



College of Engineering
University of Nevada, Reno



University of Nevada, Reno
School of Medicine

Human Performance and Biosystems Program Review

Nanoelectropulse-based electrostimulation to enhance muscle strength and mitigate musculoskeletal disorders in military pilots

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Background

AFOSR HUMAN PERFORMANCE AND BIOSYSTEMS

Goal: Improve human performance capabilities by establishing *new electric stimulation methods* to augment human performance

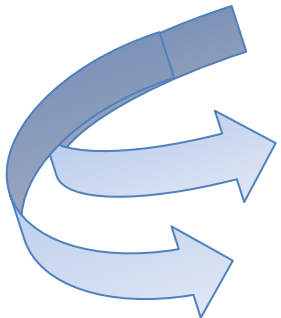
THE ELECTROSTIMULATION APPROACH BEING EXPLORED

NANOSECOND DURATION ELECTRIC PULSES (NEP)

Have the ability to modulate/fine-tune cellular responses by altering the electric pulse waveform

Have the potential to be delivered to a targeted tissue *non-invasively*

TWO MAIN APPLICATIONS



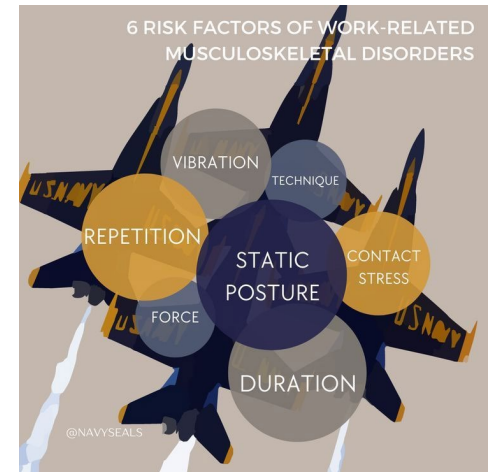
Evoking a *transient* “adrenaline burst” on demand (i.e., triggering a “fight or flight” response)

Mitigating flight-induced musculoskeletal disorders by enhancing skeletal muscle force and increasing endurance

Mitigating flight-induced musculoskeletal disorders by enhancing skeletal muscle force

Musculoskeletal disorders are a common problem in armed forces worldwide, especially in Air Force personnel

- To counteract these disorders, resistance exercise is supplemented with various forms of external electrical stimulation (e.g., neuromuscular electrical stimulation or NMES) to elicit involuntary contractions of skeletal muscle, thus enhancing skeletal muscle force and increasing endurance.



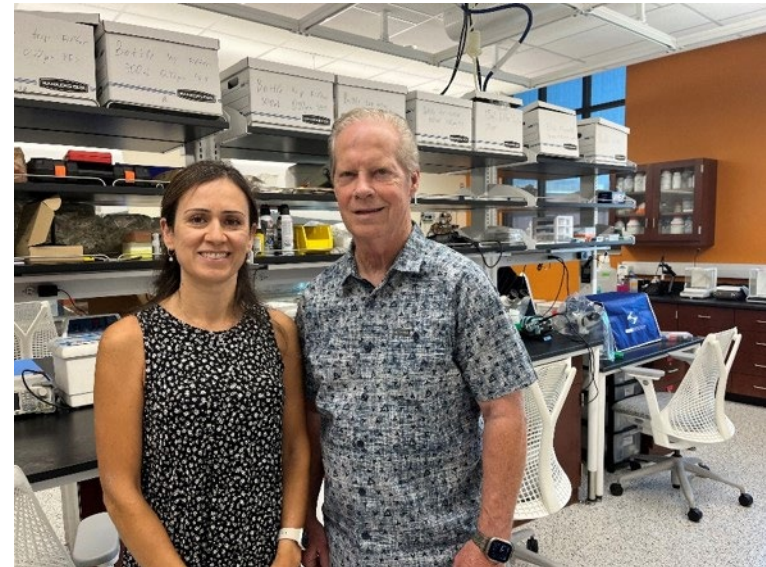
<https://www.pinterest.com/pin/factors-for-workrelated-musculoskeletal-disorder-within-the-navy--913315999404361844/>

Limitations of NMES

Rapid onset of NMES-induced fatigue, reducing the ability to cause repeated contraction.

- Limits the duration that NMES can be applied.
- Reduces the efficacy of treatment, particularly during maximal strength training.

J. Travis Luz, CAPTAIN (0-6) U.S. Navy (Retired)



Captain Luz in the Navy's Training Centrifuge (@ + 7.9 Gz) at Naval Air Station Lemoore, CA. Navy's Subject Matter Expert for G-Induced Loss of Consciousness (GLOC).

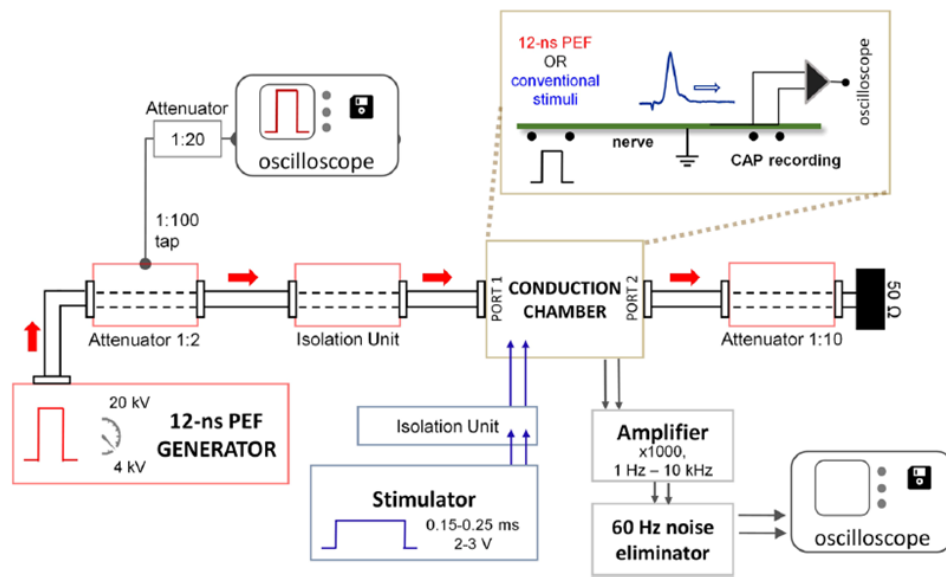
“... you can certainly interpolate what skeletal muscle fatigue is introduced within the environment of High Onset Rate of +9 GZ / per second on the body as experienced in the Attack/Fighter Jet Communities.”

