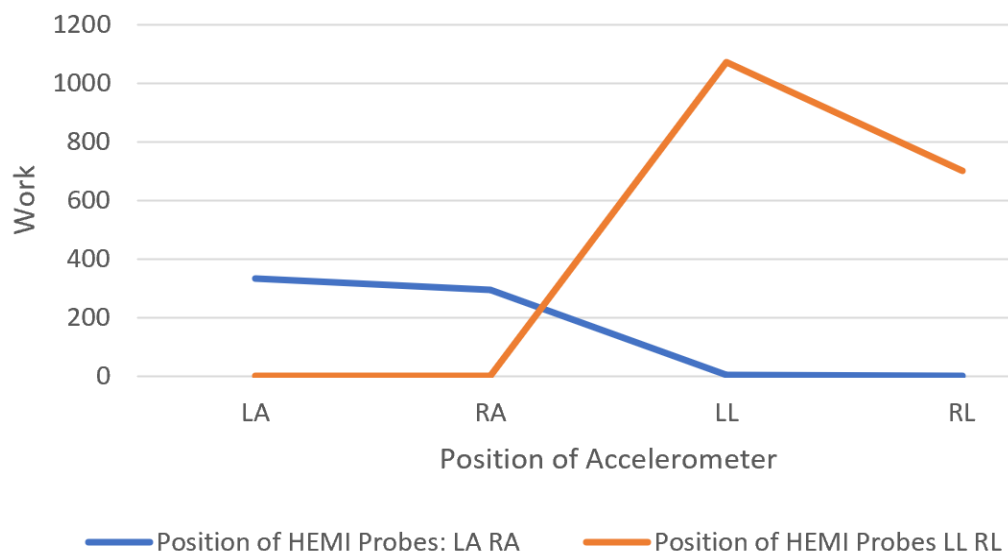


Figure 46. Work per 5 second intervals – probes placed on the hind limbs (LL and RL). The figure shows the total 3-D work as measured by each accelerometer over each train of 5 seconds. The probes are placed close to the lower limbs (LL and RL). Note that the work generated by the limbs proximal to the HEMI probes (LL and RL) is much greater than the work measured at the limbs distal to the probe positions (LA and RA). Note also the minimal work (close to zero) measured at the accelerometers placed “distally” (near the fore limbs).



The next illustration, Figure 47 shows the same experiment as presented in the previous figures. In this case, we present the total, cumulative work over 35 seconds, specifically to illustrate the marked increase in the amount of work generated by the limbs in close proximity to the HEMI probes.

Figure 47. Probe placements on the upper versus the lower limbs. The figure shows the total 3-D work as measured by each accelerometer over a 35 second period of HEMI stimulation. The position of the HEMI probes are initially placed in close proximity to the forelimbs (LL and RL) (Lower line – blue). Note that the total work generated by the limbs in close proximity to the HEMI probes (LL and RL) is markedly greater than the work measured at the limbs “distal” to (further away from) the probe positions. The position of the HEMI probes are secondly placed in close proximity to the hind limbs (LL and RL) (upper line- orange). Note that the total work, in this case, generated by the hind limbs closer to the HEMI probes (LL and RL) is markedly greater than the work measured at the limbs distal to the probe positions (LA and RA).



The next table shows, in digital format, the information from the previous figure. The variations in the probe positions showed a major difference between probes that were closer to a limb (ispi lateral) compared to where the probes were further away from the limb (contra-lateral).

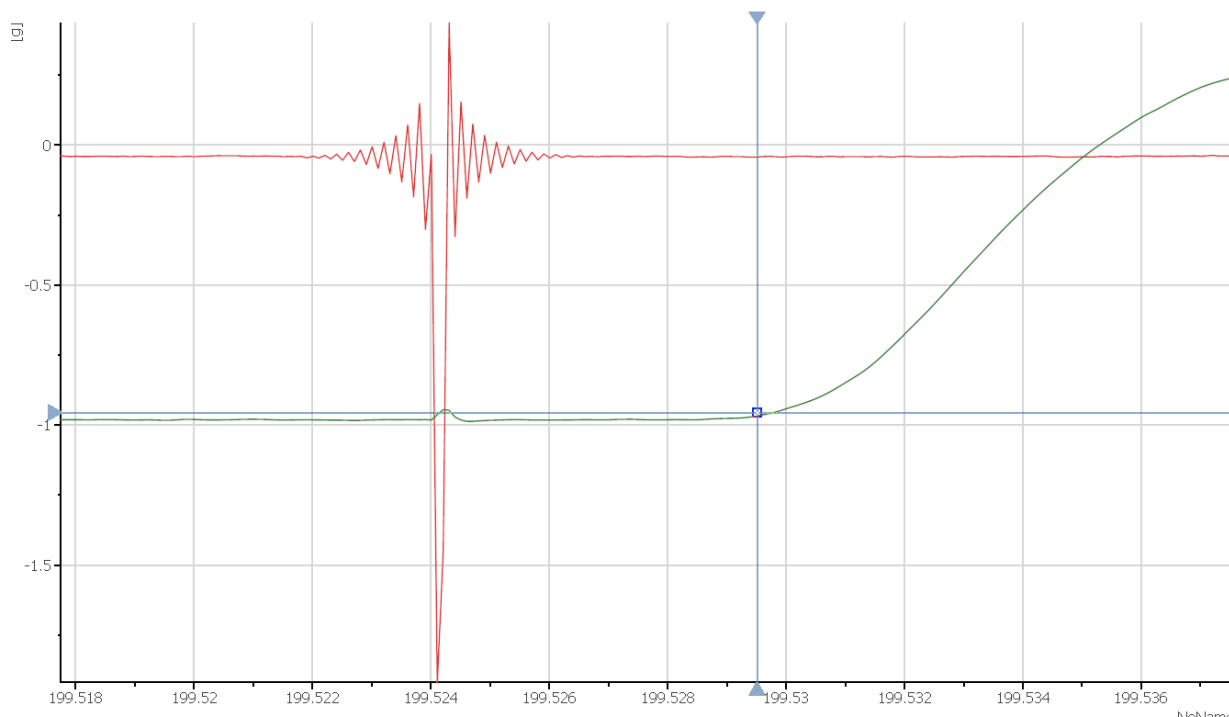
Table 25. Total Work Generated with Probe placements upper versus lower limbs. The table shows the total 3D work as measured by each accelerometer over a 35 second period of HEMI stimulation. The position of the HEMI probes are initially placed in close proximity to the forelimbs (LL an RL). Note that the total work generated by the limbs in close proximity to the HEMI probes (LL and RL) is markedly greater than the work measured at the limbs “distal” to (further away from) the probe positions. The position of the HEMI probes are secondarily placed in close proximity to the hind limbs (LL an RL). Note that the total work, in this case, generated by the hind limbs closer to the HEMI probes (LL and RL) is markedly greater than the work measured at the limbs distal to the probe positions (LA and RA).

	LA	RA	LL	RL
Position of HEMI Probes: LA RA	333.1	295.6	2.4	2.1
Position of HEMI Probes LL RL	0.6	2.0	1071.5	700.8

Phase delay between HEMI stimulus and muscle movement as a measure of deleterious effects

We present a pilot investigation as to the feasibility of measuring the Phase delay between the onset of the HEMI stimulus and the onset of muscle movement as a measure of deleterious effects

Figure 48. Phase delay between HEMI stimulus and onset of muscle movement as a measure of deleterious effects. 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). Note the phase delay as measured manually is 0.006 msec.



HEMI exposures of 360 seconds

We had noticed clinically that animals seemed to have less deleterious effects, with the second and third HEMI exposures (“runs”). We therefore decided to increase the data set from our previous HEMI report of animals exposed to 360 seconds after several prior HEMI applications.

Table 26. HEMI Exposures of 360 seconds. The table shows the series of HEMI exposures of 180 and 360 seconds, where Ax represents the consecutive number of the animals. The probe positions are LA – Left forelimb and RL-Right hind limb. The first of the long durations was 180 seconds, all the other exposures were for 360 seconds. All of the animals continued to breathe throughout the 360 second exposure, as well as after the exposure, except animal A11 where respiration ceased shortly after the 240 second mark.

Number	Train (msec)	Interruption (msec)	Total Duration	Probe positions	
A9	5000	3000	180	LA	RL
A10	5000	3000	360	LA	RL
A11	5000	1000	360	LA	RL
A12	5000	1000	360	LA	RL
A13	5000	1000	360	LA	RL
A14	5000	1000	360	LA	RL

Arterial Blood Gas (ABG) Results

The ABG and electrolyte data were analyzed in several different ways to determine if there were statistically significant differences due to the duty cycles. For instance, comparisons of shorter and longer interruptions between the trains of 5 seconds of HEMI stimuli. There were three runs of the same duty cycle in an attempt to magnify/elucidate/demonstrate any differences between the duty cycles.

The changes in the parameters of the ABGs were analyzed as follows:

- From Pre-Run 1 to Post-Run 1, Pre Run 2 to Post Run 2, and Pre-Run 3 to Post Run 3.
- During each rest period, e.g., Post Run 1 to Pre-Run 2, Post Run 2 to Pre-Run 3, as well as Post Run 3 to the next run.
- From Pre-Run 1 to Post Run 2, and **Pre-Run 1 to Post Run3**.

There was a statistically significant difference between the HEMI stimuli with “short” and “long” interruptions for the PCO₂ from pre-Run 1 to post-Run 3. Specifically, there was a statistically greater increase in PCO₂ for the duty cycles with longer interruption compared to the shorter interruptions ($p=0.049$, Fisher Exact Probability Test. Two tailed). See Table below and Discussion for more details.

There was a trend ($p=0.09$) towards an increase in the Hct from the pre-Run 1 value to the Post-Run 3 value. Even though the HEMI exposures were only 35 seconds in duration, an interesting phenomenon we noticed with this project was that the development of deleterious effects continued during the “rest/recovery” period. For instance, the pH continued to decrease, and the Hct as well as the K⁺ continued to increase.

