

AWARD NUMBER: W81XWH-17-1-0513

TITLE: Pulsed Electromagnetic Field Therapy for Accelerating Peripheral Nerve Regeneration and Preserving Neuromuscular Junctions

PRINCIPAL INVESTIGATOR: Thomas L. Smith, PhD

CONTRACTING ORGANIZATION: Wake Forest University Health Sciences

REPORT DATE: September 2020

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
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13. SUPPLEMENTARY NOTES									
14. ABSTRACT Major accomplishments include receipt of IACUC and ACURO approvals for all animal protocols, receipt and installation of a PEMF cage system providing commercially available PEMF waveforms, and initiation of experiments in Specific Aims 1 and 2. The PEMF cage system is installed in a dedicated room in the Wake Forest vivarium. Caging does not interfere with the PEMF waveform. Rats were placed on study to address Specific Aims 1, 2, 3. Initial gait analysis was performed, the sciatic nerve was transected and repaired, and the animals received PEMF treatment for 4, 3, or 2 months. Animals underwent functional testing utilizing EMG and muscle force generation following their last gait analysis. Specific Aim 3 (ability of PEMF to sustain neuromuscular junctions) was initiated – Animals were placed on study following nerve transection without repair in December 2018. They were treated for 4 months. A second cohort was placed on study for a treatment period of 3 months.									
15. SUBJECT TERMS None listed.									
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TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	3
2. Keywords	3
3. Accomplishments	3
4. Impact	10
5. Changes/Problems	12
6. Products	13
7. Participants & Other Collaborating Organizations	15
8. Special Reporting Requirements	17
9. Appendices	17

1. INTRODUCTION:

Peripheral nerve injuries often accompany blast and shrapnel wounds. Repair of peripheral nerves requires a relatively long period of recovery. Pulsed electromagnetic field (PEMF) exposure offers an FDA approved intervention that has the potential to accelerate nerve recovery. The proposed studies will study PEMF treatments in rats with transected and repaired sciatic nerves. The rate and degree of functional motor recovery will be assessed in animals with and without treatment. In addition, the ability of PEMF to maintain neuromuscular junction integrity and function following denervation will be studied.

2. KEYWORDS:

Nerve injury, pulsed electromagnetic fields, motor nerve function, neuromuscular junctions, sciatic nerve, gait.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major goals:

Specific Aim 1- In-vivo effects of Pulsed Electromagnetic Fields (PEMF) on peripheral nerve axonal regrowth will be examined in rats subjected to sciatic nerve injury and repair.

- Major Task 1: Obtain regulatory approval for animal studies
 - o Milestone Achieved November 8, 2017
- Major Task 2: Construct apparatus to deliver PEMF to test subjects –
 - o Milestone achieved November 20, 2018. Second apparatus constructed by Wake Forest Center for Nanotechnology June 2018.
- Major Task 3: Perform surgeries on animals and enroll in PEMF protocol. – First cohort of 8 rats initiated in protocol on May 14, 2018. Second cohort of 8 animals was subsequently enrolled bringing the number of animals to 8 animals exposed to PEMF and 8 animals exposed to sham PEMF for 4 months

Specific Aim 2 - Assess functional outcomes of PEMF exposure on peripheral nerve injury/repair

- Major Task 4: Perform gait analysis on animals in Specific Aim 1. – First cohort of 8 rats initiated on May 1, 2018. These animals came off PEMF exposure on September 14, 2018. Second cohort initiated 5/23/2019 and completed exposure 9/25/2019.

Specific Aim 3 – Determine whether PEMF exposure has the ability to sustain neuromuscular junction morphology following denervation of muscle.

- Major task 1: subject exposed to PEMF/sham PEMF following denervation of muscle. – Initiated 5/09/2019 and completed 6 month exposure 11/9/2019

What was accomplished under these goals?

Specific Aim 1 - In-vivo effects of Pulsed Electromagnetic Fields (PEMF) on peripheral nerve axonal h will be examined in rats subjected to sciatic nerve injury and repair.

- Major Task 1: Obtain regulatory approval for animal studies
 - o Milestone Achieved November 8, 2017
 - o Replacement IACUC protocol initiated in May 2020.
- Major Task 2: Construct apparatus to deliver PEMF to test subjects –
 - o Milestone achieved November 20, 2018.
- Major Task 3: Perform surgeries on animals and enroll in PEMF protocol. – First cohort of 8 rats initiated in protocol on May 14, 2018
 - o These aseptic surgeries consist of transecting the sciatic nerve followed by immediate primary repair. Rats then are pair housed within the PEMF exposure cages and are subjected to specific PEMF waveforms for 6 hours per day, five days per week.

Specific Aim 2 - Assess functional outcomes of PEMF exposure on peripheral nerve injury/repair

- Major Task 4: Perform gait analysis on animals in Specific Aim 1. – First cohort of 8 rats initiated on May 1, 2018. These animals came off 4 months of PEMF exposure on September 25, 2018. Second cohort initiated 5/23/2019 and completed exposure 9/25/2019.
 - o Gait analysis was performed prior to nerve injury/repair using a DigiGait system for gait analysis. Rats were subsequently studied at 10 weeks, and 4 months post-injury. Gait analysis determined that while there was impairment following nerve transection and repair, there was no significant difference in gait parameters between PEMF exposure and Sham-exposure treated groups of rats.(Fig 1,2, &3).