“Each and every Aviation Community, whether it be Rotary Wing, Heavy Transport, or the Attack and Fighter type aircraft has its own environmentally and physiologically induced fatigue factors.”

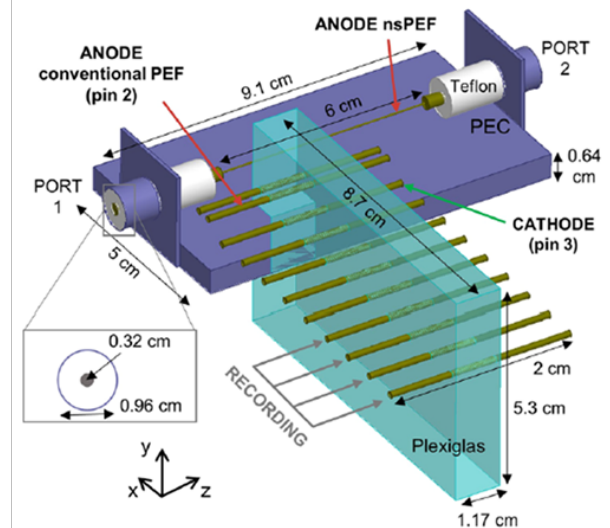
NEP have been shown to stimulate intact nerves without any damage

Approach

- Stimulate frog sciatic nerve
- Apply 12 ns stimuli at 4.1-11 kV (3.3-8.8 kV/cm)
- Apply conventional stimuli 100-250 μ s at 1-5 V (103-515 V/m)
- Record compound action potentials (CAP)



Block diagram of the setup for 12 ns stimulation

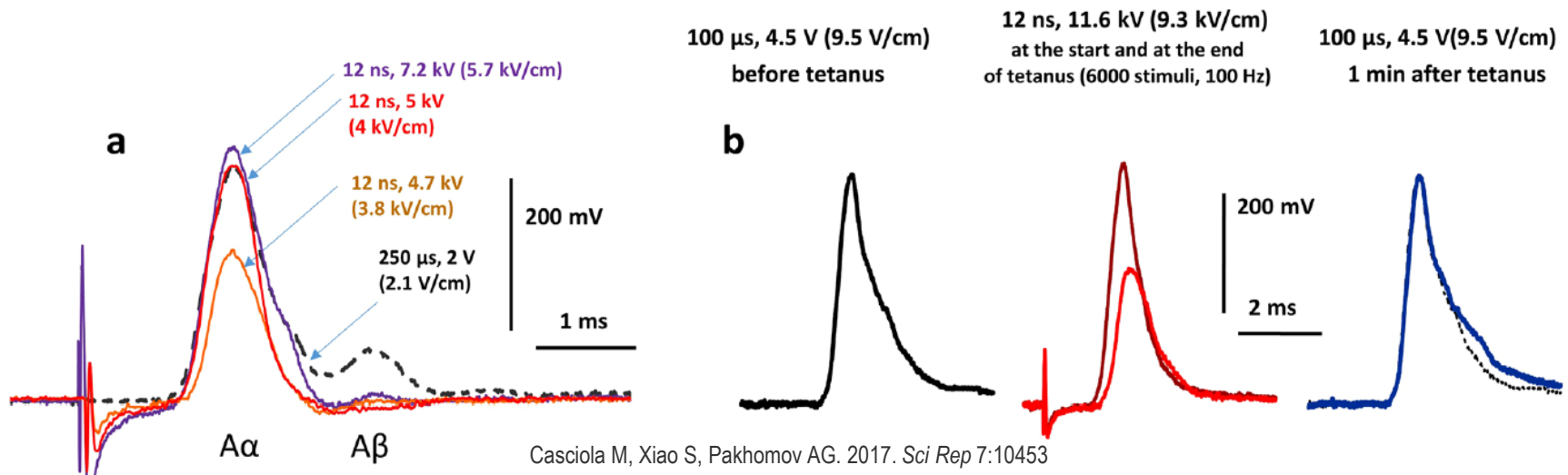


Conduction chamber design

NEP have been shown to stimulate intact nerves without any damage

What they found:

- NEP elicit CAP similar to conventional stimuli, but are selective for the excitation of fast fibers ($A\alpha$)
- Nerves sustain repeated tetanic stimulations (50 Hz or 100 Hz for 1 min) alternately by 12 ns pulses and by conventional duration electric pulses (CEP)



CAP traces evoked by NEPs at three different pulse voltages (solid lines) and by conventional stimulation (dashed line)

A 1-min, 100 stimuli/s tetanus using 12-ns, 11.6 kV pulses did not cause nerve damage

Can NEP be used as a novel electrical stimulation approach that triggers muscle contraction minimizing/without causing fatigue?

Specific Objectives

1. A. Establish the range of NEP parameters (amplitude, duration, frequency) that generate optimal stimulation of muscle (i.e., best combination of force and fatigue) via cuff-like electrodes placed around the phrenic nerve and compare these effects to those induced by standard CEP

B. Elucidate the mechanism(s) responsible for any difference in fatigue caused by CEP vs. NEP

2. Establish the range of NEP parameters that stimulate the diaphragm directly and/or indirectly via transmural electrodes placed on the muscle, as well as determine the mechanisms underlying fatigue caused by such stimulation

