

AWARD NUMBER: W81XWH-16-1-0161

TITLE: Rescuing Our Warriors from Chronic Pain: A Battlefield-to-Nondeployment Means to Prevent Opioid-induced Amplification of Neuropathic Pain from Traumatic Injury

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14. ABSTRACT Based on our preliminary data and a thorough review of the available scientific/clinical literature to date, <i>we hypothesize that:</i> (a) Trauma and opioids combine to amplify the intensity and duration of trauma-induced chronic pain. (b) This combined exposure to trauma plus opioids amplifies the creation and release of endogenous danger signals in spinal cord that create enduring release of TLR4 stimulatory substances as a consequence of cell stress/damage/death, leading to amplified trauma induced chronic pain. <u>Objective 1.</u> Define the response to opioids commonly used for acute pain management, when these are administered early after trauma, prior to development of neuropathic pain <u>Objective 2.</u> Define the response to opioids & non-opioids commonly used for neuropathic pain management, when these treatments are administered later after trauma, after development of neuropathic pain <u>Objective 3.</u> Define whether the deleterious effects on neuropathic pain observed in Aims 1 & 2 can be prevented by targeting TLR4 and P2X7 <u>Objective 4.</u> Define whether the deleterious effects of analgesics, and positive effects of co-administered TLR4/P2X7 antagonists, extend beyond neuropathic pain to other indices of disability		

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1. Introduction

In year three of the project we are exploring whether co-administration of the TLR4 antagonist (+)-Naloxone, and the P2X7 antagonist A438079 can improve the neuroinflammatory consequences of morphine, oxycodone and fentanyl, leading to an expedited resolution of neuropathic pain.

2. Keywords

Neuropathic pain, opioid analgesics, non-opioid analgesics, return to duty, morphine, oxycodone, fentanyl, toll-like receptor 4, P2X7R

3. Accomplishments:

What were the major goals of the project?

1. Test if the opioids Fentanyl and Oxycodone induced suppression of voluntary wheel running. Rats were habituated to running wheels with free access for 7 days, and baseline running data were collected. Rats then received 4 suture CCI surgeries of the sciatic nerve. At day 10 post CCI rats received a 5-day course of morphine (5 mg/kg b.i.d.), Fentanyl (0.5 mg/kg), Oxycodone (2 mg/kg) or equivolume saline vehicle. Running data were collected by computer 24 hours per day, 7 days a week. Task 13, Aim 4B
2. Test if the non-opioid Amitriptyline given at day 10 post trauma induce potentiation of neuropathic pain. All rats received four-suture CCI surgeries of the sciatic nerve. At day 10 post CCI rats began a 5-day course of Amitriptyline (10 mg/kg, 2x daily), or saline control. Assessment of mechanical allodynia by Von Frey testing occurred at day one post dosing completion and weekly thereafter. Task 6, Aim 2B
3. Test if blockade of TLR4 or P2X7R can prevent the exaggerated allodynia induced by fentanyl or oxycodone. Morphine is not tested here, as we have previously reported these results. Rats receive 1 suture CCI surgeries of the sciatic nerve. At day 9 post CCI, rats are implanted with intrathecal catheters attached to a subcutaneous osmotic minipump delivering (+)-naltrexone (60 ug/h), A438079 (30 ng/h) or vehicle for 5.5 days. At day 10 post CCI rats receive a 5-day course of fentanyl (0.5 mg/kg) or oxycodone (2 mg/kg). Von Frey testing

occurs prior to injury, prior to drug treatments, and weekly after drug treatments end. Task 10, Aim 3B

What was accomplished under these goals?