Figure 1. Gait parameters at baseline (prior to PEMF[dark bars] /Sham PEMF [lighter bars], also included as an attachment)

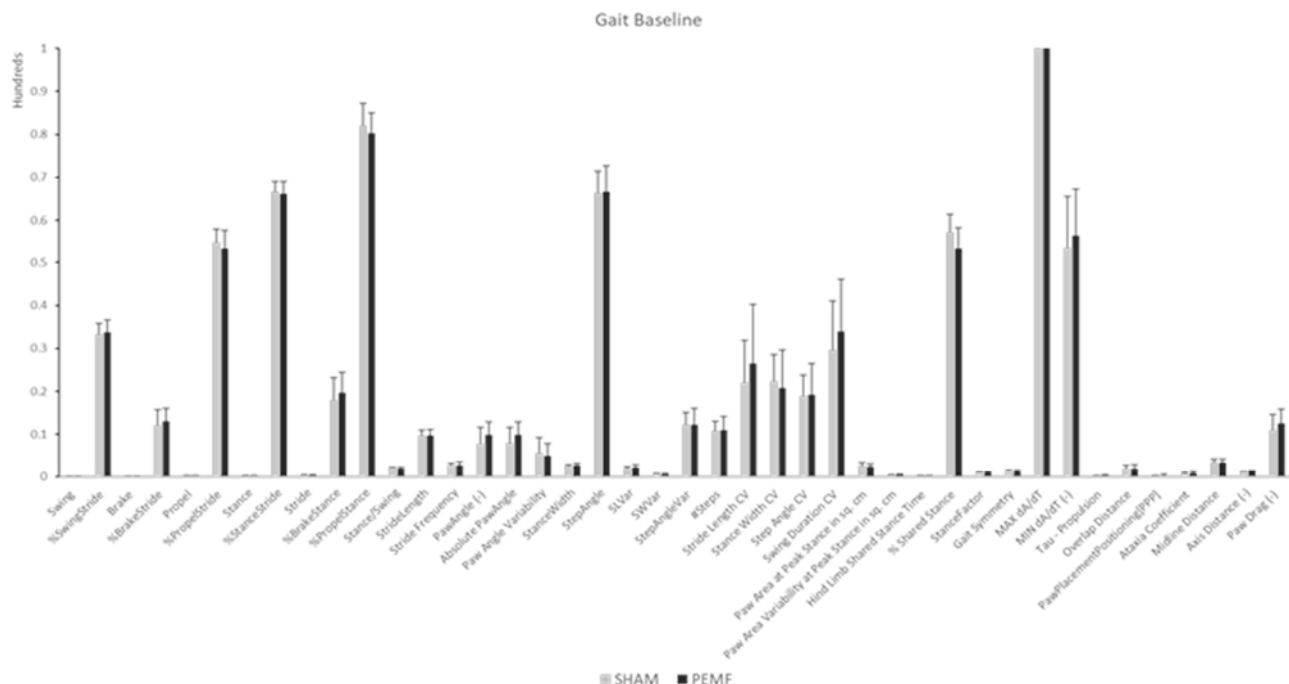


Figure 2. Gait at 10 weeks of PEMF/Sham PEMF (also included as an attachment)