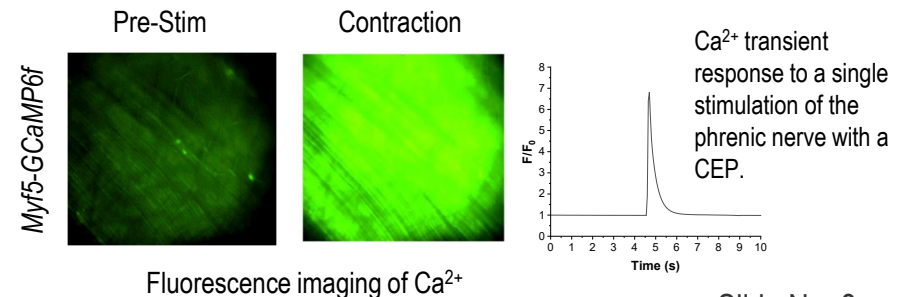
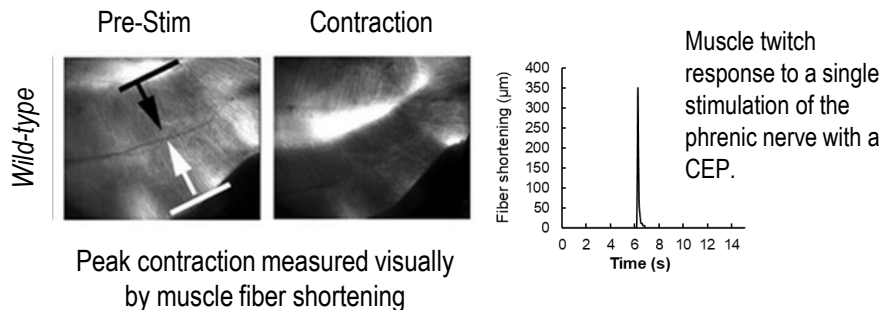
A. Determine whether delivery of NEP via transmural electrodes stimulates muscle directly or indirectly via peripheral nerve branches

B. Identify mechanisms underlying differences in peak muscle force and fatigue, if any, between CEP and NEP delivered via transmural electrodes

C. Determine whether NEP delivered via transmural electrodes can also trigger the contraction of a muscle covered by skin

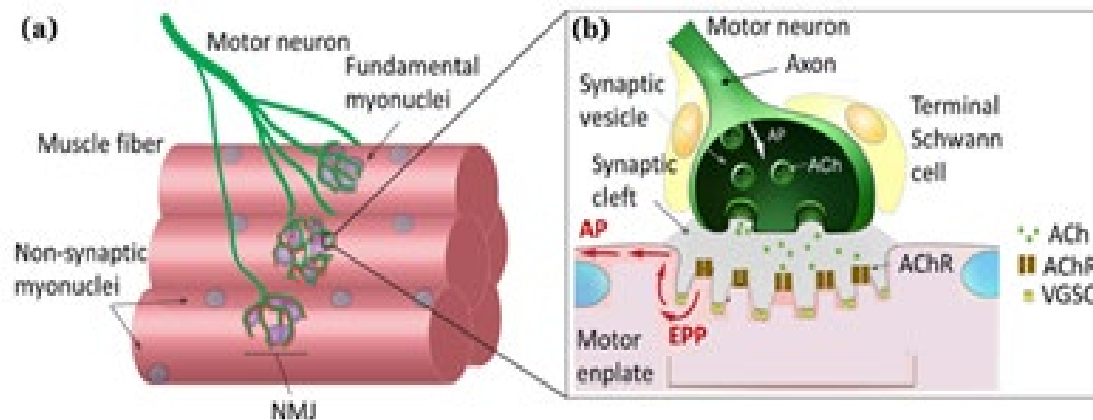
Primary Approach

- Phrenic nerve and diaphragm muscle: a model system of excitation-contraction coupling
 1. A murine neuromuscular tissue preparation in which the phrenic nerve that innervates the diaphragm (skeletal) muscle can be stimulated.
 2. A well-established preparation used to study the mechanisms underlying neuromuscular function.
- Methods
 - Employ wild-type mice to measure, by optical recording of fiber shortening, muscle contraction and fatigue.
 - Fluorescence imaging of muscle intracellular Ca^{2+} levels or $[\text{Ca}^{2+}]_i$ in mice expressing a genetically-encoded calcium indicator in skeletal muscle cells.



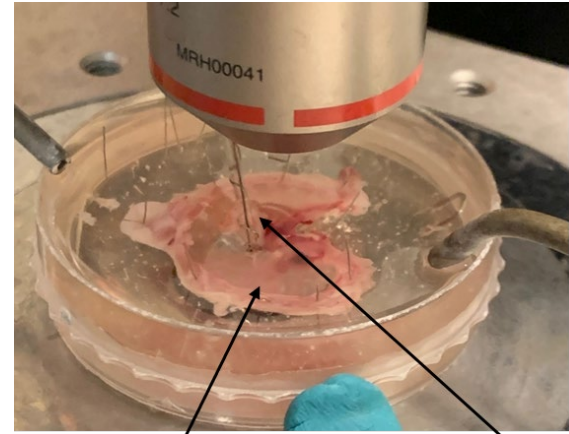
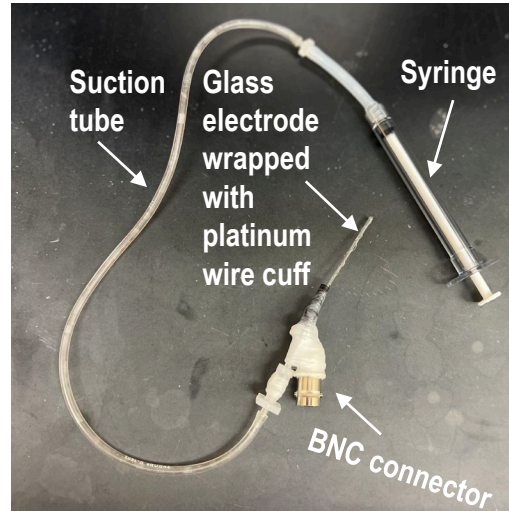
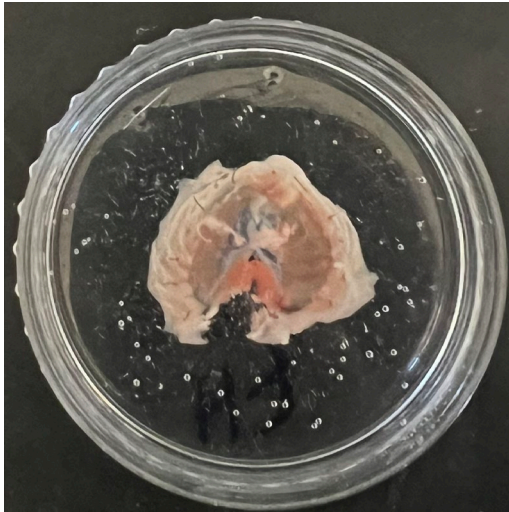
Anatomy of the neuromuscular junction