1. Test if the opioids Fentanyl and Oxycodone induced suppression of voluntary wheel running. Task 13, Aim 4B

Our previous data testing whether morphine suppresses voluntary wheel running (return to duty behavior) in rats has been inconclusive, with pilot studies showing suppression of running after morphine administration but failing to replicate this effect in follow up experiments after the research lab was required to move to a new building and animal facility. Given these inconclusive results, we conducted a study testing the opioids fentanyl, oxycodone, and morphine for side by side comparison on voluntary wheel running following nerve injury. Rats were habituated to running wheels with free access for 7 days, and baseline running data were collected. Rats then received 4 suture CCI surgeries of the sciatic nerve. At day 10 post CCI rats received a 5-day course of morphine (5 mg/kg b.i.d.), fentanyl (0.01 mg/kg), oxycodone (2 mg/kg) or equivolume saline vehicle. Running data were collected by computer 24 hours per day, 7 days a week. The results of this experiment did not show a suppression of voluntary wheel running in rats treated with the opioids morphine, fentanyl or oxycodone following the end of drug administration. These data, combined with failure to replicate reduction in voluntary wheel running following treatment with morphine after traumatic injury indicate that this outcome measure may not be affected long term by opioid treatment, despite increases in pain sensitivity and duration. Results of this experiment are presented in Figure 1.