We present the ABGs and electrolytes as measured before and after each of the 3 runs, followed by derived and calculated parameters.

ABGs Before and After each Run

We present the results of the ABG values as measured before and after each of the three runs of 35 seconds of interrupted HEMI stimuli.

Table 27. ABG Pre-run 1. Arterial blood gases before the first run of HEMI stimulation. Avg short is the average of the shorter interruptions – 0, 100 and 200 msec. Avg long is the average of the longer interruptions – 1,000 and 3,000 msec.

Pre 01	pH	PCO2	P02	BEecf	HCO3	TCO2	sO2	Lac
Avg short	7.412	47.200	124	5.125	29.663	31.250	98.500	1.585
count	8	8	8	8	8	8	8	8
StDev	0.056	9.430	20.396	1.959	2.242	2.659	0.535	0.754
SE	0.021	3.564	7.709	0.741	0.848	1.005	0.202	0.285
Min	7.307	40.6	99	2	27	28	98	0.88
Max	7.466	67.2	153	7	34	36	99	3.25
Avg long	7.427	47.083	120.167	6.333	30.933	32.167	98.667	1.372
count	6	6	6	6	6	6	6	6
StDev	0.050	3.340	27.455	2.805	2.274	2.401	0.816	0.429
SE	0.022	1.494	12.278	1.254	1.017	1.074	0.365	0.192
Min	7.376	41.4	101	3	28	29	98	0.85
Max	7.487	50.4	173	10	34	35	100	2.16

Table 28. ABG Post-run 1. Arterial blood gases after the first run of HEMI stimulation. For further details, see Table 27.

Post 01	pH	PCO2	P02	BEecf	HCO3	TCO2	sO2	Lac
Avg short	7.104	75.038	129	-6.250	23.363	25.625	95.286	18.016
count	8	8	7	8	8	8	7	8
StDev	0.061	14.934	41.392	3.370	3.197	3.583	5.155	3.232
SE	0.023	5.645	16.898	1.274	1.208	1.354	2.104	1.222
Min	6.994	59.7	68	-14	16	18	86	15.07
Max	7.183	101.0	167	-3	27	30	99	23.00
Avg long	7.059	92.100	80.200	-4.333	26.083	28.833	74.600	16.840
count	6	6	5	6	6	6	5	6
StDev	0.096	12.242	43.894	5.317	3.931	3.920	30.295	4.003
SE	0.043	5.475	21.947	2.378	1.758	1.753	15.148	1.790
Min	6.878	75.5	25	-13	21	24	25	11.10
Max	7.155	110.0	138	1	31	34	96	23.00

Table 29 : ABG Pre-run 2. Arterial blood gases before the second run of HEMI stimulation. For further details, see Table 27.

Pre 02	pH	PCO2	P02	BEecf	HCO3	TCO2	sO2	Lac
Avg short	7.090	51.525	157	-13.375	16.300	17.875	95.250	20.313
count	8	8	8	8	8	8	8	8
StDev	0.066	13.438	111.276	7.190	6.247	6.707	4.862	3.075
SE	0.025	5.079	42.058	2.718	2.361	2.535	1.838	1.162
Min	6.973	31.4	68	-24	7	8	86	16.13
Max	7.183	70.0	417	-5	24	26	100	23.00
Avg long	7.011	58.520	200.250	-16.000	14.980	16.600	98.250	22.228
count	5	5	4	5	5	5	4	4
StDev	0.219	14.099	124.535	7.176	3.976	3.578	1.258	1.545
SE	0.110	7.050	71.900	3.588	1.988	1.789	0.726	0.892
Min	6.644	42.5	135	-28	9	11	97	19.91
Max	7.169	78.9	387	-9	20	21	100	23.00

Table 30. ABG Post-run 2. Arterial blood gases after the second run of HEMI stimulation. For further details, see Table 27.

Post 02	pH	PCO2	P02	BEecf	HCO3	TCO2	sO2	Lac
Avg short	6.911	61.325	222	-19.375	12.638	14.286	97.000	22.333
count	8	8	6	8	8	7	6	7
StDev	0.077	12.825	126.153	5.370	3.752	4.348	3.033	1.765
SE	0.029	4.847	56.417	2.030	1.418	1.775	1.356	0.721
Min	6.826	35.4	111	-28	6	7	92	18.33
Max	7.085	74.4	413	-12	18	20	100	23.00
Avg long	6.883	70.467	121.000	-19.500	13.683	15.833	92.833	23.000
count	6	6	6	6	6	6	6	6
StDev	0.148	4.281	19.142	5.612	3.417	3.430	4.070	0.000
SE	0.066	1.915	8.560	2.510	1.528	1.534	1.820	0.000
Min	6.630	64.8	101	-29	8	10	88	23.00
Max	7.028	75.7	152	-14	17	19	97	23.00

Table 31. ABG Pre-run 3. Arterial blood gases before the third run of HEMI stimulation. For further details, see Table 27.

Pre 03	pH	PCO2	P02	BEecf	HCO3	TCO2	sO2	Lac
Avg short	7.006	37.700	130	-20.375	10.713	12.000	83.750	17.409
count	8	8	8	8	8	8	8	8
StDev	0.195	6.380	79.372	10.993	7.818	7.856	34.007	10.359
SE	0.074	2.411	30.000	4.155	2.955	2.969	12.854	3.915
Min	6.815	29.3	0	-29	6	7	0	0.00
Max	7.456	48.9	284	6	30	31	100	23.00
Avg long	6.990	42.680	122.200	-19.800	11.080	12.400	78.000	17.724
count	5	5	5	5	5	5	5	5
StDev	0.226	7.933	70.425	6.380	4.115	4.336	43.635	10.016
SE	0.113	3.966	35.213	3.190	2.057	2.168	21.817	5.008
Min	6.626	34.8	0	-30	4	5	0	0.00
Max	7.140	55.9	170	-15	14	16	99	23.00

Table 32. ABG Post-run 3. Arterial blood gases after the third run of HEMI stimulation. For further details, see Table 27.

Post 03	pH	PCO2	P02	BEecf	HCO3	TCO2	sO2	Lac
Avg short	6.897	47.763	140	-23.375	9.500	11.000	94.125	23.000
count	8	8	8	8	8	8	8	8
StDev	0.097	10.908	31.964	4.069	2.879	3.071	7.791	0.000
SE	0.037	4.123	12.081	1.538	1.088	1.161	2.945	0.000
Min	6.760	32.7	72	-30	5	6	75	23.00
Max	7.066	66.7	172	-19	13	15	99	23.00
Avg long	6.842	62.117	138.833	-21.833	11.517	13.500	94.000	23.000
count	6	6	6	6	6	6	6	6
StDev	0.181	5.495	25.795	6.765	4.488	4.506	3.162	0.000
SE	0.081	2.457	11.536	3.025	2.007	2.015	1.414	0.000
Min	6.589	54.9	94	-30	5	7	90	23.00
Max	7.026	70.7	165	-15	16	18	98	23.00

Table 33. PCO2 Change from Pre-run 1 to Post-run 3. The Table shows the PCO2 change from Pre-Run 01 to Post-Run 3 (where Avg short is the average of the shorter interruptions – 0, 100 and 200 msec; Avg long is the average of the longer interruptions – 1,000 and 3,000 msec; Interruption is in msec; PCO2 is in mmHg; and delta is the PCO2 before Run 1 minus the PCO2 value after Run 3). The increase in PCO2 with the longer durations of interruptions was statistically significantly greater than with shorter interruptions (p=0.049, Fisher Exact Probability Test, two tailed.)

	Interruption	PCO2 Pre-Run 1	PCO2 Post Run 3	delta
A13	0	67.2	47.8	19.4
A14	0	45.2	32.7	12.5
A11	100	41.7	58	-16.3
A12	100	55.8	66.7	-10.9
A3	200	42.2	42.5	-0.3
A4	200	41.9	38.7	3.2
A6	200	43	51.9	-8.9
A8	200	40.6	43.8	-3.2
A1	1000	41.4	61.9	-20.5
A2	1000	49.9	54.9	-5
A5	1000	45.7	70.7	-25
A7	1000	50.4	60.5	-10.1
A9	3,000	46.5	65.7	-19.2
A10	3000	48.6	59	-10.4
Avg short		47.200	47.763	-0.562
count		8	8	8
StDev		9.430	10.908	12.038
SE		1.161	1.248	1.311
Min		40.6	32.7	-16.3
Max		67.2	66.7	19.4
Avg long		47.083	62.117	-15.033
count		6	6	6
StDev		3.340	5.495	7.656
SE		0.817	1.048	1.237
Min		41.4	54.9	-25
Max		50.4	70.7	-5

Table 34. Continued Development of Deleterious Effects during the Rest Periods. Post 01 to Pre 02 indicates the change in the ABG and electrolytes values from after Run 1 to before Run 2, which consisted of the rest and recovery period (the calculation was Value Run 1 minus Value Run 2). The gray highlighted values (also in bold, italics) showed continued development of deleterious effects during the rest/recovery period. For instance, values decreasing (e.g., pH 6.983 to 6.831, or increasing (e.g., lactate from 14.34 to 19.6) both indicate continued development of deleterious effects. For further details, see Table 27.