- Stimulation of the phrenic nerve with CEP using a “cuff-like” electrode, triggers the release of the neurotransmitter acetylcholine (ACh) and thus the excitation and contraction of diaphragm skeletal muscle.
- This electrical excitation includes the initial endplate potential (EPP), a 20-40-mV depolarization of the muscle membrane potential mediated by the influx of cations through ACh receptors (AChR), and the subsequent action potential (AP), a larger depolarization mediated by voltage-gated sodium channels (VGSC) that become activated by the EPP.
- Muscle AP in turn trigger the release of Ca^{2+} from intracellular stores, which leads to muscle contraction via myosin-actin interactions.

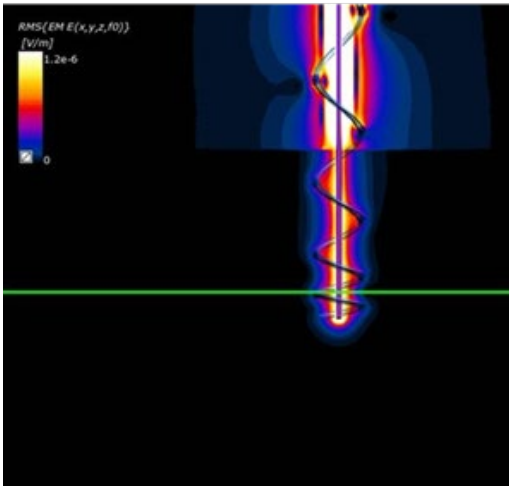


Ex vivo preparation of phrenic nerve and diaphragm muscle

Diaphragm muscle pinned to a Sylgard-coated dish



Pulse delivery electrode

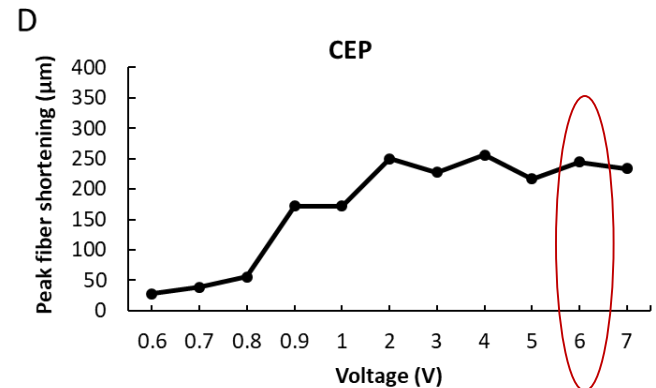
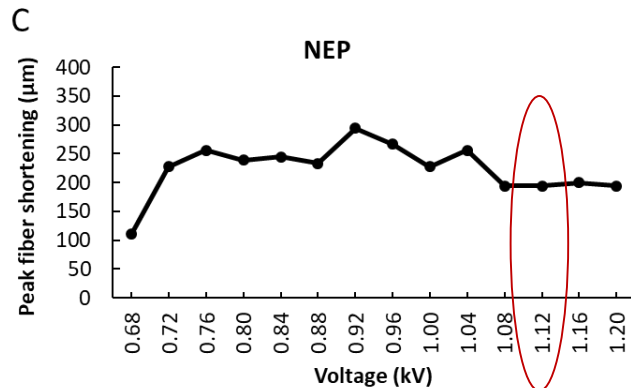
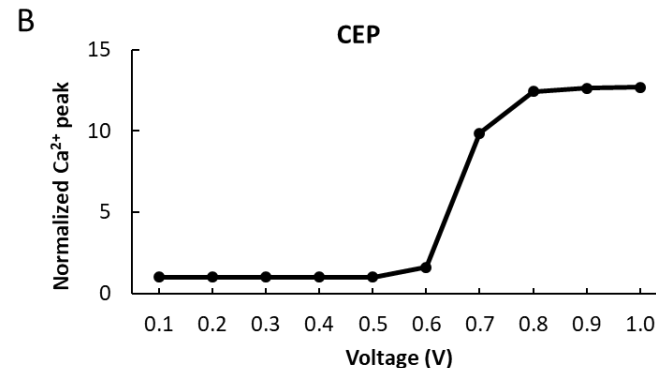
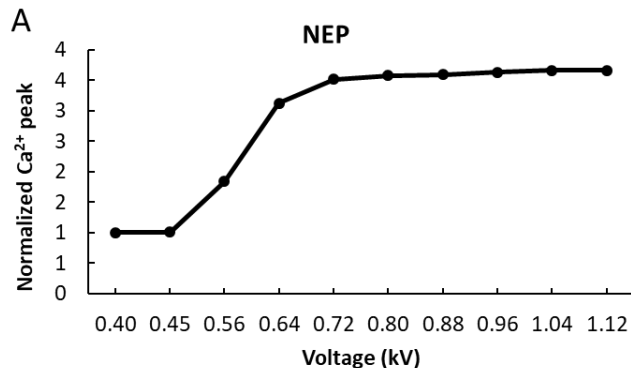


Electrical stimulation parameters investigated:

- CEP: 0.3 ms at suprathreshold (6 V)
- NEP: 30 ns at suprathreshold (1.1 kV)

Seven consecutive trains of CEP or NEP applied at 40 Hz for 30 s (i.e. 1,200 pulses/train), with 15 min inter-train interval