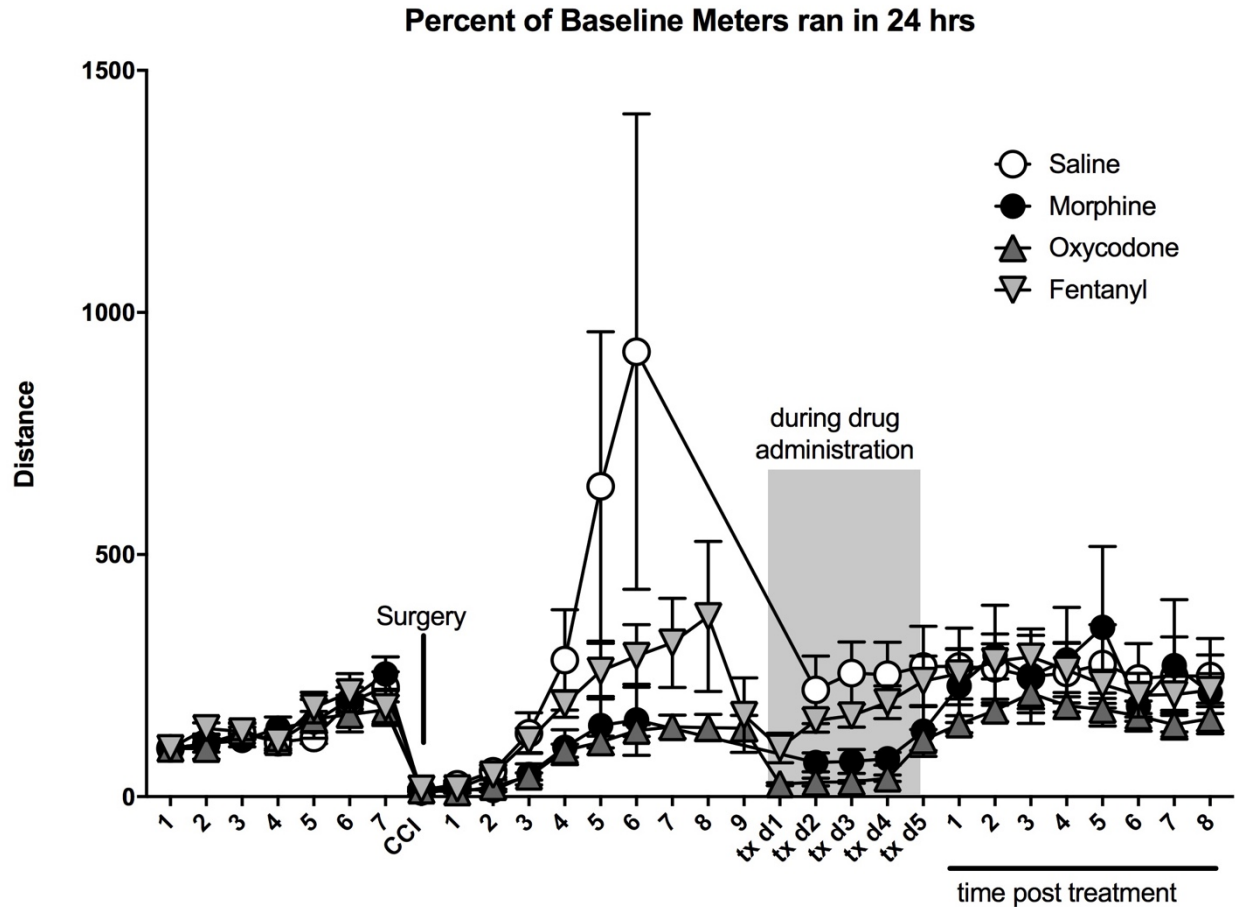


Figure 1. At day 10 post-surgery a 5 day course of morphine (5 mg/kg b.i.d.), fentanyl (0.01 mg/kg/hr), oxycodone (2 mg/kg) or saline was given. Running data of meters ran were collected by computer 24 hours per day. No significant differences were observed in running after treatment, n=6/group.

2. Test if the non-opioid amitriptyline given at day 10 post trauma potentiates neuropathic pain. Task 6, Aim 2B

Given that we predict the long term deleterious effects of opioids to be a result of their action on the TLR4 receptor in glial cells, we expect that we will not see an increase in neuropathic pain when non opioid analgesic drugs are used. In this experiment Amitriptyline was used as a negative control to opioids to determine whether the potentiation of neuropathic pain is opioid specific. Rats received four-suture CCI surgeries of the sciatic nerve, vs sham surgery. At day 10 post CCI, injured rats began a 5-day course of amitriptyline (10mg/kg), morphine (5 mg/kg b.i.d.), or saline control. Sham control rats received a 5-day course of amitriptyline (10mg/kg). Assessment of mechanical allodynia by von Frey testing occurred at day one post dosing completion and weekly thereafter. Results of this experiment are presented in Figure 2. Area under the curve is presented in Figure 3.

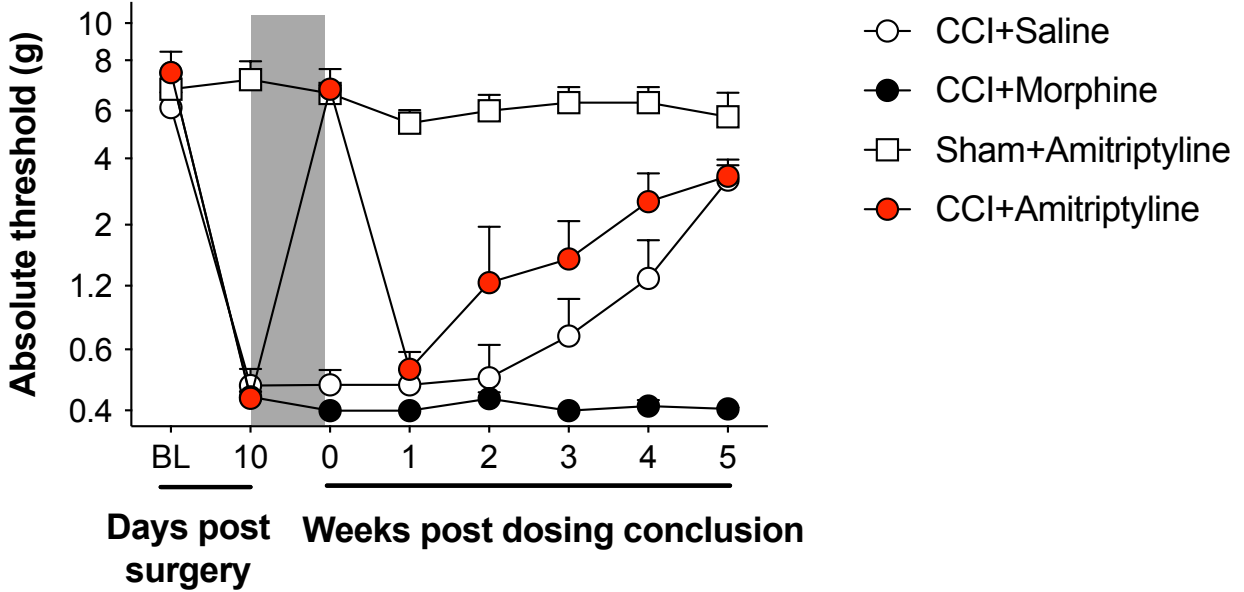


Figure 2. Chronic Constriction Injury (CCI) or sham surgeries were performed with four 4-0 suture. Amitriptyline (10mg/kg), morphine (5 mg/kg b.i.d.), or saline were administered at day 10 post-surgery for 5 days. (Main effect drug $p < 0.0001$, main effect time $p < 0.0001$, interaction $p < 0.0001$, Tukey post hoc analysis CCI+Morphine vs CCI+Amitriptyline $p < 0.0001$ week 3, 4, and 5, Two Way ANOVA, $n = 6/\text{group}$)