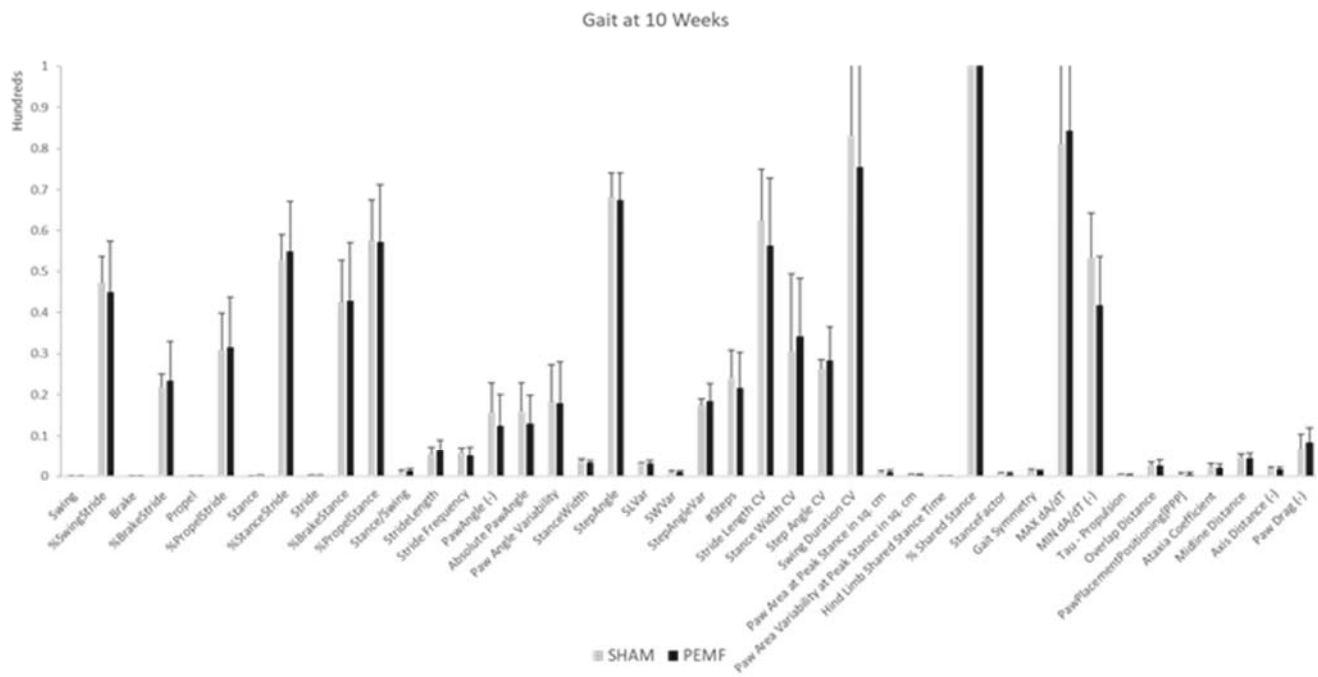
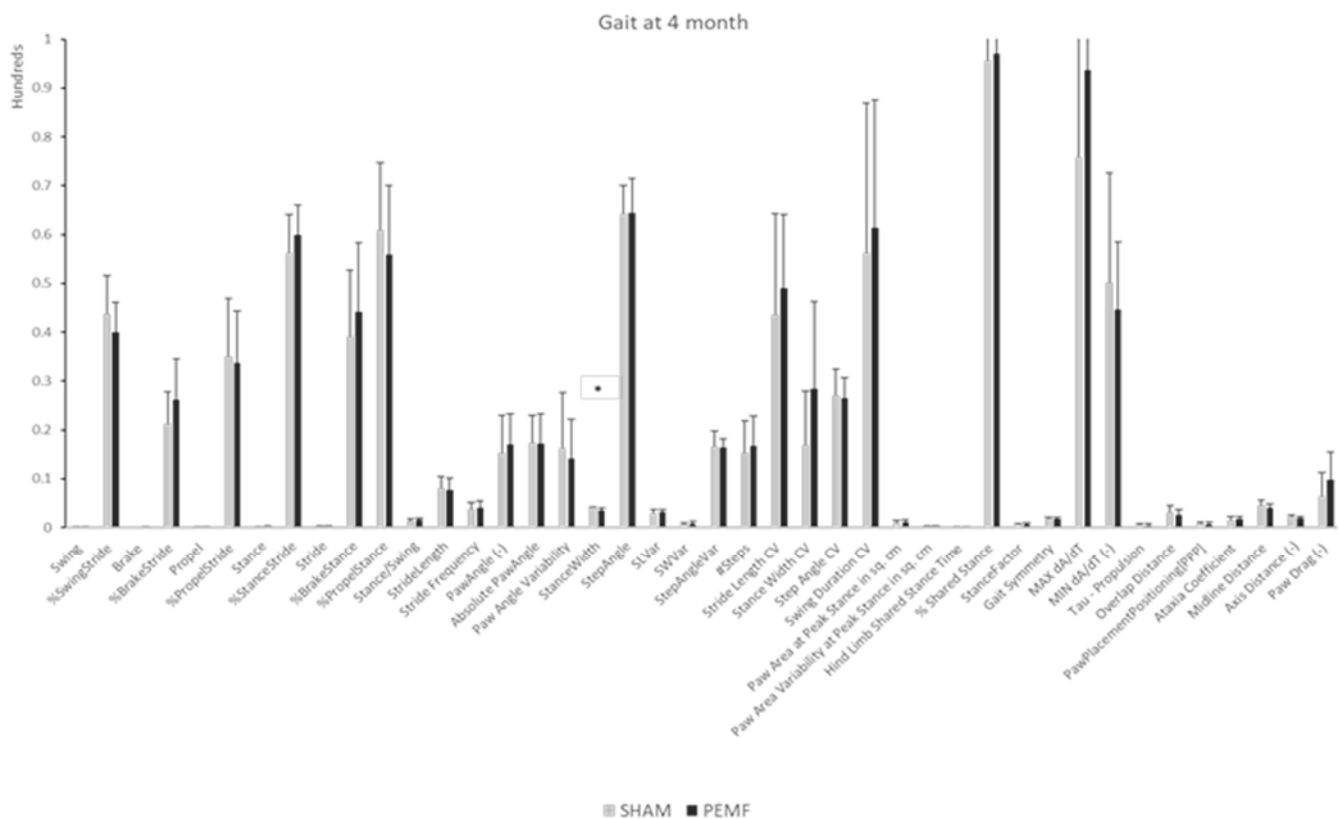
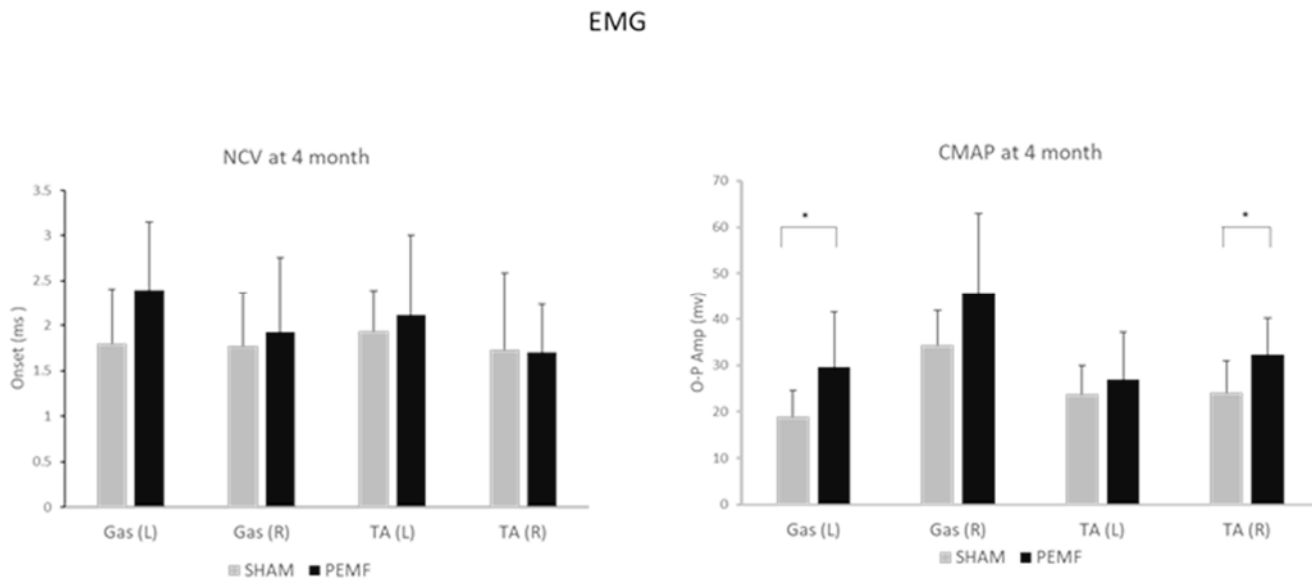


Figure 3. Gait at 4 months of PEMF/Sham PEMF (also included as an attachment)



After 4 months of treatment, electromyography was performed on all test subjects. Nerve conduction velocity (NCV) and compound motor action potentials (CMAP) were obtained and are represented below in figure 4.