Identify the threshold to stimulate the phrenic nerve with NEP and CEP

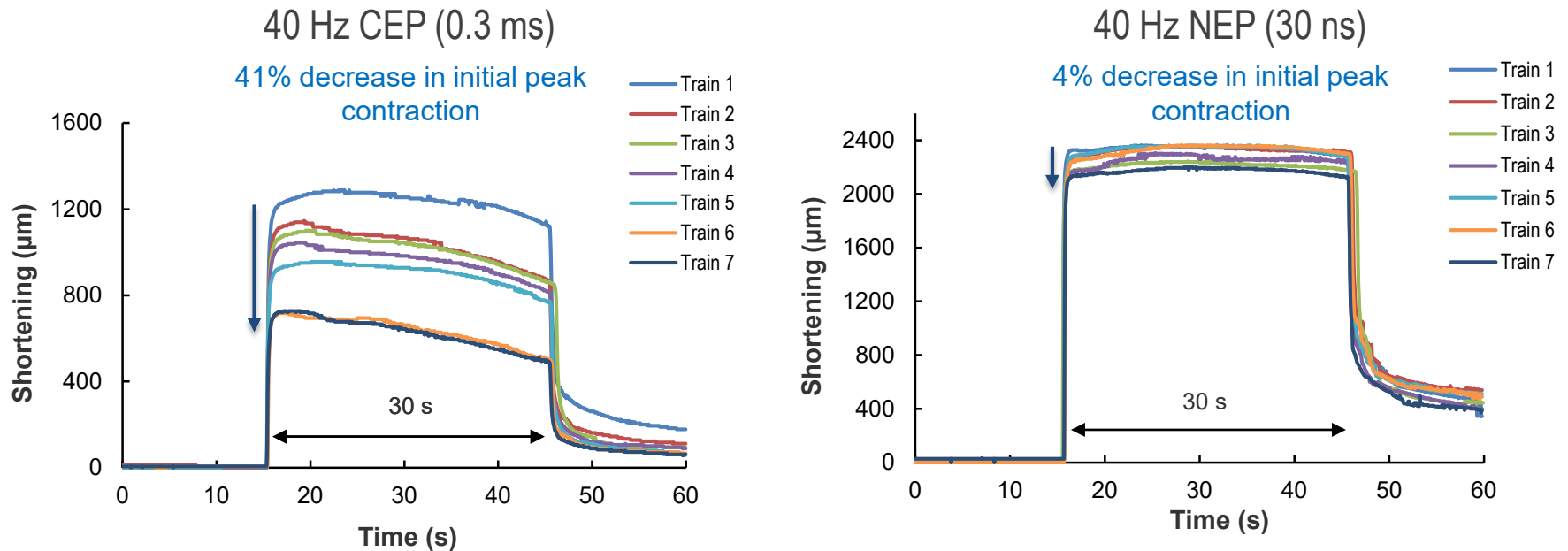


These voltage values generated the supramaximal contraction during NEP and CEP trains at 40 Hz

- Stimulation of the phrenic nerve with graded voltage steps from ~0.7 kV to 1.2 kV for NEP (30 ns) produced contractile responses, with peak responses near 0.9 kV that plateaued at 1.12 kV.
- Stimulation with graded voltage steps from 0.6 V to 7 V for CEP (0.3 ms) produced contractile responses, with peak responses near 1 V that plateaued at 2 V.

Evaluate the effect on muscle contractile force and fatigue of seven consecutive high-frequency stimulation (HFS) trains of the phrenic nerve with CEP and NEP

Representative experiments

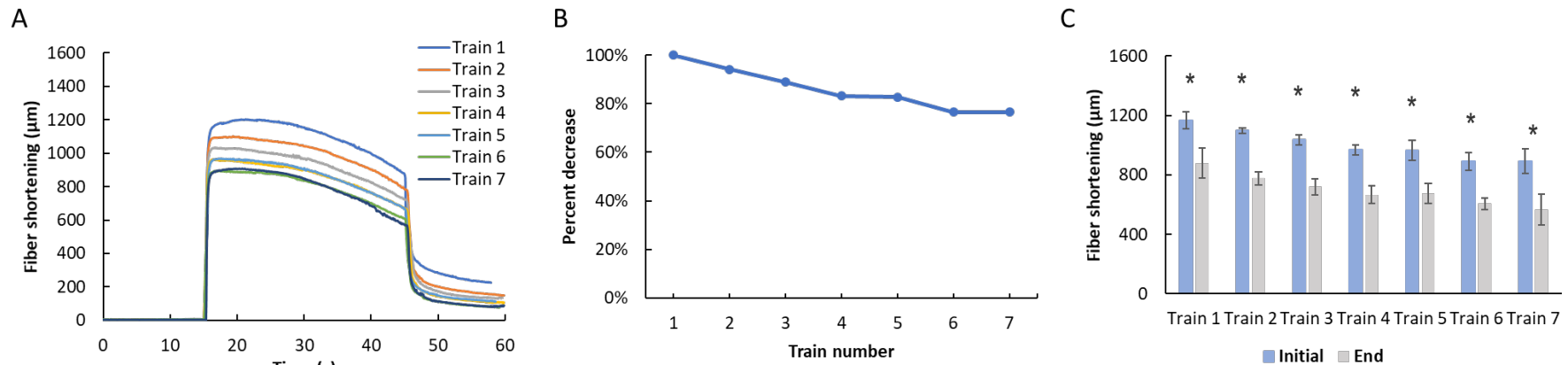


NEP are as effective as CEP in evoking muscle contraction but with less fatigue.