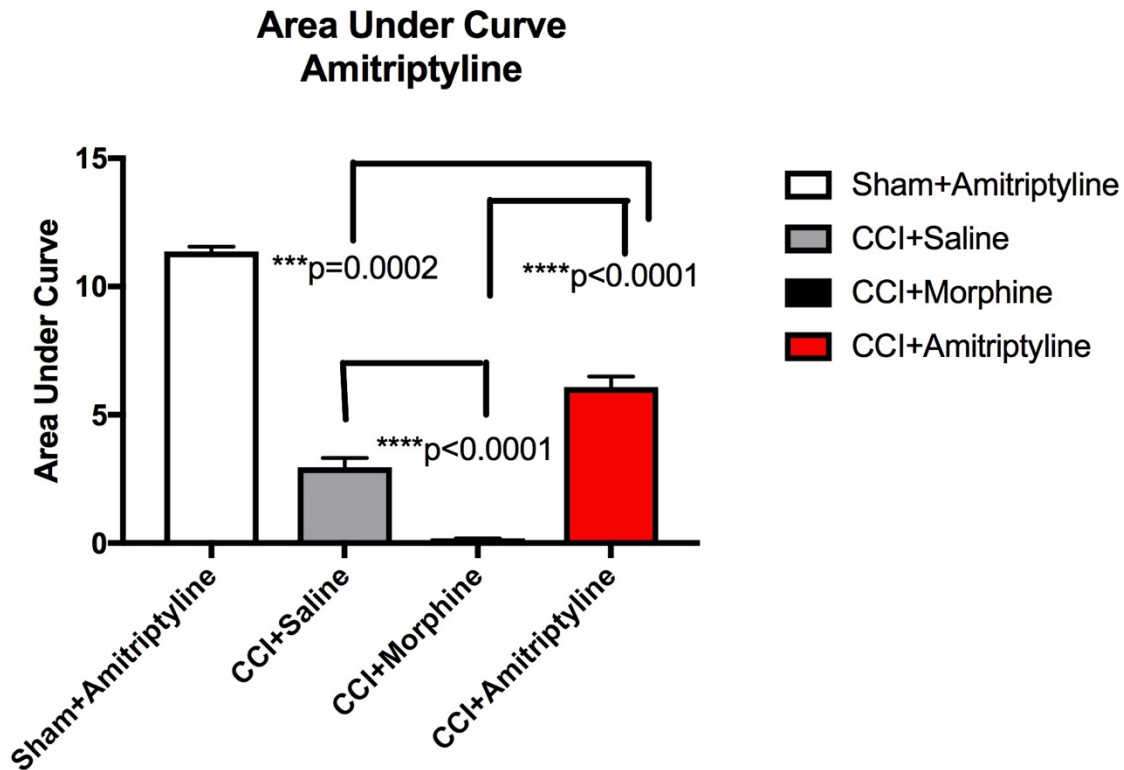


Figure 3. Chronic Constriction Injury (CCI) or sham surgeries were performed with four 4-0 suture. Amitriptyline (10mg/kg), morphine (5 mg/kg b.i.d.), or saline were administered at day 10 post-surgery for 5 days. (CCI+Saline vs CCI+Morphine $p < 0.0001$, CCI+Saline vs CCI+Amitriptyline $p = 0.0002$, CCI+Morphine vs CCI+Amitriptyline $p < 0.0001$, unpaired t test of Area Under Curve, $n = 6/\text{group}$)

3. Test if blockade of TLR4 or P2X7R can prevent the exaggerated allodynia induced by fentanyl or oxycodone. Task 10, Aim 3B

In figure 4, we report results of an ongoing experiment in which all animals had CCI surgery, followed by treatment with oxycodone. We have previously reported that this combination profoundly exacerbates neuropathic pain, compared to vehicle/sham. Here, we show that the exaggerated allodynia can be prevented if TLR4 or P2X7 are blocked (by (+)-naloxone or A438079, respectively) during oxycodone treatment.