Post 01 to Pre 02	Interruption	pH	PCO2	BEecf	HCO3	Lac	K	Hct
A2	1000	0.4	14.1	23	16.8	-7.53	1.6	
A3	200	0.129	30.8	16	13.8	-4.98	1.2	-5
A4	200	0.055	9.1	7	5.6	-2.2	0.8	-1
A5	1000	-0.014	29.9	10	9.9		2.3	0
A6	200	0.034	22.1	9	8.5	-4.71	1	-3
A7	1000	-0.075	33	6	7.6	-2.44	0	0
A8	200	0.196	28	18	15.1	-6.47	1.1	-3
A9	3000	0.117	35	17	15.5	-11.9	-4.8	-8
A10	3000	-0.005	38	11	11.3	-4.14	0.7	1
A11	100	-0.052	60.3	12	13.4	0	0.7	-3
A12	100	0.060	28.4	12	11.3	-5.73	0.4	-2
A13	0	-0.007	49	14	14.2	-7.93	1.2	0
A14	0	0.074	28.3	10	9.1	0	0.2	-4
Avg short		0.061	32.000	12.250	11.375	-4.003	0.825	-2.625
count		8	8	8	8	8	8	8
StDev		0.077	15.840	3.655	3.352	2.958	0.373	1.598
SE		0.029	5.987	1.381	1.267	1.118	0.141	0.604
Min		-0.052	9.100	7.000	5.600	-7.930	0.400	-5.000
Max		0.196	60.300	18.000	15.100	0.000	1.200	0.000
Avg Long		0.085	30.000	13.400	12.220	-6.503	-0.040	-4.600
count		5	5	5	5	4	5	5
StDev		0.190	9.365	6.656	3.851	4.174	2.801	7.335
SE		0.095	4.683	3.328	1.925	2.410	1.400	3.667
Min		-0.075	14.1	6	7.6	-11.9	-4.8	-16
Max		0.4	38	23	16.8	-2.44	2.3	1

Electrolytes Before and after each Run

Table 35. :Electrolytes Pre-run 1. The table shows Electrolytes Pre-run 1 is measured from arterial blood gas sampling before the first run of HEMI stimulation. Avg short is the average of the shorter interruptions – 0, 100 and 200 msec and Avg long is the average of the longer interruptions – 1,000 and 3,000 msec.

Pre 01	Na	K	Cl	TCO2	BUN	Glu	Hct	AnGap	Hb*
Avg short	139.125	3.975	101.000	32.500	10.750	85.500	26.375	11.375	9.2
count	8	8	8	8	8	8	8	8	8
StDev	1.885	0.225	1.773	4.472	2.315	21.974	3.777	3.852	7.1
SE	0.712	0.085	0.670	1.690	0.875	8.305	1.428	1.456	9.5
Min	136	3.6	98	29	7	54	21	3	10.9
Max	142	4.3	103	42	14	124	33	15	8.5
Avg long	139.500	3.817	101.000	31.333	9.167	64.833	26.833	12.500	9.133
count	6	6	6	6	6	6	6	6	6
StDev	1.378	0.387	4.195	2.582	2.041	20.478	3.764	1.871	1.288
SE	0.616	0.173	1.876	1.155	0.913	9.158	1.683	0.837	0.576
Min	137	3.3	97	27	6	32	22	10	7.5
Max	141	4.4	108	34	12	92	32	15	10.9

Table 36. Electrolytes Post-run 1. The table shows Electrolytes Post-run 1 is measured from arterial blood gas sampling before the first run of HEMI stimulation. Avg short is the average of the shorter interruptions – 0, 100 and 200 msec and Avg long is the average of the longer interruptions – 1,000 and 3,000 msec.

Post 01	Na	K	Cl	TCO2	BUN	Glu	Hct	AnGap	Hb*
Avg short	150.875	5.475	112.000	26.250	12.625	98.500	27.750	20.500	11.9
count	8	8	8	8	8	8	8	8	9
StDev	1.959	0.341	0.926	4.432	2.504	29.530	2.315	2.726	8.5
SE	0.741	0.129	0.350	1.675	0.946	11.161	0.875	1.030	9.2
Min	148	4.9	111	17	9	58	25	18	9.5
Max	154	6.1	114	30	16	140	31	26	8.5
Avg long	150.500	5.540	110.167	27.333	11.333	73.833	29.000	21.800	9.850
count	6	5	6	6	6	6	6	5	6
StDev	2.168	0.773	2.563	3.777	1.633	26.589	3.225	5.450	1.104
SE	0.970	0.387	1.146	1.689	0.730	11.891	1.442	2.725	0.494
Min	147	4.5	107	23	9	32	26	15	8.8
Max	153	6.4	113	33	13	99	35	30	11.9

Table 37. Electrolytes Pre-run 2. The table shows electrolytes measured from the arterial blood gases before the second run of HEMI stimulation. Avg short is the average of the shorter interruptions – 0, 100 and 200 msec and Avg long is the average of the longer interruptions – 1,000 and 3,000 msec.

Pre 02	Na	K	Cl	TCO2	BUN	Glu	Hct	AnGap	Hb*
Avg short	145.875	5.038	108.875	19.000	12.125	100.500	29.250	24.750	0
count	8	8	8	8	8	8	8	8	14
StDev	5.743	0.665	3.796	8.018	2.167	34.430	2.712	4.367	8.5
SE	2.171	0.251	1.435	3.030	0.819	13.013	1.025	1.651	9.2
Min	140	4.3	102	9	9	55	25	18	9.5
Max	154	6.1	114	30	14	140	33	30	9.5
Avg long	144.000	4.500	106.400	16.000	12.000	79.000	32.400	28.000	11.020
count	5	5	5	4	5	5	5	4	5
StDev	4.690	0.616	4.336	3.559	1.225	28.705	6.189	4.761	2.111
SE	2.345	0.308	2.168	2.055	0.612	14.353	3.094	2.749	1.056
Min	140	3.8	101	11	10	36	27	25	9.2
Max	152	5.2	113	19	13	112	42	35	14.3

Table 38. Electrolytes Post-run 2. The table shows electrolytes measured from the arterial blood gases after the second run of HEMI stimulation. Avg short is the average of the shorter interruptions – 0, 100 and 200 msec and Avg long is the average of the longer interruptions – 1,000 and 3,000 msec.

Post 02	Na	K	Cl	TCO2	BUN	Glu	Hct	AnGap	Hb*
Avg short	149.875	6.013	112.500	14.625	13.875	109.250	33.000	30.625	14.3
count	8	8	8	8	8	8	8	8	14
StDev	4.155	0.577	3.891	3.503	2.800	38.795	3.928	4.033	10.5
SE	1.571	0.218	1.471	1.324	1.058	14.663	1.485	1.524	9.5
Min	141	5.3	104	7	10	59	28	25	13.6
Max	155	6.7	116	18	18	152	39	36	9.5
Avg long	152.000	5.883	112.333	15.833	13.333	84.500	37.000	31.667	12.583
count	6	6	6	6	6	6	6	6	6
StDev	2.683	0.445	2.503	2.927	1.966	30.032	5.177	4.131	1.766
SE	1.200	0.199	1.120	1.309	0.879	13.431	2.315	1.848	0.790
Min	149	5.3	110	11	11	43	30	28	10.2
Max	157	6.4	117	19	16	128	42	39	14.3

Table 39. Electrolytes Pre-run 3. The table shows electrolytes measured from the arterial blood gases before the third run of HEMI stimulation. Avg short is the average of the shorter interruptions – 0, 100 and 200 msec and Avg long is the average of the longer interruptions – 1,000 and 3,000 msec.

Pre 03	Na	K	Cl	TCO2	BUN	Glu	Hct	AnGap	Hb*
Avg short	142.375	5.313	108.125	12.000	12.750	112.625	33.750	28.875	11.475
count	8	8	8	8	8	8	8	8	14
StDev	3.204	0.751	3.357	7.540	2.659	51.818	3.495	7.180	11.2
SE	1.211	0.284	1.269	2.850	1.005	19.585	1.321	2.714	9.5
Min	138	4.2	101	7	10	61	28	13	10.2
Max	149	6.2	112	30	17	212	39	38	10.5
Avg long	142.800	5.240	86.080	12.800	13.000	86.800	34.600	30.200	11.760
count	5	5	5	5	5	5	5	5	5
StDev	4.324	0.598	41.792	4.087	1.581	29.811	4.879	4.764	1.641
SE	2.162	0.299	20.896	2.043	0.791	14.906	2.439	2.382	0.821
Min	139	4.5	11	6	11	50	30	26	10.2
Max	150	5.8	107	16	15	131	41	38	13.9

Table 40. Electrolytes Post-run 3. The table shows electrolytes measured from the arterial blood gases after the third run of HEMI stimulation. Avg short is the average of the shorter interruptions – 0, 100 and 200 msec and Avg long is the average of the longer interruptions – 1,000 and 3,000 msec.

Post 03	Na	K	Cl	TCO2	BUN	Glu	Hct	AnGap	Hb*
Avg short	146.500	6.800	110.750	10.750	15.000	129.500	36.250	33.000	15.300
count	8	8	8	8	8	8	8	8	14
StDev	3.625	0.812	2.375	2.712	3.505	60.844	2.712	4.140	11.9
SE	1.370	0.307	0.898	1.025	1.325	22.997	1.025	1.565	11.2
Min	139	5.7	107	6	10	64	33	27	12.9
Max	152	7.9	114	14	20	230	41	40	11.6
Avg long	148.833	6.433	110.667	13.333	14.167	88.667	40.167	32.500	13.667
count	6	6	6	6	6	6	6	6	6
StDev	3.189	0.484	3.615	4.367	2.229	32.315	4.446	3.728	1.527
SE	1.426	0.217	1.617	1.953	0.997	14.452	1.988	1.667	0.683
Min	145	5.8	106	7	11	58	33	28	11.2
Max	154	6.9	117	18	17	145	45	37	15.3

Table 41. Changes in Hct from Pre-Run 1 to Post Run 3. The table shows the change in Hct from baseline (pre-Run 1) to after Run 3 of the HEMI stimuli. Several prior publications (including our own) have found increases in Hct following exposure to HEMI stimuli. This was again evident in this study. There was a trend ($p=0.09$ Wilcoxon Rank Sum Test) towards a greater increase in the Hct with longer durations of interruptions

Hct	Short	Long
-19		1
-18		2
-17		3
-16		4.5
-16	4.5	
-14	6	
-14	7	
-13	8	
-9	9	
-7	10	
-6		11
-5	12	
-4		13
-1	14	
	70.5	34.5
		0.09

DISCUSSION

Introduction and Goals

The Need for HEMI Research. There remains an unmet operational requirement to extend the safety and duration of the HEMI effect of interest, as well as the range at which it might be applied (i.e., the distance between the targeted subject and the operator). Our project is to focus on the safety and efficacy of the HEMI stimuli, thereby enabling extension of the duration. There are multiple potential directions of research here. We have **focused on the safety of HEMI stimuli**. We do think that we can simultaneously elucidate multiple aspects of safety, thereby further expanding the knowledge base of deleterious effects.