- Peak force fatigues less between trains for NEP compared to CEP
- Peak force declines less within a train for NEP compared to CEP

Tetanic muscle contraction evoked by 30-s trains of CEP applied at 40 Hz to the phrenic nerve produces fatigue

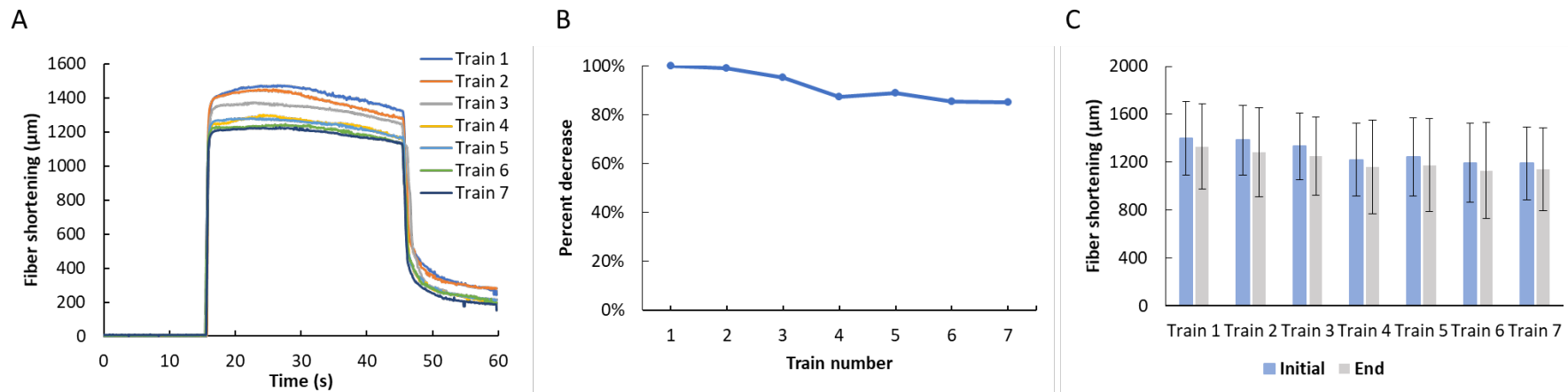
Average of each Train across 5 CEP experiments



- 23% reduction in initial peak contraction in response to the seventh train of CEP compared to the first.
- Significant decrease in the magnitude of fiber shortening at the beginning of the train (initial) vs. the end of the train (end), ranging on average from 25% to 36% ($n = 5$), for all seven trains.

Tetanic muscle contraction evoked by 30-s trains of NEP applied at 40 Hz to the phrenic nerve produces less fatigue than CEP

Average of each Train across 4 NEP experiments



- 15% reduction in initial peak contraction in response to the seventh train of NEP compared to the first.
- No significant decrease in the magnitude of fiber shortening at the beginning of the train (initial) vs. the end of the train (end), ranging on average from 4% to 7% ($n = 4$), for all seven trains.

Summary of Findings

Comparison of twitch and tetanic muscle contractile responses to phrenic nerve stimulation with CEP and NEP

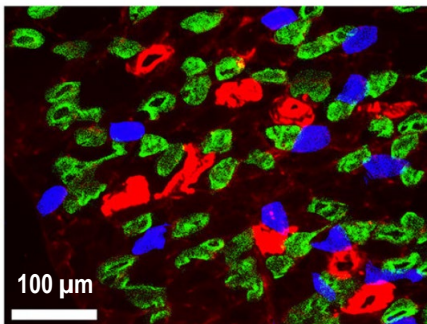
- We identified the range of voltages of NEP that produced muscle responses, as well as the voltage at which peak and plateaued responses were observed.
- There was less of a reduction in initial peak force from the first to seventh train in response to phrenic nerve stimulation with HFS with NEP vs. CEP.
- There was also less of a reduction in ending vs. initial peak force within a train in response to HFS of the phrenic nerve with NEP vs. CEP.
- Muscle fatigue, therefore, whether measured by the loss of initial peak contractile response between trains, or measured by the loss of peak contractile force within an individual train, was enhanced in response to HFS of the phrenic nerve with CEP vs. NEP.

Is motor unit recruitment affected differently by NEP vs. CEP?

Identify cellular mechanisms by which trains of HFS with NEP cause less fatigue

- Investigate NEP and CEP effects on Ca^{2+} signals in muscle using transgenic mice expressing GCaMP6f in the muscle.
- Examine whether distinct patterns of muscle fiber recruitment occur in response to HFS trains of NEP vs. CEP.

Immunohistochemistry

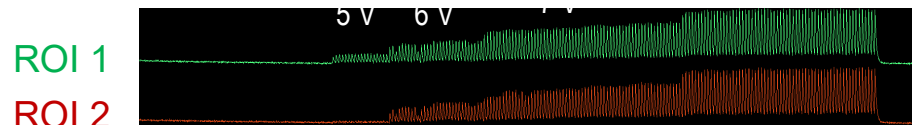


Heredia et al. Front Cell Neurosci. 2016.
10:276

Type I (slow)

Type IIB (fast-fatigable)

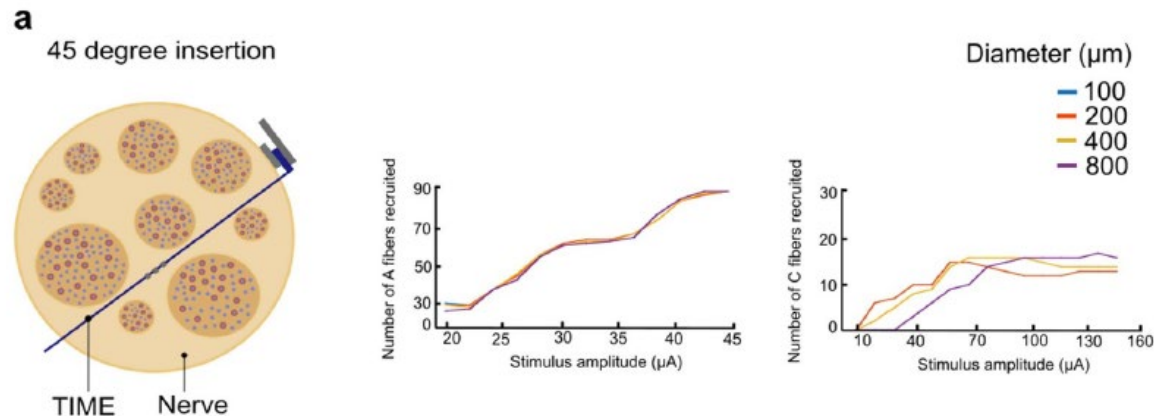
Type IIA (fast fatigue-resistant)



Stimulation of phrenic nerve with graded intensities of voltage (CEP, 1 Hz) sequentially activates distinct motor units.