Figure 4. Electromyographic data collected following 4 months of PEMF/Sham PEMF exposure in rats subsequent to sciatic nerve transection and repair.



These data are interesting in that compound motor action potentials are elevated in the PEMF – treated animals in both the gastrocnemius and tibialis anterior muscles.

In interpreting these results, it is important to remember that the nerve repairs employed in these studies are the current “gold standard” clinically; i.e. a tension-free end-to-end anastomosis. Repairs were performed by individuals with extensive experience. Therefore, similar outcomes in gait between PEMF treated and Sham PEMF treated are not unexpected. PEMF apparently did not accelerate recovery between treated groups because there are not significant differences between 10 week treated groups or 4 month treated groups. The difference in electromyographical results are surprising because they were not accompanied by comparable changes in gait.

Specific Aim 3 – Determine whether PEMF exposure has the ability to sustain neuromuscular junction morphology following denervation of muscle.

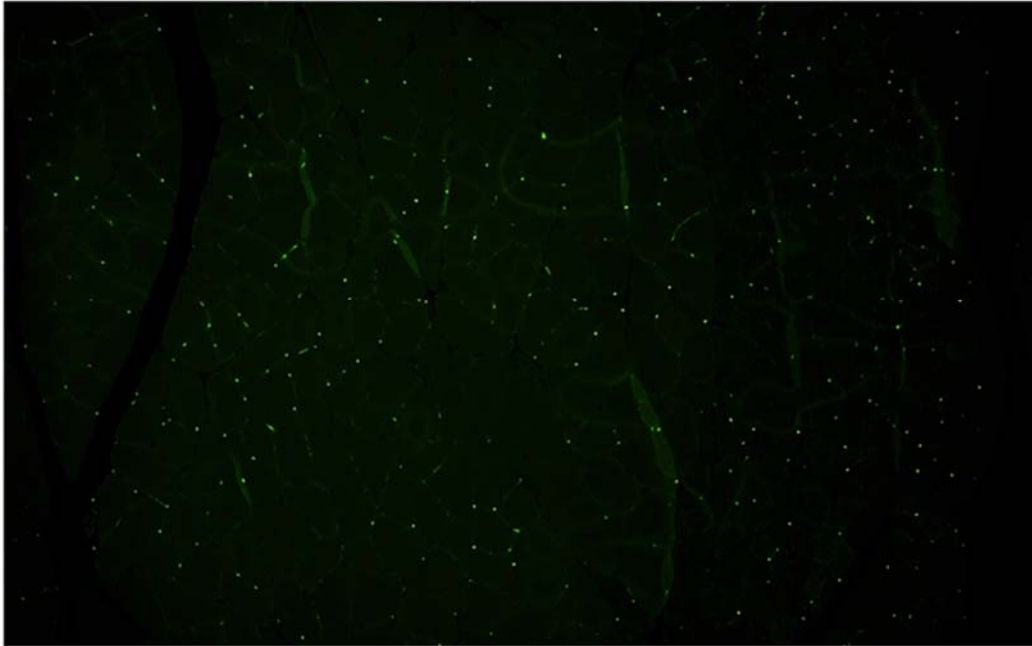
Major task 1: subject rats to PEMF following denervation of muscle. – Initiated in 5/19/2019.

- Exposure completed 11/02/2019.
- Animals euthanized for recovery of Tibialis anterior and gastrocnemius to assess neuromuscular junction morphology and muscle mass.

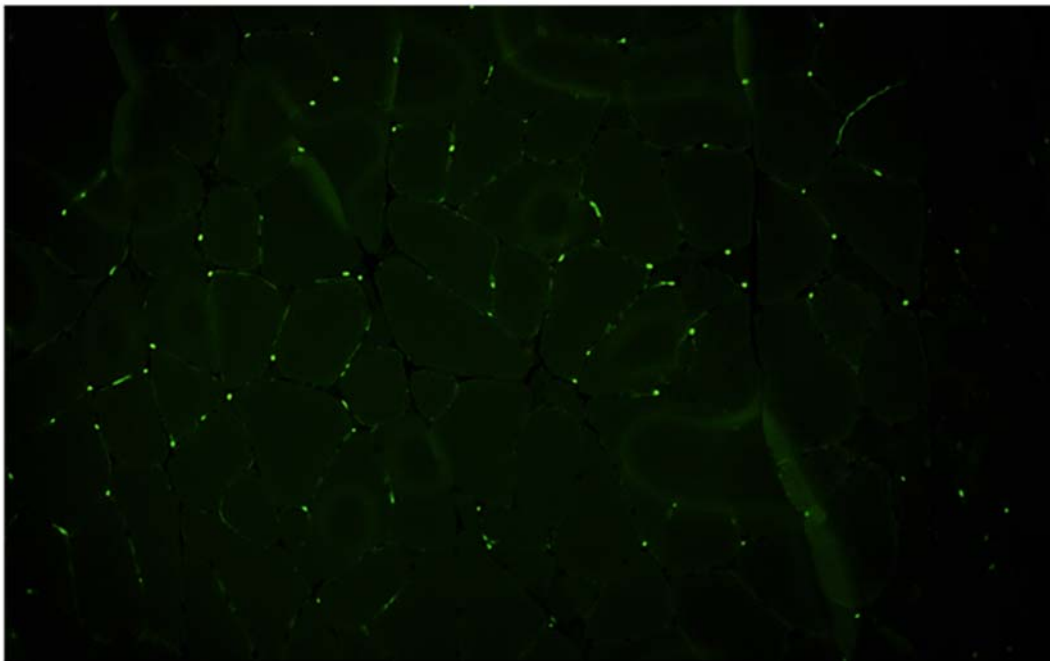
Histology is still being completed. However, representative images are included below.

Panel A. 6 months of Sham PEMF treatment following complete denervation of the gastrocnemius muscle. Cross section of muscle with the neuromuscular junctions stained with a fluorescent marker (alpha bungarotoxin). Images at 10 and 20 X magnification.