The goal is to understand and thereby mitigate deleterious effects associated with changes to the duty cycle. We set out to study the safety of a range of duty cycles by identifying and measuring deleterious effects caused by HEMI applications. The usual ranges of biochemical parameters (ABG, lactate, and electrolytes) are typically exceeded by applications of HEMI stimuli. Therefore, we elected to use the “TOTAL WORK GENERATED” by 35 second applications of HEMI stimuli as the main measure of deleterious effects, while varying the duty cycles. The total work imposed on the research subject is believed to be the main driver of the deleterious effects. We were also interested to see, over and above the total work, if there were patterns in the work signals that could be used to indicate differences in deleterious effects.

Further, in order to understand the effects of deleterious physiology on the generation of work, we varied the sites of the HEMI probe insertions. We also measured the **decrease of the amount of work** generated by consecutive three consecutive runs of HEMI stimulation, which we expected would be able to be used as a measure of deleterious effects.

We performed the research study as planned, as described in Methods. We used a standard duty cycle (“train”) of 5 seconds.

In the pilot studies, we varied the probe positions and we varied the patterns of HEMI applications. We also confirmed and expanded prior findings as well as found several potential new measures of deleterious effects.

In the main study, we varied the OFF periods (“interruptions”) from zero to 3,000 msec. We found that the decrement of the amount of work generated in a subsequent HEMI application was a useful measure of deleterious effects. We also demonstrated the value of interruptions to mitigate the deleterious effects: zero interruptions lead to statistically significant more deleterious effects compared to any duration of interruptions. Interruptions of 3,000 msec had the least deleterious effects.

In the following section, we provide background regarding the relevant physiology pertaining to the measurement of work as well as the rationale for choices in the design of the study.

Background Physiology – Perfusion of Muscles

We first provide a background for understanding the nature of the tetanic contractions on the perfusion of the contracting muscles, followed by other factors leading to variations in the deleterious effects of HEMI contractions caused by variations in the duty cycle.

Coronary artery perfusion during diastole: similarities with tetanic muscle contractions due to HEMI stimuli. In cardiac physiology, it is well known and accepted that coronary artery blood flow occurs mainly during diastole. Cardiac muscle perfusion (Oxygen “**Supply**”) occurs mainly during the relaxation phase (diastole) of the cardiac muscle, and is exquisitely balanced to provide for the Oxygen “**Demand**.” The cardiac muscle perfusion and oxygen supply is critically dependent upon the diastolic blood pressure as

well as the duration of the diastolic phase. We postulate that the tetanic muscle contraction of HEMI exposure decreases (maybe even prevents) perfusion (“Supply”) of the peripheral muscles during the tetanic phase of muscle contractions. “Tetanic” muscle contractions imply and indicate that the muscles do not relax between stimuli. The tetanic muscle contractions also imply a large oxygen “Demand”

Similar to cardiac physiology, during the HEMI stimulus, due to the intense, tetanic muscle contractions, the intra-muscular pressure of skeletal muscles is presumed to be higher than the blood pressure. Therefore, minimal skeletal muscle perfusion occurs during the HEMI stimulus. Peripheral muscle perfusion therefore occurs mainly during the interruption phase.

A phenomenon noted with HEMI stimulation, that is not present in cardiac physiology, is that the total systemic vascular resistance increases dramatically during the periods (“trains”) of the HEMI stimulation. This increased resistance further decreases the blood supply, leading to deleterious effects.

With our previous HEMI 3 study, following the increased blood pressure during the HEMI stimulation, an interesting phenomenon noted during the interruption phase of the HEMI stimulation was the major decrease in blood pressure (systolic, mean and diastolic). This was noted during the interruption phase of the HEMI stimuli. In instances where the interruption coincides with the diastolic phase of the blood pressure, there was an even steeper drop-off of the diastolic blood pressure during the interruption. There are now several competing physiological phenomena related to longer durations of interruption:

- May improve the perfusion of muscle;
- May lead to diastolic blood pressure rapidly decreasing;
- May lead to larger stroke volumes and tidal volumes;
- May allow the subject to pull off the HEMI probes.

We believe that the blood pressure values during the interruption are critical to minimize the potentially deleterious effects due to poor perfusion of the peripheral muscles. A future study should specifically measure intra-muscular pressure to be able to study and calculate the perfusion pressure of the muscle (i.e., the difference between the mean BP and the intra-muscular perfusion pressure) during the contraction (HEMI stimulus phase) compared to the relaxation phase (interruption phase) of the interruption.

There are complex interactions of the physiology that include competing effects. For instance:

- The tetanic contractions may support and increase the blood pressure and therefore provide an increased perfusion pressure for muscle tissues (similar to Phase I of the Valsalva Maneuver);
- In opposition to this supporting effect, the tetanic contractions may cause an effect of a decreased perfusion of muscles with a failure of wash-out of intermediary metabolites. The metabolites are only taken up by the capillary circulation to the central circulation once the tetanic contractions totally end, and muscle perfusion is re-established.
- The clinical impression of decreased muscle movements (“vibrations” or oscillations at 18 Hz) is presently interpreted as the development of “muscle weakness” with possibly less powerful contractions resulting in less compression of the muscle blood vessels and therefore better muscle perfusion, but at a lower blood pressure. It will be important to continuously measure intra-muscular pressures for the entire period of intermittent HEMI stimulation.

Variables that were studied. There were multiple aspects and variables in the tasking which were taken into consideration during the planning phase of the study. We briefly summarize the variables and the terminology. For a full explanation, see Methods and Results.

The first variable is the **“total application time”** for 35 seconds for the main study, and from a single twitch up to 360 seconds (6 minutes) for the exploratory studies.

The second variable in the approach to interrupting the standard duty cycle is finding the **“ideal” interruption time**. We planned to begin with the extremes of the interruptions of the duty cycles (i.e., from 0 to 3,000 msec) where we expected the maximum differences in deleterious effects to be found.

In our previous HEMI studies, we believe that we have bounded the interruption time fairly well between 400 and 750 msec. Selecting a single interruption time, however, may not ultimately be the “best” tactical approach. For example, we may find that it is better to alternate a shorter and longer interruption time, or that we should start with a shorter interruption time, and then migrate to a longer interruption time after a period, when the subjects’ muscles have fatigued.

The third variable is the duration of the period of HEMI stimuli at 18 Hz (“train” of HEMI stimuli). The train can also be thought of as the length of **time between interruptions**. For standardization purposes, we studied mainly “trains” of 5 seconds continuous HEMI stimuli. As shown in our previous (HEMI 3) study, each interruption after each train of 5 seconds, lead to a respiratory rate of 12 breaths per minute.

Rationale for Choice of “0 to 3,000 msec” interruptions during the HEMI stimulus of 35 sec. We initially explored interruptions of 1,000 msec and up to 3,000 msec. We found no discernable difference in the amount of work, so we believed we had reached the upper end of the envelope (upper bounding).

We then explored interruptions down to 200 msec. We had shown (HEMI 3 study) that interruptions ARE beneficial in terms of a “good breath” in an anesthetized swine model. Human studies have shown increased respiration with HEMI probes in the anterior chest during a continuous HEMI exposure of 30 seconds. We had not shown that interruptions and a “good breath” are beneficial (less deleterious) in terms of biochemistry compared to continuous HEMI stimuli (i.e., zero interruptions)

We had also previously not shown the effect of interruptions on **“work”** performed (i.e., not explored the very lowest part of the work envelope, i.e., zero interruptions.) There is a possibility that the interruptions lead to more work performed based on the phenomenon of post-tetanic facilitation, i.e., the large contractions following the interruptions. Clinically, we had noticed that the contractions are initially stronger after each interruption.

The neuro-muscular junction is known to produce more acetyl-choline after a period of tetanic stimulation, leading to stronger muscle contractions. We use this phenomenon (tetanic contraction for 5 seconds) in human practice to test the remaining effects of muscle relaxants. Remaining muscle relaxants lead to weaker muscle contractions, and the need for reversal of the muscle relaxant.

We therefore decided to study the work generated in an animal during a **continuous HEMI stimulus of 35 seconds without any interruptions**.

The Influence of Duty Cycles on Deleterious Effects

We postulated that the **work** imposed by the application of HEMI stimuli would be the generator of the development of deleterious effects. We measured the standard biochemical parameters (lactate, ABG, electrolytes, etc.) to compare the biochemical deleterious effects with our prior studies. We did not expect the biochemical measurements to be sensitive enough to differentiate between various duty cycles. We therefore decided to measure movement via accelerometers and calculate the “work”

performed by each of the limbs. We calculated the work performed by each limb – the calculation actually resulted in units of power. However, as the conversion of power to work is via a constant factor, and the units of work are much better understood, we use the term work throughout the report.

As shown in Methods, we varied the duty cycles to include interruptions of 0, 100, 200, 1,000 and 3,000 msec. We defined the interruptions of 0, 100 and 200 msec as “SHORT” interruptions, while interruptions of 1,000 and 3,000 msec were defined as “LONG” interruptions.