Use of numerical modeling to help identify basic mechanisms of NEP- and CEP-induced nerve fiber responses

- The use of computational methods could help identify basic mechanisms of NEP- and CEP-induced nerve fiber responses and shed light on how NEP and CEP are interacting with the various types of fibers in the nerve to cause fatigue.
- Approach similar to the one described by Xie et al. (J. Neural Eng. 2023. 20:046032) wherein we will create phrenic nerve models that solve nerve responses using cable equations and computes extracellular potentials using finite element methods.
- We will include various types of fibers to study fiber responses (i.e., recruitment) to a variety of pulse parameters and waveforms.



Xie et al. J. Neural Eng. 2023. 20:046032

Significance and Plans for next year

Significance

- The experiments proposed under Objective 1 will provide a comprehensive analysis of the impact of different NEP parameters on nerve stimulation-induced muscle contraction, and how this excitation can be precisely manipulated such that less fatigue is observed.
- Objective 1 will also delve into potential mechanistic avenues to explain how NEP produces less fatigue than CEP of similar frequencies and pulse train durations.

Future studies

- Establish the range of NEP parameters (amplitude, duration) that generate optimal stimulation of muscle through indirect stimulation of the phrenic nerve with cuff-like electrodes.
- Finish replicates, data analysis and stats.
- Perform similar fatigue studies in GCaMP6f mice.
- Elucidate the mechanisms that underly fatigue using experimental and computational approaches.

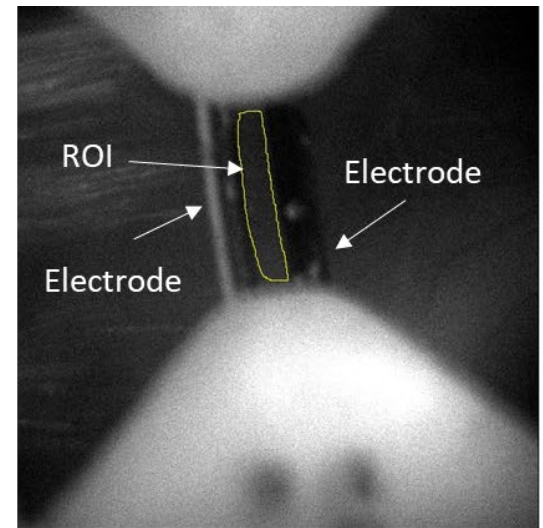
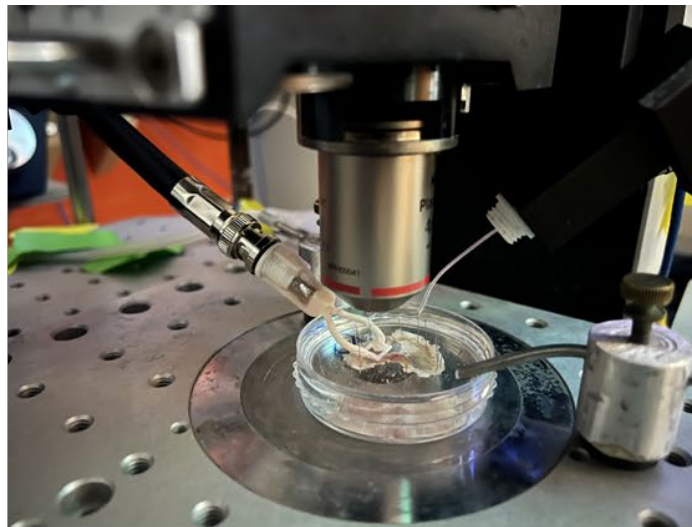
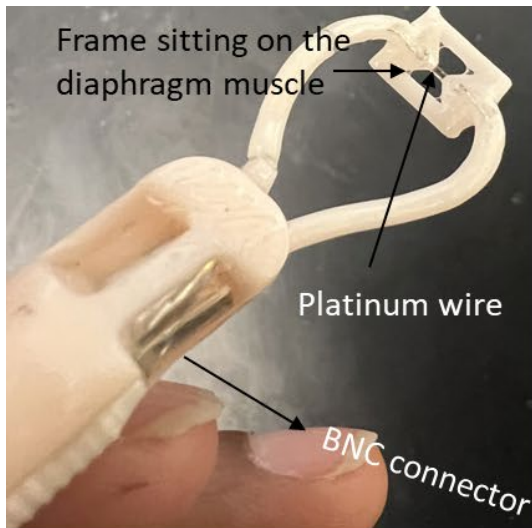
Specific Objectives

1. A. Establish the range of NEP parameters (amplitude, duration, frequency) that generate optimal stimulation of muscle (i.e., best combination of force and fatigue) via via cuff-like electrodes placed around the phrenic nerve and compare these effects to those induced by standard CEP
B. Elucidate the mechanism(s) responsible for the lack of fatigue
2. Establish the range of NEP parameters that stimulate the diaphragm directly and/or indirectly via transmural electrodes placed on the muscle, as well as determine the mechanisms underlying fatigue caused by such stimulation
 - A. Determine whether delivery of NEP via transmural electrodes stimulates muscle directly or indirectly via peripheral nerve branches
 - B. Identify mechanisms underlying differences in peak muscle force and fatigue, if any, between CEP and NEP delivered via transmural electrodes
 - C. Determine whether NEP delivered via transmural electrodes can also trigger the contraction of a muscle covered by skin

Approach

Goal: Examine whether NEP delivered to the surface of the muscle via transmural electrodes selectively activate peripheral nerve branches and/or whether NEP directly activates muscle.

Approach: Record muscle Ca^{2+} responses evoked by 1 Hz NEP or CEP stimulation in the absence and presence of the nicotinic acetylcholine receptor blocker curare ($12\ \mu\text{M}$).