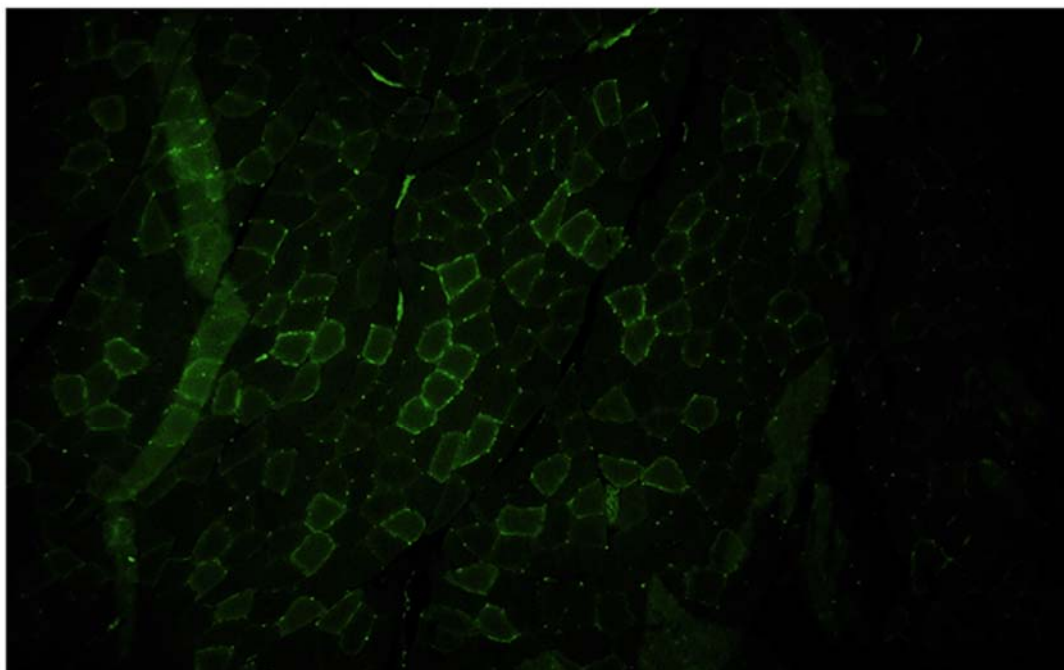
PEMF Gas 6 month 10X



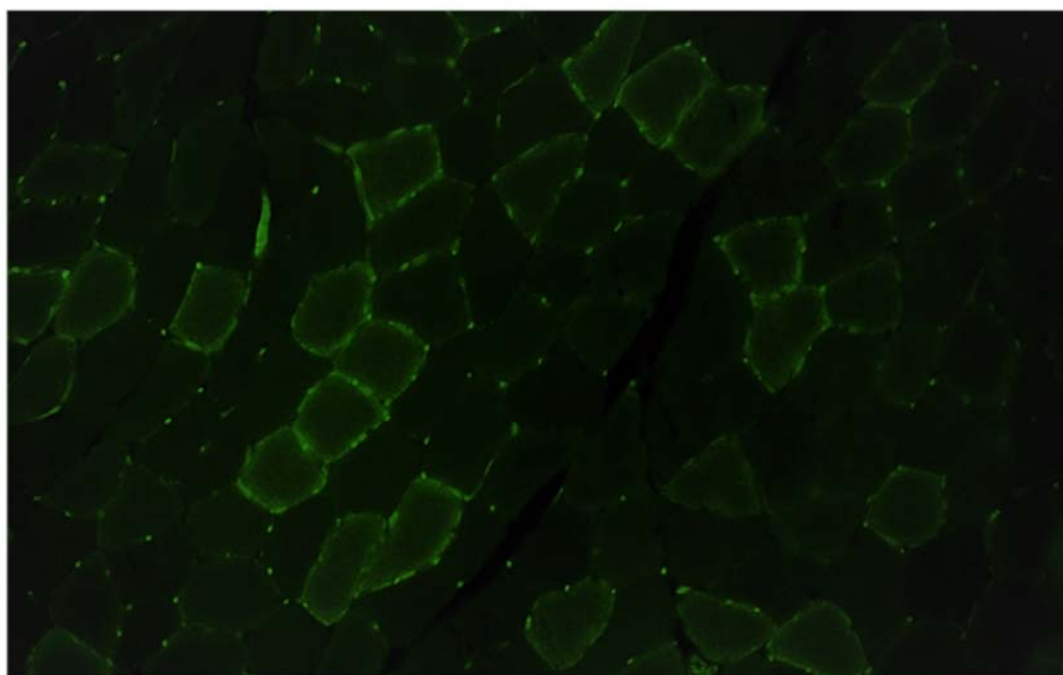
PEMF Gas 6 month 20X



Sham Gas 6 month 10X



Sham Gas 6 month 20X



Currently, these images are being quantitatively analyzed using image analysis software to define the number and density of neuromuscular junctions

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Abstract of results of initial 4 months of PEMF exposure was submitted to MHSRS.

What do you plan to do during the next reporting period to accomplish the goals?

Specific Aims 1 and 2 – Enroll rats on PEMF exposure protocols of shorter duration. This sequence will allow us to complete the studies in the upcoming year.

Specific Aim 3 – These studies to examine the potential for PEMF to maintain neuromuscular junction integrity will be completed and the histology analyzed.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Present results suggest no improvement in gait with PEMF exposure following sciatic nerve injury and repair. The technique employed in these studies yields optimal results and is considered the “Gold Standard” for peripheral nerve repair. Improvements to these outcomes would be clinically important because they could further optimize recovery of function in this patient population. The application of PEMF therapy post-operatively is non-invasive and cost effective and could be readily implemented because PEMF therapy currently is FDA approved. Studies to examine earlier time points following PEMF exposure may reveal group differences but that is yet to be determined. These time periods will be studied in the upcoming year.