For each animal, we applied the same HEMI duty cycle for three sequential exposures, termed Run 1, Run 2 and Run 3. As a baseline, we analyzed the total work performed by all four limbs of each animal for each run. We calculated the decrement of the work performed in Run 2 and Run 3 as a percentage of the work generated in Run 1. We considered the decrement of work in the subsequent runs as indicators of deleterious effects.

We clearly demonstrated the value of an interruption in mitigating the deleterious effects as indicated by a decreased ability to generate work in subsequent runs.

- A duty cycle with no interruption (5000-0) had a statistically significant decrement in subsequent runs when compared to the other animals where interruptions were present.
- Comparing animals with zero interruptions to animals with the longest interruptions of 3,000 msec: The animals with no interruptions again had significantly less ability to generate work in subsequent runs.
- We analyzed the group with short interruptions (n=8) compared to the group with long interruptions (n=6). Both groups, short and long interruptions, had statistically significant reductions in the ability to perform work in subsequent runs. There was no significant difference between the two groups.
- When comparing the combined data of the front limbs, the group with the long interruptions generated statistically significantly less work in Run 2 compared to the group with the short interruptions. (Table 20)
- When comparing the combined data for the hindlimbs, the group with the long interruptions had a significant decrement in the amount of work generated from Run 1 to Run 2 and from Run 1 to Run 3. The group with the short interruptions had a significantly decreased generation of work from Run 1 to Run 3. There were no significant differences in the amount of work generated by the hindlimbs in any of the runs. (Table 21)
- The duty cycle with the longest interruption (3,000 msec) showed the least decrement in the work generated with Run 2 and Run 3.
- A duty cycle of 3,000 msec also demonstrated the preservation of work up to 30 seconds, while the shorter duty cycles demonstrated almost total lack of work by 20-25 seconds.

We had expected and predicted that the longer duty cycles would lead to less decrement of work in Run 2 and Run 3. This was demonstrated with the interruptions of 3,000 msec. Interestingly, the reverse occurred with some of the intermediate interruptions, where interruptions shorter than 3,000 msec had better preservation of work generated in subsequent runs. A possible explanation is that relaxation is an active process, requiring energy stores, Ca²⁺ and oxygen. With relatively short interruptions, there is no time for relaxation to occur, thereby saving energy stores, while with the very long interruptions, there is time for relaxation to occur, but also for perfusion of the muscle tissues. Further studies will be needed to elucidate these aspects.

Aspects of Work – Measuring Deleterious Effects

In this section, we describe

- The correlation of measured work with muscle bulk, i.e., the larger hind limbs generate more work than the front limbs, thereby demonstrating that the methodology we employed can identify and differentiate between levels of work, and therefore, is a valid measure of work.
- Similarly, the distance from the HEMI probes to the limbs correlate with the amount of work generated. Specifically, a greater distance results in less work. This also supports our methodology as validly measuring work performed.
The position of the probes relative to the spinal cord (unilateral, lumbar nerve roots, etc.) had a major effect on the amount of work generated.
- We found a new measure of deleterious effects, which is useful because the standard biochemical parameters (lactate, ABG, electrolytes, etc.) are overwhelmed and cannot measure the high ranges of parameters caused by the HEMI stimuli. We found that the work produced by the second and third runs of HEMI exposure could differentiate between various durations of interruptions for the groups. Individuals had large variations which needed group averages for detection and significance. For instance, we found that the ability to perform work was statistically significantly less with zero interruptions compared to interruptions of 3,000 msec.
- The ability to perform work at the 30 second mark was also better maintained with longer interruptions, specifically with interruptions of 3,000 msec.
- The external work as measured by movements (“isotonic”) provided useful intra-animal comparisons from Run 1 to Run 2 as well as from Run 1 to Run 3. A future study should be designed to measure the tetanic tension work (“isometric”).

New findings and data that support “work” as a valid measure. The larger hind limbs generated statistically significantly more work than the front limbs. This correlation of measured work with muscle bulk was expected, and the front limbs and hind limbs were evaluated separately in the statistical analysis.

The hind limbs also had a greater percentage decrement in the amount of work generated in Run 2 and 3. We consider that this supports our hypothesis that more work is correlated with more deleterious effects as demonstrated by the decrement of work in the subsequent runs. This also supports the hypothesis that the muscle is an important “effect site” for the deleterious effects over and above the central hemodynamics and biochemistry.

An interesting phenomenon we noted was the development of “double peaks” of work (see Results.) There was an initial peak of work at 1-2 seconds, followed a period of less work (5-10 seconds), with the development of a second peak of work between 10-20 seconds, followed by the slow onset of fatigue. The forelimbs had a statistically significantly larger incidence of double peaks compared to the hind limbs. The origin and significance of the “double peak” is not fully understood. A more detailed discussion is presented in the next section.

Distance of the HEMI probes from the limb. Based on clinical observations from prior HEMI studies, we postulated that the position of the probes would have a major influence on the total work generated by the animals. The distance from the HEMI probes to the limbs correlate with the amount of work generated. Specifically, a greater distance (e.g., probes in the upper thorax) results in statistically significantly less work in the hind limbs. Similarly, probes in the groins near the hind limbs, result in statistically significantly less work measured in the fore limbs.

As noted in Figures D32 – D38, the position of the HEMI probes have a major influence on the amount of work generated due to the HEMI stimuli. With this study, it is possible to confirm the prior clinical observations, as well as quantify the influence of the positions of the HEMI probes.

We measured the effect of placing the probes bilaterally high on the torso 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 shown in Figure 36 and LL and RL = Left hind limb and right hind limb shown in Figure 44).

With the standard positions of the probes, left forelimb and right hind limb, there is a decreased amount of work measured in the contralateral limb. This is statistically significant difference for the fore limbs, but not for the hind limbs, probably to large variances. We believe these results also support our methodology as validly measuring work performed.

The amount of work generated does not necessarily mean that the HEMI stimuli is ineffective and does not lead to incapacitation. The fact that there is some effect on the distal limbs implies that there was conduction of 18 Hz impulses in the spinal cord. This conclusion is based on the following reasoning:

- From our earlier work with muscle relaxants, we showed that the HEMI stimuli does not propagate via tissue-to-tissue and/or muscle-to-muscle conduction.
- HEMI research in general has strongly indicated that propagation of HEMI stimuli is conducted via the spinal cord.
- With HEMI probes placed in positions that lead to muscle stimulation effects at a distance from the nerve roots closest to the HEMI probes, the impulses must have traversed the spinal cord.
- Of note is that, with symmetrical probe positions on either side of the spinal cord, conduction of vertical (up and down) signals in the spinal cord will be expected to be greatly reduced, including and especially signals related to proprioception. However, given the interruption of the postural (proprioceptive) spinal cord signals, the subject will most likely not be able to remain in an upright posture, in other words, fall down, being incapacitated (unable to move forwards).
- We postulate that HEMI stimuli being propagated via the spinal cord at 18 Hz would inhibit postural proprioception and therefore limit the ability to remain upright.

Based on the concept of less muscle activity with probe positions that stimulate less of the spinal cord, the Risk of Significant Injury (RSI) might also be dependent upon the extent of the spinal cord involved (e.g., the number of spinal roots “traversed” by the HEMI current from the probes) in conjunction with the duty cycle.

We propose a study with consideration of placing the HEMI probes in positions to cause less whole body physiological impairment, e.g., stimulate only the hind limbs, or only the front limbs, or a unilateral position which might only stimulate a single limb. The biochemical parameters would then be unlikely to exceed the upper limits, and the biochemistry could be used to check for deleterious effects. The muscle being stimulated would also only be influenced by the work performed, without the complication factors of central biochemical derangements.

A study of videos of the use of HEMI devices in the community would be useful to study the postulate that HEMI movements in limbs distal to (further away from) the HEMI probes would indicate that proprioception would be inhibited. The videos would be used to compare the positions of the HEMI probes with the ability to remain upright.

Simultaneously, it would be useful to compare videos of the position of the HEMI probes with the ability to maintain respiration. A study should be designed to specifically analyze the effect of probe positions on the maintenance of respiration with probe positions distal (inferior) to the intercostal muscles and thoracic nerve roots, e.g., lower abdomen. The effects of such positions on the blood pressure should also be studied.

Decrement of work with Sequential Runs. In previous studies, we had noticed that there was less movement in the limbs during the second and third HEMI runs. With the objective measures of work, the clinical impression of less work was confirmed. We used this as a new measure of deleterious effects, which was needed as the standard biochemical parameters (lactate, ABG, electrolytes, etc.) are overwhelmed and cannot measure the high ranges of parameters caused by the HEMI stimuli. We found that the work produced by the second and third runs of HEMI exposure could differentiate between various durations of interruptions for the groups. Individuals had large variations which needed group averages for detection and significance. For instance, we found that the ability to perform work in the second and third HEMI exposures was statistically significantly less with zero interruptions compared to interruptions of 3,000 msec.

The hind limbs, with greater muscle bulk performing more work, had a greater decrease in the percentage of work generated during Run 2 compared to the forelimbs, with less muscle bulk. This supports our hypothesis that the work generated is a harbinger of deleterious effects, such as a decreased capability to perform work in subsequent runs.

Figure 14 shows the expected result where Run 2 generates less work than Run 1, and Run 3 generates less work than Run 2. In contrast, Figure 27 shows instances where Run 3 generated more total work than Run 1. This again demonstrates the variability between animals.

Overall, as a group, the average of total work generated by Run 1 was greater than Run 2, and Run 2 was greater than Run 3 (see Table 16).

Work preserved longer with longer durations of interruptions (3,000 msec). The ability to do work for a longer period is better preserved in duty cycles with a longer period of interruption. Figure 26 shows an example of work still being performed at 30-35 seconds with interruptions of 3,000 msec, while Figure 23 and D15 show examples where close to zero work is being performed at 20 seconds with shorter interruptions. Furthermore, with longer interruptions, there is less decrement in the amount of work generated in Run 2 and Run 3, and the ability to generate work is preserved for a longer period.