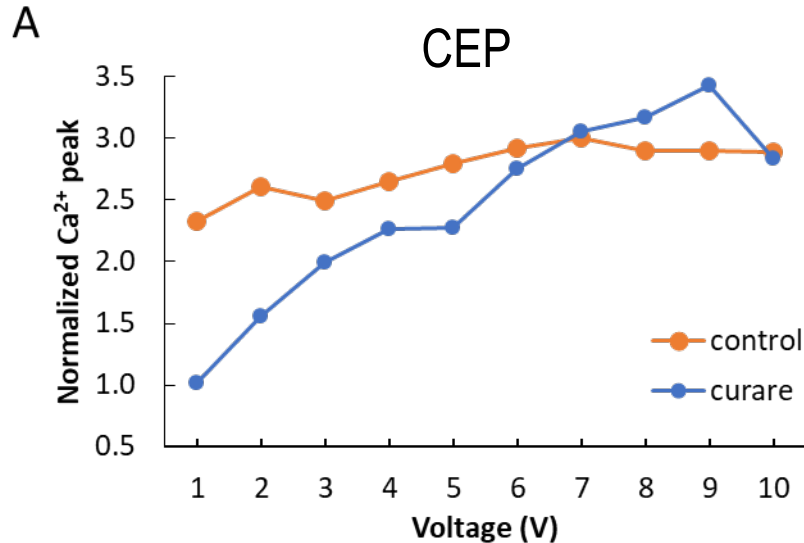


Transmural electrode used to stimulate the diaphragm muscle with NEP or CEP

Determine whether CEP stimulates muscle directly or indirectly via peripheral nerve branches

- Kuei et al. (J Appl Physiol. 1990. 68:174-80) reported that a 0.2-ms pulse stimulated the muscle via the nerve, whereas a 2-ms pulse stimulated the muscle directly.

Preliminary Data

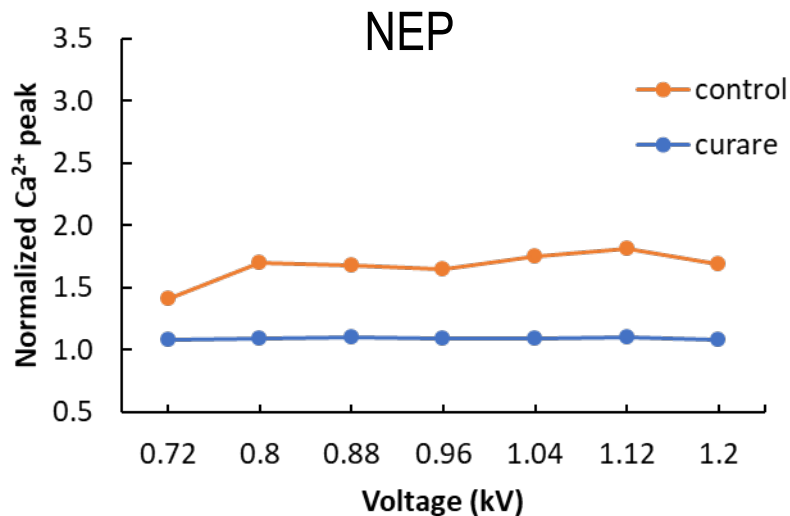


For a pulse duration of 0.3 ms, CEP delivered via transmural electrodes stimulated the muscle through a combination of indirect effects on nerve and direct effects on muscle, depending on the voltage.

Determine whether NEP stimulates muscle directly or indirectly via peripheral nerve branches

Preliminary Data

B



NEP (25 ns) delivered via transmural electrodes stimulated the muscle through an indirect effect on the nerve.

The response observed in the absence of curare resulted from stimulation of peripheral nerve branches only.

- Need to perform control experiments to confirm that curare effectively blocks the nerve input throughout the entire duration of the experiment.

Significance and Plans for next year

Significance

- Characterizing the efficacy of NEP directly applied to diaphragm muscle will provide essential information for determining the parameters of NEP delivery (e.g., pulse number, pulse repetition rate, electric field intensity) that trigger muscle contraction, with the goal to develop more effective NMES protocols that delay fatigue while maximizing muscle response.
- Establish whether the advantages of NEP over CEP with respect to muscle fatigue, observed with cuff-like electrode stimulation of nerve trunks, extend to a less invasive approach using transmural electrodes laying on top of the muscle.

Future studies

- Build on the preliminary findings to optimize the range of NEP and CEP parameters that cause direct stimulation of the diaphragm using transmural electrodes, and compare the effects of HF trains of CEP and NEP on peak force, Ca^{2+} signals, and fatigue.
- Future studies will also interrogate the mechanism by which this occurs.

Acknowledgments



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Professional Personnel

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Graduate Students

Farhana Hossain
Jose Moreno Duran
Sung Hae Yun



<https://www.unr.edu/nevada-today/news/2024/josette-el-zaklit-gets-grant-for-nanoelectrical-stimulation-research>

Nanoelectropulse-based electrostimulation to enhance muscle strength and mitigate musculoskeletal disorders in military pilots

Objectives:

Develop practical uses of nanosecond electric pulses (NEP) to augment human performance.

- Establish the range of NEP parameters that generate optimal stimulation of muscle either directly or indirectly via the nerve.
- Establish the efficacy of NEP to evoke muscle contraction without causing fatigue.
- Elucidate the mechanism(s) responsible for any difference in fatigue caused by NEP vs conventional-duration electric pulses (CEP).

Accomplishments:

Identified the range of voltages that produced muscle responses, as well as the voltage at which peak and plateaued responses were observed.

Evaluated the effect on muscle contractile force and fatigue of seven consecutive high-frequency stimulation trains of the phrenic nerve with CEP and NEP.

Designed and tested a transmural electrode to stimulate the diaphragm muscle with NEP or CEP.

Hired a new PhD student; One conference abstract (BMES 2024); One presentation at a national meeting; One presentation at the GSA poster symposium at UNR; One manuscript in preparation; One NSF proposal under review.

Technical Approach:

A murine *ex vivo* neuromuscular preparation (phrenic nerve and diaphragm muscle).

Muscle contraction: Brightfield microscopy to measure muscle fiber shortening to measure muscle contraction.

Muscle Ca²⁺ transients: Fluorescence imaging of muscle intracellular Ca²⁺ levels in transgenic mice expressing a genetically-encoded calcium indicator (GCaMP6f) in skeletal muscle cells.

DoD Benefit:

Mitigate flight-induced musculoskeletal disorders by applying NEP externally to enhance skeletal muscle force and increase endurance.

Reduced risk for musculoskeletal injuries.

Portable NEP delivery device for muscular training programs.

Thank You for Your Attention!

Questions?