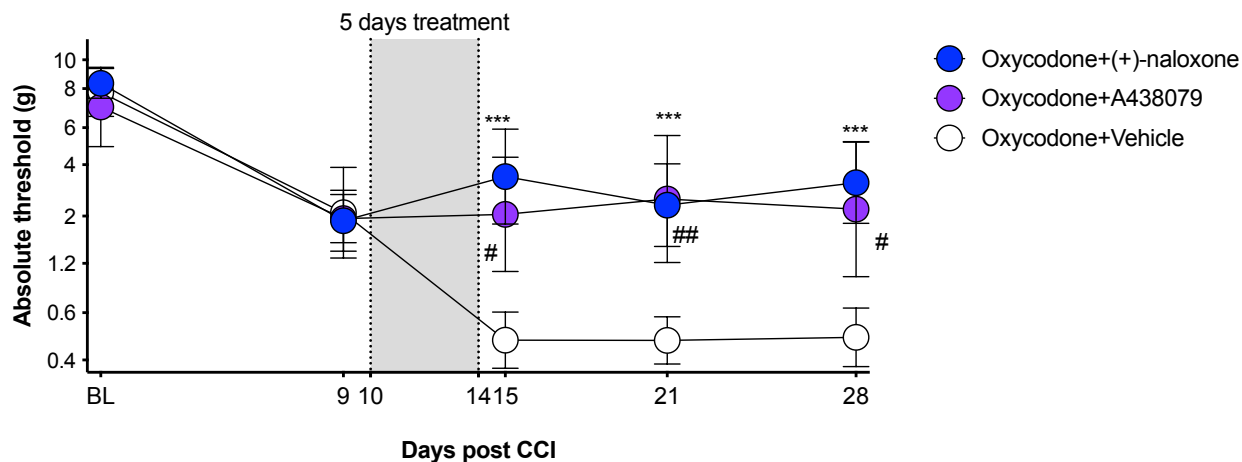


Figure 4. CCI surgery was performed in male Sprague Dawley rats. Nine days later, rats were implanted with intrathecal osmotic minipumps to administer vehicle, (+)-naloxone (60 ug/h) or A438079 (30 ng/h). On day 10 post CCI, a 5-day course of oxycodone (2 mg/kg b.i.d.) treatment began. Von Frey testing was performed at baseline, prior to pump implantation, and at regular intervals after treatment concluded.

In figure 5, we report results of an ongoing experiment in which all animals had CCI surgery, followed by treatment with fentanyl. We have previously reported that this combination profoundly exacerbates neuropathic pain, compared to vehicle/sham. Here, we show that the exaggerated allodynia can be prevented if TLR4 or P2X7 are blocked (by (+)-naloxone or A438079, respectively) during fentanyl treatment.