Experimental findings of increased muscle force potential following 4 months exposure to PEMF is of interest because it may suggest that the muscle responds to PEMF treatment. This could be through preservation of neuromuscular junctions following treatment with PEMF after denervation accompanying nerve injury and repair. This concept is reinforced by preliminary histology of muscle fibers in the two treatment arms of the experiment in which muscle is denervated and the animal is treated with PEMF or Sham PEMF.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

No major changes in approach were experienced. A minor change was made to utilize a solenoid-typed PEMF coil rather than a Helmholtz-type coil. This change was made upon the recommendation of Orthofix, a major supplier of medical instrumentation for PEMF therapy. By utilizing a solenoid coil around the animal's home cage, a more consistent PEMF field was obtained, insuring uniform exposure of the animal to the correct waveform intensity, regardless of where they are located within their cage.

Actual or anticipated problems or delays and actions or plans to resolve them

Challenges have arisen with collecting gait data in trained rats. Although all rats enrolled in the studies have provided gait data, some animals have refused to walk or run on the treadmill after undergone extensive PEMF exposure. A variety of methods have been employed to obtain gait information from these animals, but some animals could not provide gait data.

The COVID-19 pandemic has significantly impacted the timeline of the current studies. The research endeavors at our teaching hospital were halted by our administration on March 17, 2020. This suspension continued until June 1, 2020. Furloughs for some personnel remained in place, however, for an addition few months. Renewal of our animal care and use committee approval for this research protocol was required and was initiated 5/08/2020. Because of furloughs, final approval of this protocol was not achieved until 8/11/2020, at which time the protocol was forwarded to the ACURO for review. Further research was suspended pending their approval.

Changes that had a significant impact on expenditures

Suspension of research activities due to the COVID-19 pandemic has had a negative impact on our expenditures. We will continue the remainder of the studies without additional salary support for the required extension of time.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **Website(s) or other Internet site(s)**

Not applicable

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Thomas L. Smith, PhD

Project Role: Principal Investigator

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1.2 calendar months

Contribution to Project: Dr. Smith developed the animal model for use in this application and completed all animal surgeries. He has worked closely with his team to review, analyze, and interpret experimental results.

Funding Support: W81XWH-17-1-0513

Name: Zhongyu Li, MD, PhD

Project Role: Co-Investigator

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 0.24 calendar months

Contribution to Project: Dr. Li's role for this project has been to provide expertise as needed with respect to experimental design, nerve injury and repair surgeries, and data interpretation.

Funding Support: W81XWH-17-1-0513

Name: Xue (Amy) Ma, MD, PhD

Project Role: Co-Investigator

Researcher Identifier (e.g. ORCID ID): N/A

Nearest person month worked: 6 calendar months

Contribution to Project: Dr. Ma's role for this project has been to assist Dr. Smith in nerve injury and repair as well as functional testing at specific time points. She also is the primary person responsible for histologic preparation of the tissues being studied.

Funding Support: W81XWH-17-1-0513

Name: Eileen Elsner

Project Role: Laboratory Technician

Researcher Identifier (e.g. ORCID ID): N/A

Nearest person month worked: 6 calendar months

Contribution to Project: Ms. Elsner has assisted Dr. Smith in acquisition of animals and general animal husbandry. She has prepared the surgical suites and ensured appropriate supplies have been available for animal surgeries. She has also monitored the animals during daily PEMF exposure.

Funding Support: W81XWH-17-1-0513

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

Zhongyu Li, MD, PhD

No changes in active support

Xue (Amy) Ma, MD, PhD

What other organizations were involved as partners?

Organizational name: Orthofix

Location of Organization: 345 Plano Parkway
Lewisville, TX 75056

Partner's contribution: Orthofix, a major supplier of PEMF equipment in the medical device industry, graciously loaned Wake Forest School of Medicine a complete, functional cage system for delivering the PEMF waveform approved in our grant. This system utilizes solenoid coils for generation of the PEMF signal. This system compliments but does not replace the PEMF system constructed by the Wake Forest Center for Nanotechnology and Molecular Materials.

- Collaboration : Jane Jacob, PhD, Vice President for Research and Clinical Affairs
 - Erik Waldorf, PhD, Principal Scientist and Research Manager
 - Dr. Waldorf oversaw the transportation and installation of the Orthofix PEMF system