External “movement” work as measured. Muscle work can be classified as external, whereby the muscle shortens and causes movement. This is called isotonic work, and can be measured by analyzing the movements of the muscle or the relevant limb, in our case. Muscles can also perform work by contracting (“tensioning”) against an immovable object. This is called isometric work. We elected to measure external, isotonic work. The external work as measured by movements (“isotonic”) was deemed to be able to provide useful **intra-animal comparisons** from Run 1 to Run 2 and to Run 3. We recommend a future study should be designed to measure the tetanic tension work (“isometric”) to determine if such measurements would further increase the sensitivity to differentiate between various duty cycles.

Patterns of Work – Double Peaks

Clinically, in prior studies, we had noticed that in some animals, after an initial few seconds of vigorous contractions, exhibited a period (several seconds) of less active contractions, followed by increased contractions again for multiple seconds. The clinical impression of this pattern was confirmed by the objective measurements of work in the present study. This phenomenon was **termed a “double peak”** (see Figures D14 and D15).

This interesting phenomenon of “double peaks” was noted in some, but not all, limbs, and also not in all animals and not in all runs. The initial peak of work occurred in the first 1-5 seconds, followed by a secondary peak, typically in the range of 10-25 seconds. In some animals, the first peak was lower than the second peak (Figure 24), while in other animals, the first peak was higher (Figure 23).

We present the following hypotheses and discussion related to the Double Peaks:

- The cause of the first peak is possibly due to the immediately available energy stores and oxygen stores being used initially. Due to strong muscle contractions, the blood supply is cut off and aerobic energy stores are rapidly exhausted. The first peak possibly indicates the initial existence and the duration of “aerobic” metabolism.
- Once the initial immediately energy stores have been depleted, relaxation follows. The relaxation following the initial peak possibly enables improved perfusion. This concept of relaxation (due to weakness or fatigue) leading to improved perfusion, is supported by the runs where there were zero interruptions, and still a second peak developed.
- After the interruption, the first few contractions are mostly larger than the contractions at the end of the prior “train.” These increases in the size of the contractions are explained by the concept of post-tetanic facilitation (PTF).
- The development of the second peak (indicating more work being performed) is possibly explained by increased perfusion as mentioned above, which provides only a temporary boost to the contractions. Another possible explanation for the second peak is that the contraction is not as strong, and therefore lasting longer. However, this explanation is only relevant and applicable when the second peak is lower (less work) than the first peak. An alternative explanation is the onset of anaerobic metabolism, which is also rapidly exhausted.
- The size of the second peak, which is often larger than the first peak, is a previously undescribed phenomenon, which could be studied in a future study which would include measurement of the tonic work of contraction, as well as muscle biopsies. This study would be necessary to provide data to be included in building a mathematical simulation model of work by muscles, where the work was imposed by electrical tetanic stimulation.
- In a human study of supra-maximal stimulation of the quadriceps muscle of human volunteers, a multi-phasic response was found. Initially, there was strong muscle contraction, followed by weakening – attributed to the immediate aerobic energy stores being used and depleted – and improvement. An improvement in muscle strength followed – attributed to the anaerobic mechanism being stimulated. Once the anaerobic stores were depleted, aerobic mechanisms were stimulated, but only at about 25% of the initial strength. It would be necessary to perform muscles biopsies to compare the HEMI stimulation with the human studies.

Long Duration HEMI Applications

Literature describing longer duration HEMI exposures in animal models demonstrated the feasibility of a three minute exposure (Ziriak 2011⁵). The “X26 lethality study” (Lu 2008⁶) used 30 seconds of continuous exposure followed by 5 seconds on and 5 seconds off. The authors found: “... the parameters forced an inadequate rate of breathing (6 breaths per minute) given the level of muscular exertion forced by the X26 stimulation.” The shortest time to induction of shock was 6.23 minutes, and the shortest time to death was 10.47 minutes. The authors state: “It is also worth noting that animals were breathing room air without any mechanical assistance.” The focus in the latter study was cardiovascular collapse during periods of forced breathing, rather than the presence of breathing or apnea after the HEMI exposure.

In a human study with a 30 second exposure to HEMI stimuli on the anterior thorax (Dawes 2010⁷), an increase in tidal volume was found with continuous spirometry. In a review article of human studies with various durations of exposure (Kunz 2018⁸), increases in minute volume were found during the exposures.

We had noted clinically that the animals seemed to have less deleterious effects on the second and third runs of the day. In the present study, the amount of work caused by the second and third runs was significantly less than during the first HEMI application of the day. We therefore studied the effect of 360 seconds of HEMI applications to the animals as the last run of the day. The value of the 360 second series of HEMI stimuli lies in the hypothesis that the deleterious effects are proportional to the amount of work generated by the HEMI stimulation. Five of the six animals with HEMI applications of 360 seconds continued breathing throughout and after the exposure. One animal developed a BP of over 300 mmHg in the early phases of the HEMI application, with hypotension developing at the 4 minute mark, followed by apnea.

ABG and Biochemistry

The ABG and electrolyte data was analyzed in several different ways to determine if there were statistically significant differences due to the duty cycles; specifically, shorter and longer interruptions between the trains of 5 seconds of HEMI stimuli. There were three runs of the same duty cycle in an attempt to “magnify” any minor differences between the duty cycles. The changes in the parameters of the ABGs were analyzed as follows:

- From Pre-Run 1 to Post-Run 1, Pre Run 2 to Post Run 2, and Pre-Run 3 to Post Run 3.
- During each rest period, e.g., Post Run 1 to Pre-Run 2, Post Run 2 to Pre-Run 3, as well as Post Run 3 to the next run.
- From Pre-Run 1 to Post Run 2, and **Pre-Run 1 to Post Run 3.**

⁵ Ziriak JM (2011) Human electromuscular incapacitation (HEMI) bioeffects project: final report. Naval Medical Research Unit, San Antonio. 31 January 2011

⁶ Lu ST, Ziriak JM, Klages C, Crane C, Comeaux J, Cox DD, Fines D, D'Andrea JA (2008). Acute biological effects of extended Taser®-X26 stimulation in swine (*Sus Scrofa domestica*). Naval Detachment Directed Energy Bioeffects Research Laboratory, Brooks City-Base, TX. Technical Report NHRC-DEBL-TR-2008-01.

⁷ Dawes DM, Ho JD, Reardon RF, Miner JR (2010). The cardiovascular, respiratory, and metabolic effects of a long duration electronic control device exposure in human volunteers. *Forensic Sci Med Pathol* 6(4):268–274. <https://doi.org/10.1007/s12024-010-9166-9>. Epub 2010 May 26.

⁸ Kunz SN, Calkins HG, Adamec J, Kroll MW

Adrenergic and metabolic effects of electrical weapons: review and meta-analysis of human data *Int J Legal Med.* 2018;132(5):1469-1475

There was a statistically significant difference between the HEMI stimuli with “short” and “long” interruptions for the PCO₂ from **Pre-Run 1 to post-Run 3**. Specifically, there was a statistically greater increase in PCO₂ for the duty cycles with longer interruption compared to the shorter interruptions ($p=0.049$, Fisher Exact Probability Test. Two tailed). Metabolic acidosis leads to hyperventilation as a compensatory mechanism. More marked/severe metabolic acidosis leads to more hyperventilation with a lower PCO₂. Even though the pH is not statistically significantly different, the physiology detects and responds to the differences in acidosis. The animal’s physiology (in terms of the PCO₂) reacts as if there is a greater effect of acidosis present with shorter interruptions, i.e., a decreased PCO₂ due to hyperventilation caused by acidosis.

Several publications (including our own) have found increases in Hct following exposure to HEMI stimuli. In this study, there was a trend ($p=0.09$) towards an increase in the Hct from the pre-Run 1 value to the Post-Run 3 value.

Even though the HEMI exposures were only 35 seconds in duration, an interesting phenomenon we noticed with this project, was that the development of deleterious effects continued during the “rest/recovery” period. For instance, the pH continued to decrease, and the Hct as well as the K⁺ continued to increase.

In our previous study (HEMI 3⁹), we noticed that there was continued development of deleterious effects during the rest/recovery period. This was evidenced by the development of delayed apnea, the development of abnormal T-waves several minutes after the end of the HEMI exposure as well as by the continued decrease of blood pressure for variable periods after the end of the HEMI exposure. In this study we noticed the continued development of biochemical signs of deleterious effects, as noted in the Results. For instance, the pH after the rest period was often lower than the pH immediately after the end of the prior application of the HEMI stimulus.

In the Results, we presented the ABGs and electrolytes as measured before and after each of the 3 runs, followed by derived and calculated parameters. As expected, and predicted, the biochemical parameters were not sensitive enough to, and did not significantly differentiate between the various duty cycles, which was the reason to focus on alternative indicators of deleterious effects such as the generation of work.

⁹ Hughes EL, Werner JR, Murray WB, Jenkins DM, Kennett MJ, Bienus DA, Welker ML
Human Electro-Muscular Incapacitation (HEMI) Mechanisms Study 3: An Examination of the Deleterious Physiological Effects of Variations in the Duty Cycle Parameters, Applied Research Laboratory, The Pennsylvania State University, 22 October 2018, [USMC Contract #17F8307 of NAVSEA Contract N00024-12-D-6404]

New methods to detect deleterious effects

Train-of-Four (TOF) Nerve Stimulation as a potential measure of deleterious effects. The “Train of Four” is a test of the function of the neuro-muscular junction that is used on a daily basis in human anesthesia practice. The TOF methodology tests for residual muscle weakness, specifically due to remaining muscle relaxants. The test consists of four supra-maximal, single twitches delivered 500 msec apart. A decrement of the size of the 4th twitch as a percentage of 1st twitch is an indicator of muscle weakness which would require reversal of the muscle relaxants (“curare”).