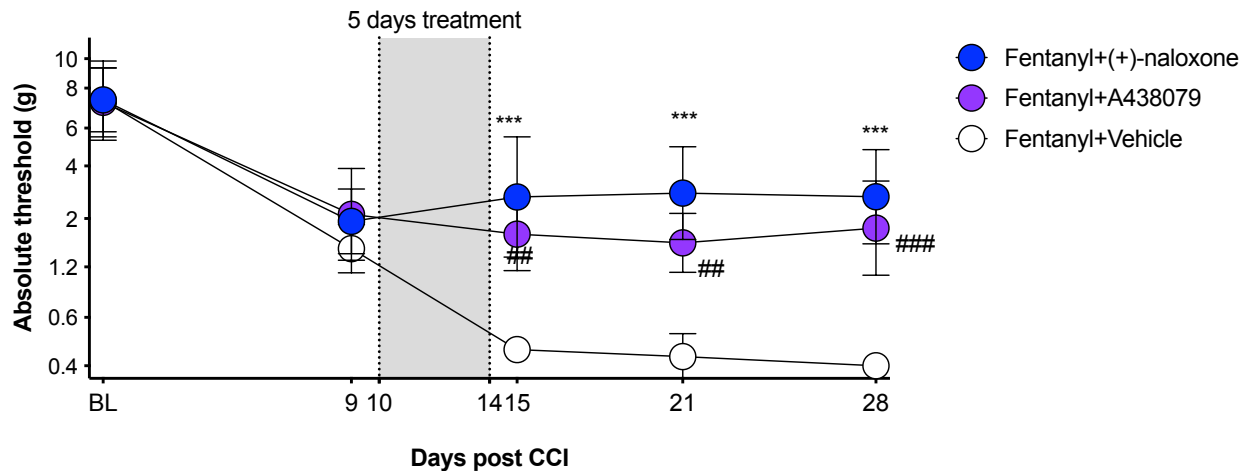


Figure 5. CCI surgery was performed in male Sprague Dawley rats. Nine days later, rats were implanted with intrathecal osmotic minipumps to administer vehicle, (+)-naloxone (60 ug/h) or A438079 (30 ng/h). Subcutaneous osmotic minipumps were also implanted to administer fentanyl for 5 days (0.01 mg/kg/h). Von Frey testing was performed at baseline, prior to pump implantation, and at regular intervals after treatment concluded.

CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

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

Task 6 and Task 8 were both delayed, awaiting ACURO approval for the work to be initiated and pursued at MD Anderson. A copy/paste of the initial portion of the letter received is included below, indicating December 27th as receipt date allowing the work to now begin.

The laboratory of Dr. Grace moved to a new facility in August 2019. This caused a ~2 week delay in beginning the experiments.

Changes that had a significant impact on expenditures

Nothing to report.

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

Nothing to report.

- **Significant changes in use or care of human subjects**

Nothing to report.

- **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.

PRODUCTS:

The following article is in press:

Green-Fulgham SM, Ball JB, Fabisiak T, Maier SF, Watkins LR and Grace PM. Oxycodone, fentanyl, and morphine amplify established neuropathic pain in male rats. PAIN. 2019.

This work was supported under this grant, and reports our findings that the deleterious effects of morphine generalize to other clinically relevant opioids, oxycodone and fentanyl. Enduring exacerbation of pain was also observed when these opioids were administered 28 days after nerve injury, but surprisingly not when administered one day after traumatic injury.

In addition, we presented the research at the 2019 American Pain Society meeting:

Green-Fulgham, S.M., Ball, J.B., Kwilasz, A.J., Maier, S.F., Watkins, L.R. and Grace, P.M., The opioids oxycodone, fentanyl, and morphine amplify neuropathic pain when given after chronic pain is established, Proc. American Pain Society, 2019.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name: Linda R. Watkins, Ph.D.

Project Role: Principal Investigator

Researcher Identifier (e.g. ORCID ID): none

Nearest person month worked: 10% effort for this quarter (no funds utilized)

Contribution to Project: Principal Investigator

Name: Peter M. Grace, Ph.D.
Project Role: Co- Principal Investigator
Researcher Identifier (e.g. ORCID ID): orcid.org/0000-0002-8999-1220
Nearest person month worked: 5% effort for this quarter
Contribution to Project: Co-Principal Investigator

Name: Michael Lacagnina, Ph.D.
Project Role: Postdoctoral Researcher
Researcher Identifier (e.g. ORCID ID): none
Nearest person month worked: 30% effort for this quarter
Contribution to Project: Conducted experiments and analyzed data

Name: Sabina Lorca, B.A.
Project Role: Research Assistant II
Researcher Identifier (e.g. ORCID ID): none
Nearest person month worked: 10% effort for this quarter
Contribution to Project: Conducted experiments and analyzed data

Name: Suzanne M. Fulgham, M.S.
Project Role: Professional Research Assistant
Researcher Identifier (e.g. ORCID ID): none
Nearest person month worked: 3
Contribution to Project: Suzanne analyzed data and prepared the manuscript

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.

What other organizations were involved as partners?

Nothing to report.

1. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from **BOTH** the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.*

- **QUAD CHARTS:**

Quad Chart attached.

2. **APPENDICES:**

Nothing to Report.