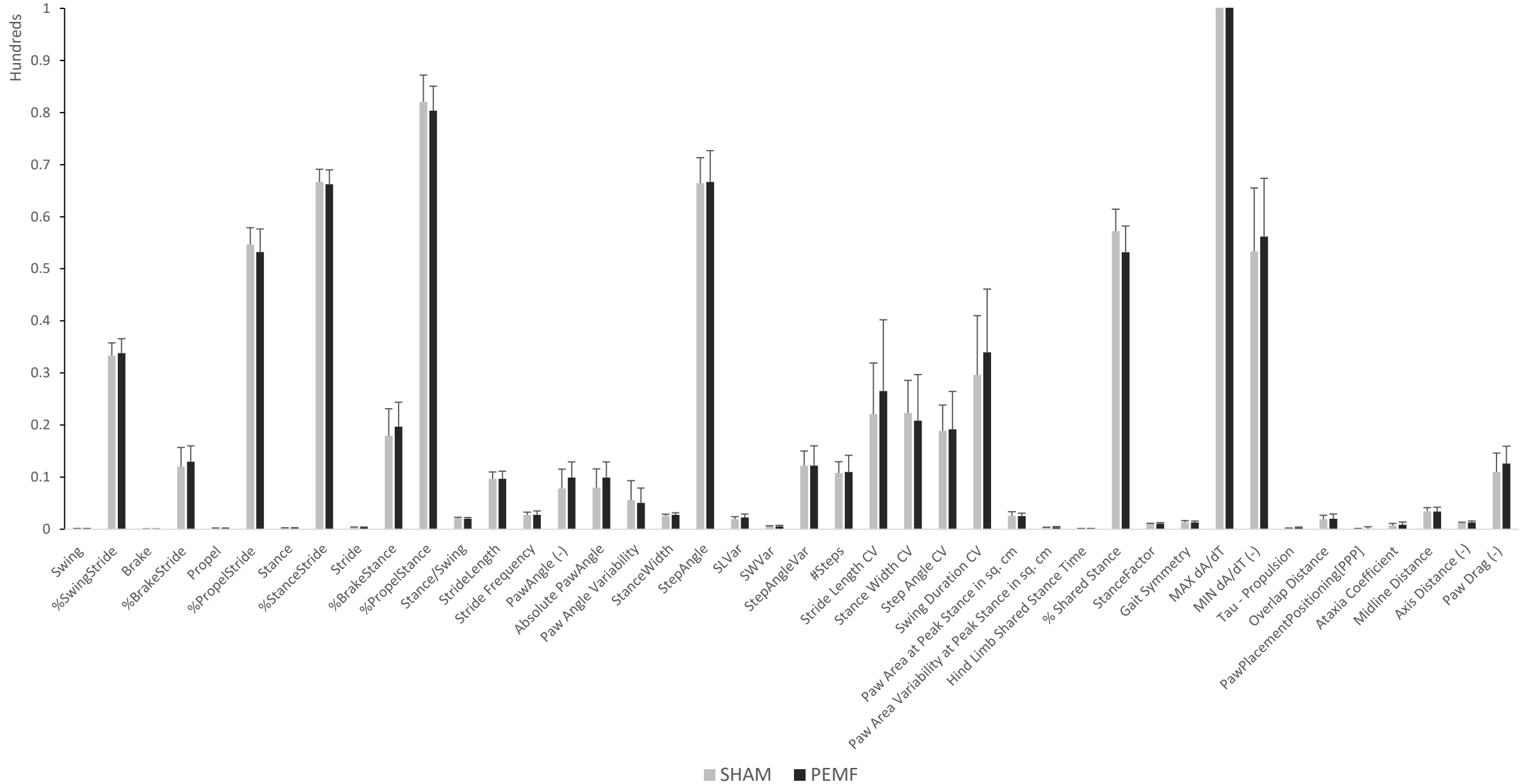
8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

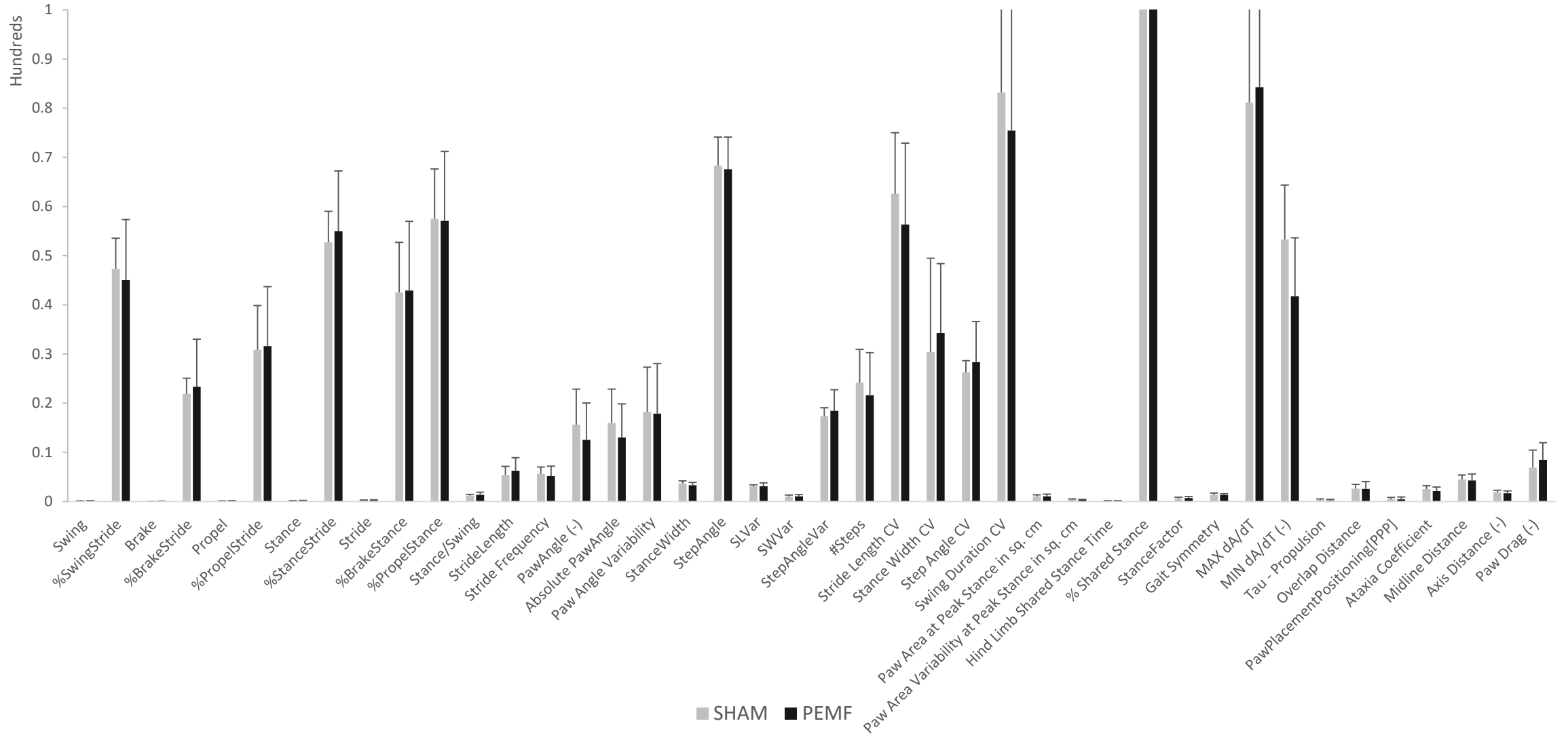
QUAD CHARTS:

9. APPENDICES:

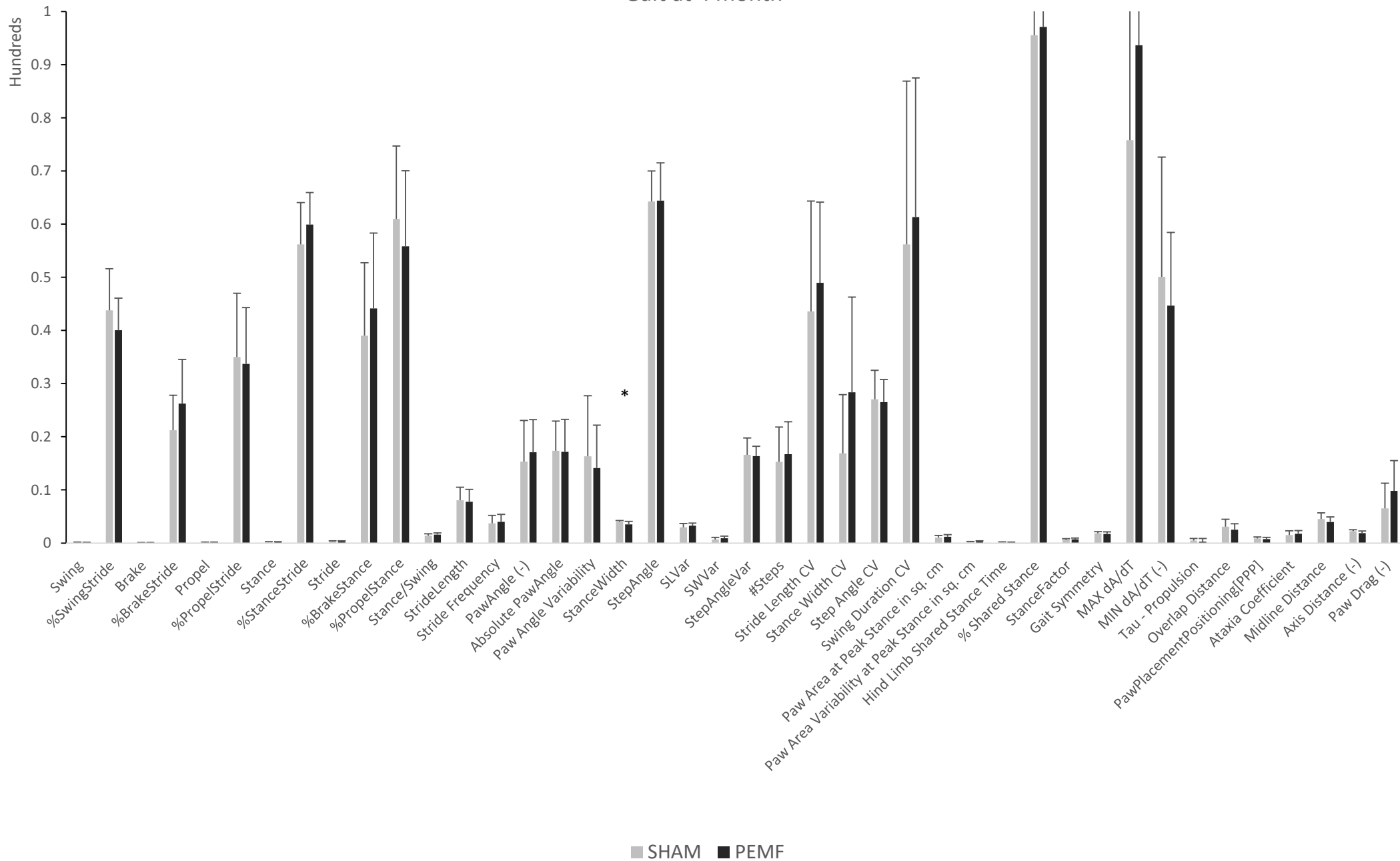
Gait Baseline



Gait at 10 Weeks

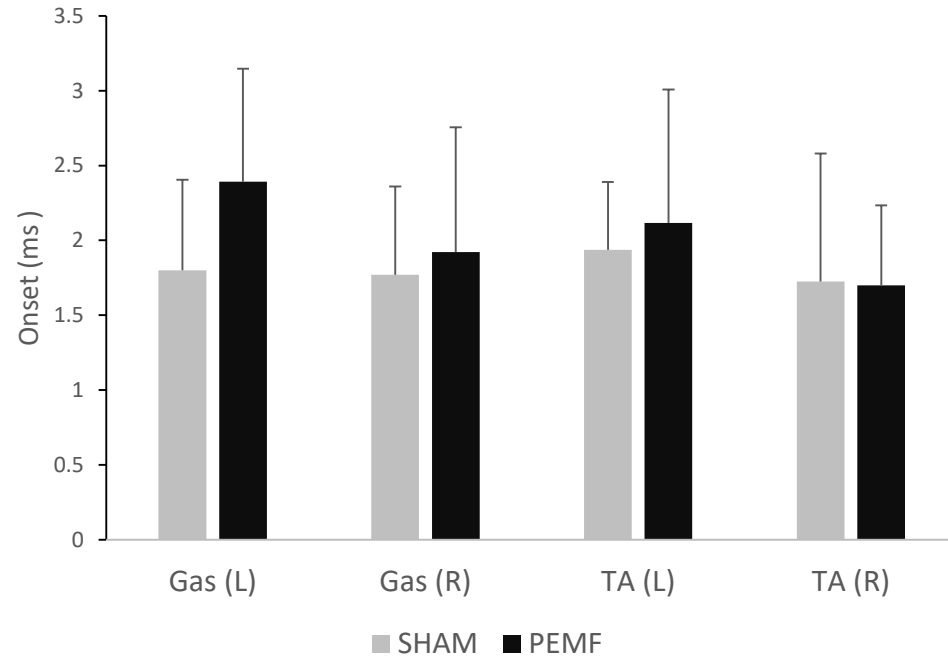


Gait at 4 month



EMG

NCV at 4 month



CMAP at 4 month