In the case of HEMI applications, 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

Our results indicate that the Post-HEMI TOF shows that all 4 the twitches are essentially the same size, which is a similar pattern to the TOF before the HEMI stimulation. See Figures D27 and D28. Further study would be indicated to determine if the muscle fatigue noted during and after HEMI applications has an element of impaired neuro-muscular function as an association.

Single Twitch (ST-1) as a potential measure of deleterious effects. The “Single Twitch” is another method of testing the neuro-muscular junction in human anesthesia practice. The Single Twitch consists of a supra-maximal twitch every one second. If the twitches become smaller in size, the phenomenon is called “fade”, again indicating abnormalities of the neuro-muscular junction.

Over and above the use described above, we propose a different use and interpretation of the single twitch based on a prior clinical observation. We had noticed clinically, that the limbs tended to be quite stiff with post-mortem examination. As noted on Figures D27 and D28, there are oscillations noted after the last twitch. The oscillations are noted to decrease in an exponential fashion. With stiffer muscles, the natural frequency of oscillation would tend to increase, and the time constant of the decay of the oscillations would decrease. We postulated that there would be an exponential decay of the magnitude of the oscillations that could potentially be used to calculate the stiffness of a given limb, before and after HEMI stimulation, and that the degree of stiffness could be used as a measure of deleterious effects.

We show in Figure 38, as a pilot, a method of automated calculation of the natural frequency of the oscillation of a limb after a single twitch. The calculation would also provide data for the exponential decay and time constant of the decay. We believe such data points might provide sensitive methods of calculating small differences in muscle physiology, useful as measures of deleterious effects.

A study should be designed with a dedicated protocol with very high frequencies of data capture, which will be required for precise detection of the peaks and valleys.

Post-tetanic Facilitation (PTF) as an indication of deleterious effects. Post-Tetanic Facilitation (PTF) is a stimulation technique which is used on a daily basis in human anesthesia practice to explore the state of the neuro-muscular junction and identify more extensive patho-physiological processes. Post-Tetanic Facilitation is used when the Train-of-Four is not sensitive enough, for instance with severe impairment of function (“deep” muscle relaxation.). We will be able to compare the effects of the HEMI stimuli to the large literature base of TOF and PTF in human medical practice.

Note in Figure 26, that after each of the 3,000 msec interruptions, the next train of HEMI stimuli rapidly builds up to a higher level than at the end of the previous train. This is the phenomenon of Post-Tetanic Facilitation (PTF.) The post-tetanic facilitation with longer durations of the interruption (3,000 msec) is still present towards the end of the run.

Single Muscle and Single Limb Stimulation. We propose using the stimulation of a single limb or single muscle to measure work and stiffness as measures of deleterious effects. This would isolate the muscle physiology from the deleterious effects of the whole body physiology having an influence on the muscle physiology.

Stunning and Relaxation: Phase delay between HEMI stimulus and muscle movement as a measure of deleterious effects. The phase delay (time period) between the delivery of the HEMI stimulus and onset of muscle movement (**electro-mechanical delay**) can be used as measure of deleterious effects. It is hypothesized that the time from the HEMI stimulus to the onset of muscle movement would increase as muscle fatigue sets in. On the other hand, it has been shown that with cardiac defibrillation during a cardiac arrest, the impedance is decreased with the second defibrillation and more energy is transferred to the heart. A study should be designed to elucidate these effects.

These are quite complex signals as shown in Figure 48. It will require signal processing to identify the exact nadir, as well as the onset of muscle movement. A consistent point of onset of movement of the accelerometer will have to be defined and identified using an automated, consistent program. It is to be expected that there will be some biological variation, hence the need to identify a consistent point for the onset of the muscle movement.

A second phase delay that would be a measure of deleterious effects would be the time of relaxation after the end of the HEMI application to the movement of the limb to the neutral (natural) position. Relaxation is an active process which requires calcium and oxygen. We postulate that fatigued muscle would be relatively depleted of energy stores, and relaxation would be slowed (“delayed”.) To measure relaxation with the accelerometers, it would be necessary to remove and negate the effect of gravity on the return of the limb to the neutral position (e.g., the animal would be lying on the side.) The study would be to determine if relaxation is prolonged in fatigued muscle compared to the control state, prior to the HEMI application. Such measurements would be needed to determine if this could be used as a measure of deleteriousness. A biological example of such “stunning” as this slow relaxation is termed, is based on studies in electric eels¹⁰ where the prey was “stunned” after an electric shock by the eel – the prey did not attempt to escape.

Onset tetanization – “predicts” is correlated with deleterious effects. A phenomenon noted in previous studies (HEMI 3) was that some animals exhibited a slower increase in the blood pressure at the start of the HEMI exposure. In contrast to the typical response described above, some animals did not have a rapid increase in BP with the onset of the HEMI exposure. With the present study, we could show objectively that the onset of tetanization, as indicated by the amount of work performed, varied with several different patterns being exhibited.

We identified and classified five unique patterns of the sequences of the peaks and nadirs (“valleys”) of the amount of work generated.

As a measure of the onset of tetanization, we calculated the work generated in the 1st 5 seconds, before the first interruption. Up to this point, all 14 animals had been exposed to the same protocol. The observed variance, as objectively measured, could therefore mainly be attributed to variations in the anatomy and physiology of the animals. We calculated the variance of the work generated in the 1st 5 seconds for each limb (Table 16). The variance is one of the determinants necessary to perform a Power Calculation to determine an appropriate number of animals for a future study.

¹⁰ Catania KC, Shock & Awe, Scientific American 2019; 320(4): 62-69

We calculated the work generated by the forelimbs (LL+RL) compared to the work generated by the hind limbs (LL+RL) and analyzed the differences.

We determined if the size (weights) of the animals had a correlation with the amount of work generated (Figure 25). There was no correlation, which indicates satisfactory selection of sizes. We determined if there were differences in the amount of work generated in the 1st 5 seconds between the male and female animals (Figure 26). A future study should be designed to correlate the BP and work signals for signal processing and analysis.

FINDINGS and RECOMMENDATIONS

In this study, we explored the deleterious effects caused by HEMI stimuli while varying the interruptions. We examined 18 Hz HEMI stimuli:

- For the main study, durations mainly of 35 seconds, with up to 360 seconds for the pilot studies
- Trains of 18 Hz for 5 seconds; and
- Interrupted by “OFF” periods from 0 to 3,000 msec.

Our data included a wide range of duty cycles, as the extremes of duty cycles (extreme lengths of interruptions) were deemed likely to show the largest differences with the most likelihood of detecting such differences. This plan was successfully implemented.

- A duty cycle with zero interruptions was found to be the most deleterious. The amount of work/power generated with subsequent runs was only about 5% of the first exposure.
- The duty cycle with the longest interruption (3,000 msec) demonstrated the ability to maintain a clinically significant amount of work up to 35-45 seconds, while the other duty cycles with shorter interruptions demonstrated that the work produced decreased to negligible amounts by 15-25 seconds.
- For some limbs, there was a trend of better preserved work/power in Runs 2 and 3 with the interruptions of 3,000 msec, indicating less deleterious effects with longer interruptions.

We analyzed the 1st 5 seconds of the HEMI stimuli, before any interruptions. This enabled a baseline of the variability that would be useful for future power calculations to determine robust group sizes for statistical purposes. We also developed a classification system for the patterns of the work generated during the first 5 seconds. The patterns should be correlated in future studies with the onset of BP increases which is most likely dependent upon the speed of development of tetanization. Measurement of intra-thoracic and intra-muscular pressures would be able to further inform the understanding of this complex physiology.

Unilateral probe positions cause much less total work (work generated by all 4 limbs) than bilateral placements, as the contra-lateral limbs produce much less work.

Bilateral probe positions on the lower abdomen cause minimal effects on the forelimbs and also do not inhibit respiration (minimal effect on chest muscles.)

Based on the concept of placing probes to cause less systemic physiological impairment (e.g., stimulate only the hind limbs with HEMI probes, only front limbs, or unilateral), then the systemic biochemical parameters might not be exceeded. Therefore, the pure, isolated muscle performance (contraction as well as relaxation) could be used to check for deleterious effects, without the complicating factors of systemic biochemical abnormalities.

The Risk of Significant Injury (RSI) may be dependent on the position of the probes as well as on the duty cycle. The statistical distribution of the probe positions on a human subject would be an important factor to study and help to calculate the RSI (Risk of Significant Injury)

A phenomenon further elucidated with this project (after being identified in the HEMI 3 project) was the continued development of deleterious effects for several minutes, and up to an hour, after the end of the HEMI exposure. This is evidenced by further deterioration of biochemical parameters.

A phenomenon that we further elucidated (after being described in the HEMI 3 report) was that prior exposure to HEMI stimuli seemed to protect against deleterious effects of subsequent HEMI stimuli, and animals survived 360 seconds (6 minutes) of HEMI exposure after prior HEMI exposures. Due to the muscles being fatigued, minimal work was performed by the muscles. This confirmed our hypothesis that deleterious effects are caused by, and mirror, the amount of work.

Objective measures of work showed differences in deleterious effects as caused by different duty cycles. The ranges of standard biochemical measurements are exceeded with longer duration HEMI stimuli, and therefore cannot be used to determine finer differences between duty cycles.

In conclusion, with this study we discovered several new features caused by variations of the duty cycles of the HEMI stimuli, and suggests multiple avenues for further study to expand the potential uses of this family of devices.

APPENDIX 1 - REFERENCES

- Andy Adler, David Dawson, Robert Evans, Laurin Garland, Mike Miller, Ian Sinclair and Richard Youmaran, Toward a Test Protocol for Conducted Energy Weapons, Modern Instrumentation, 02, 01, (7), (2013).
- H.J. Adrogué, M.N. Rashad, A.B. Gorin, J. Yacoub, N.E. Madias, Assessing acid–base status in circulatory failure: Differences between arterial and central venous blood, N. Engl. J. Med. 320 (1989) 1312–1316.
- Adrogué H. and Madias N. Management of life-threatening acid-base disorders. NEJM, 1998; 338: 26–34
- G.A. Brooks, Anaerobic threshold: review of the concept and direction for future research, Med. Sci. Sports Exerc. 17 (1985) 31–35.
- Catania KC, Shock & Awe, Scientific American 2019; 320(4): 62-69
- Dawes D, Ho J, et al. 15-second conducted electrical weapon application does not impair basic respiratory parameters, venous blood gases, or blood chemistries and does not increase core body temperature. Ann Emerg Med (Supplement), 2007; 50(3): S132
- Dawes DM, Ho JD, Reardon RF, Miner JR. 2010. The cardiovascular, respiratory, and metabolic effects of a long duration electronic control device exposure in human volunteers. (30 seconds – anterior chest wall: Spirometry showed an increase in minute ventilation during the exposure.) Forensic Sci Med Pathol. 2010 Dec;6(4):268-74. doi: 10.1007/s12024-010-9166-9. Epub 2010 May 26
<https://doi.org/10.1007/s12024-010-9166-9>.
- Dawes, D.M., Ho, J.D., Reardon, R.F. et al. The respiratory, metabolic, and neuroendocrine effects of a new generation electronic control device. Forensic Sci Int. 2011; 207: 55–60
- Dennis, A.J., Valentino, D.J., Walter, R.J. et al. Acute effects of TASER X26 discharges in a swine model. J Trauma. 2007; 63: 581–590
- Alex Golberg and Boris Rubinsky, Towards Electroporation Based Treatment Planning considering Electric Field Induced Muscle Contractions, Technology in Cancer Research & Treatment, 11, 2, (189), (2012).
- Gregory CM, Bickel CS. Recruitment patterns in human skeletal muscle during electrical stimulation. Phys Ther. 2005;85:358–64.
- Ho J D, Dawes D M, Bultman L L, Thacker J L, Skinner L D, Bahr J M, Johnson M A, Miner J R. 2006. Respiratory effect of prolonged electrical weapon application on human volunteers. Acad Emerg Med 2006;13:589-595.
- Ho J D, Dawes D M, Bultman L L, Thacker J L, Skinner L D, Bahr J M, Johnson M A, Miner J R. 2007. Respiratory effect of prolonged electrical weapon application on human volunteers. Acad Emerg Med 2007;14:197-201.
- Ho, J., Lapine, A., Joing, S., et al. Confirmation of respiration during trapezial conducted electrical weapon application. Acad Emerg Med, 2009; 15: 398.
- Ho, J.D., Dawes, D.M., Bultman, L.L. et al. Prolonged TASER use on exhausted humans does not worsen markers of acidosis. Am J Emerg Med. 2009; 27: 413–418
- Hughes, Edward L., M.J. Kennett, W.B. Murray, J.R. Werner, D.M. Jenkins, Electro-Muscular Disruption (EMD) Bioeffects: A Pilot Study on the Effects of Continuous Application of the TASER®X26 Waveform on

Swine, Applied Research Laboratory, The Pennsylvania State University, 30 November 2008. [USMC Contract M67854-05-D-5153/DO03]

Hughes EL, Werner JR, Murray WB, Jenkins DM, Kennett MJ, Bienus DA, Welker ML
Human Electro-Muscular Incapacitation (HEMI) Mechanisms Study 3: An Examination of the Deleterious Physiological Effects of Variations in the Duty Cycle Parameters, Applied Research Laboratory, The Pennsylvania State University, 22 October 2018, [USMC Contract #17F8307 of NAVSEA Contract N00024-12-D-6404]

Jauchem JR, Sherry CJ, Fines DA, et. al. 2006. Acidosis, lactate, electrolytes, muscle enzymes, and other factors in the blood of *Sus scrofa* following repeated Taser exposures. *Forensic Science Intl.* 2006;161:20-30.

Jauchem JR. Repeated or long-duration TASER® electronic control device exposures: Acidemia and lack of respiration. *Forensic Science, Medicine, and Pathology* 6: 46-53, 2010.

Jauchem JR. Muscle stimulation by TASER® conducted energy weapons: Similarities with voluntary muscle contractions during exercise. *Advances in Medicine and Biology* 7: 169-179, 2010.

James R. Jauchem 2010). An Animal Model to Investigate Effectiveness and Safety of Conducted Energy Weapons (Including TASER® Devices). *Journal of Forensic Sciences* 2010;55(2):521-526

Jauchem, James R. Increased hematocrit after applications of conducted energy weapons (including TASER devices) to *Sus scrofa*. *Journal of forensic sciences* 56 Suppl 1 (2011): S229-33.

Jauchem, J.R., Seaman, R.L., and Fines, D.A. Survival of anesthetized *Sus scrofa* after cycling (7 s on/3 s off) exposures to a TASER X26 electronic control device for three minutes. *Am J Forensic Med Pathol.* 2011; 32: 124–130.

Jauchem JR. (2015). Exposures to conducted electrical weapons (including TASER® devices): how many and for how long are acceptable? *J Forensic Sci.* 2015 Jan;60 Suppl 1:S116-29. doi: 10.1111/1556-4029.12672. Epub 2014 Nov 28.

S. N. Kunz & H. G. Calkins & J. Adamec & M. W. Kroll. Adrenergic and metabolic effects of electrical weapons: review and meta-analysis of human data. *International Journal of Legal Medicine.* <https://doi.org/10.1007/s00414-018-1771-2> First Online: 19 January 2018

T. Kurita, K. Morita, T. Kazama, S. Sato, Comparison of isoflurane and propofol-fentanyl anaesthesia in a swine model of asphyxia, *Br. J. Anaesth.* 91 (2003) 871–877.

Lu ST, Ziriach JM, Klages C, Crane C, Comeaux J, Cox DD, Fines D, D'Andrea JA (2008). Acute biological effects of extended Taser®-X26 stimulation in swine (*Sus Scrofa domestica*). Naval Detachment Directed Energy Bioeffects Research Laboratory, Brooks City-Base, TX. Technical Report NHRC-DEBL-TR-2008-01.

L. Martin, All You Really Need to Know to Interpret Arterial Blood Gases, second Ed., Lippincott Williams & Wilkins, Philadelphia, 1999.

Y-G Min, E-J Park and S-C Choi, Acute effect of simultaneous discharge of TASER X26 on anesthetized swine model. *Intensive Care Medicine Experimental*, **3**, Suppl 1, (A370), (2015).

Panescu, D., Kroll, M. W., Efimov, I. R., & Sweeney, J. D. (2006). Finite Element Modeling of Electric Field Effects of TASER Devices on Nerve and Muscle. 2006 International Conference of the IEEE Engineering in Medicine and Biology Society. doi:10.1109/iembs.2006.260376. Available at: <http://dx.doi.org/10.1109/IEMBS.2006.260376>.

Park EJ, Choi SC, Ahn JH, Min YG: Repetitive TASER X26 discharge resulted in adverse physiologic events with a dose-response relationship related to the duration of discharge in anesthetized swine model. Journal of forensic sciences. 2013, 58 (1): 179-83. 10.1111/j.1556-4029.2012.02287.x. Jan.

Ravindra P. Joshi, Ashutosh Mishra, Shu Xiao and Anderi Pakhomov, Model study of time-dependent muscle response to pulsed electrical stimulation, Bioelectromagnetics, 31, 5, (361-370), (2010).

Florin Despa Suki Basati Zhen-Du Zhang John D'Andrea J Patrick Reilly Elena N. Bodnar Raphael C. Lee. Electromuscular incapacitation results from stimulation of spinal reflexes. Bioelectromagnetics 2009;30:411–421 <https://doi.org/10.1002/bem.20489>. Bioelectromagnetics 30:411–421, 2009. 2009 Wiley-Liss, Inc.

Sebastian N. Kunz, MD. Biases in TASER research. Ludwig-Maximilians-University, Munich, Institute of Forensic Science, Munich, Germany, PlumX Metrics. DOI: <https://doi.org/10.1016/j.ahj.2011.12.015>

K. Schwarzkopf, T. Schreiber, N.P. Preussler, E. Gaser, L. Huter, R. Bauer, H. Schubert, W. Karzai, Lung perfusion, shunt fraction, and oxygenation during one-lung ventilation in pigs: the effects of desflurane, isoflurane, and propofol, J. Cardiothorac. Vasc. Anesth. 17 (2003) 73–75

Sweeney, J.D., 2009. Theoretical comparisons of nerve and muscle activation by neuromuscular incapacitation devices. 2009 Annual International Conference of the IEEE Engineering in Medicine and Biology Society. Available at: <http://dx.doi.org/10.1109/IEMBS.2009.5334537>.

Sun H, Webster J. Estimating neuromuscular stimulation within the human torso with TASER stimulus. Phys Med Biol. 2007;52:6401–11

Valentino DJ, Walter RJ, Dennis AJ, Nagy K, Loo MM, Winners J, Bokhari F, Wiley D, Merchant A, Joseph K, Roberts R. Neuromuscular effects of stun device discharges. J Surg Res. 2007 Nov;143(1):78-87.

H. Westerblad, J.A. Lee, J. Lannergren, D.G. Allen, Cellular mechanisms of fatigue in skeletal muscle, Am. J. Physiol. 26 (1991) C195–C209.

Zirix JM (2011) Human electromuscular incapacitation (HEMI) bioeffects project: final report. Naval Medical Research Unit, San Antonio. 31 January 2011.

A. Ziser, R.A. Strickland, M.J. Murray, Propofol does not induce pulmonary dysfunction in stressed endotoxemic pigs receiving Intralipid, Crit. Care. Med. 31 (2003) 2029–2033.

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