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

PRINCIPAL INVESTIGATOR: Prof. Linda Watkins, Ph.D

CONTRACTING ORGANIZATION:
Regents of the University of Colorado
Boulder, CO 80309

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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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6. AUTHOR(S)
Linda R Watkins, Ph.D.
Peter M Grace, Ph.D.
Suzanne M Fulgham, M.S.

E-Mail: linda.watkins@colorado.edu

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Regents of the University of Colorado

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Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Based on our preliminary data and a thorough review of the available scientific/clinical literature to date, *we hypothesize that:* (**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.

Objective 1. Define the response to opioids commonly used for acute pain management, when these are administered early after trauma, prior to development of neuropathic pain

Objective 2. 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

Objective 3. Define whether the deleterious effects on neuropathic pain observed in Aims 1 & 2 can be prevented by targeting TLR4 and P2X7

Objective 4. 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

15. SUBJECT TERMS chronic pain, opioid analgesics, non-opioid analgesics, toll-like receptor 4, return to duty					
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Table of Contents

Page

1. Introduction

In this year, we are exploring whether co-administration of the TLR4 antagonist (+)-naltrexone, and the P2X7 antagonist A438079 can improve the neuroinflammatory consequences of morphine, oxycodone and fentanyl, leading to an expedited resolution of neuropathic pain. We are also exploring whether non-opioids gabapentin, amitriptyline, will exacerbate neuropathic pain, similar to morphine, oxycodone and fentanyl.

2. Keywords

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

3. Accomplishments:

What were the major goals of the project?

1. Test if blockade of TLR4 or P2X7R can prevent the exaggerated allodynia induced by morphine, fentanyl or oxycodone. Rats receive 1 suture CCI surgeries of the sciatic nerve. At day 27 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 28 post CCI rats receive a 5-day course of morphine (5 mg/kg), 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 11, Aim 3C
2. Test if non-opioids gabapentin or amitriptyline exacerbate allodynia induced by CCI. Rats receive 1 suture CCI surgeries of the sciatic nerve. At day 10 or 28 post CCI, rats receive a 5-day course of gabapentin (5 mg/kg) or amitriptyline (2 mg/kg). Von Frey testing occurs prior to injury, prior to drug treatments, and weekly after drug treatments end. Tasks 10 and 11, Aims 3B,C

What was accomplished under these goals?

1. Test if blockade of TLR4 or P2X7R can prevent the exaggerated allodynia induced by morphine, fentanyl or oxycodone. Task 11, Aim 3C

In figure 1, we report results of a completed experiment in which all animals had CCI surgery, followed by treatment with oxycodone beginning 4 weeks later. We have previously reported that this combination profoundly exacerbates neuropathic pain,

compared to sham/vehicle. Here, we show that the exaggerated allodynia can be prevented if TLR4 or P2X7 are blocked (by (+)-naloxone or A438079, respectively) during oxycodone treatment.

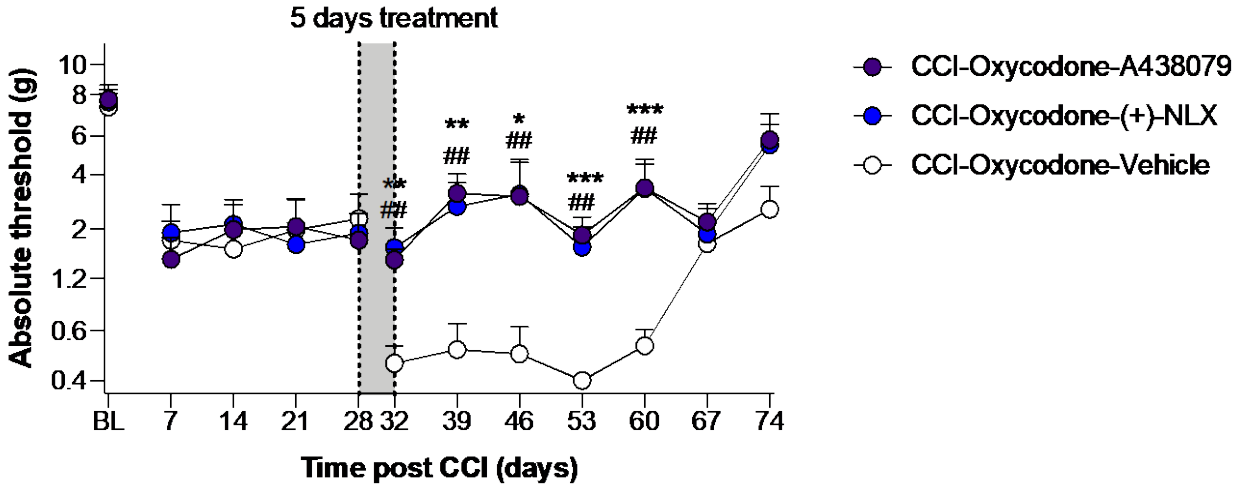

Figure 1. CCI surgery was performed in male Sprague Dawley rats. Twenty-seven days later, rats were implanted with intrathecal osmotic minipumps to administer vehicle, (+)-naloxone ((+)-NLX) (60 ug/h) or A438079 (30 ng/h). On day 28 post CCI, a 5-day course of oxycodone (2 mg/kg b.i.d.) treatment began. Von Frey testing was performed at baseline, prior to pump implantation, and at regular intervals after treatment concluded. N=6/group. Treatment x time: $F_{12, 90} = 1.86$; $P = 0.049$; treatment: $F_{3.72, 55.86} = 9.72$; $P < 0.001$; time: $F_{2, 15} = 79.99$; $P < 0.001$. A438079 vs. vehicle: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; (+)-NLX vs. vehicle: $^{\#\#}P < 0.01$.

In figure 2, we report results of a completed experiment in which all animals had CCI surgery, followed by treatment with fentanyl beginning 4 weeks later. We have previously reported that this combination profoundly exacerbates neuropathic pain, compared to sham/vehicle. Here, we show that the exaggerated allodynia can be prevented if TLR4 or P2X7 are blocked (by (+)-naloxone or A438079, respectively) during fentanyl treatment.

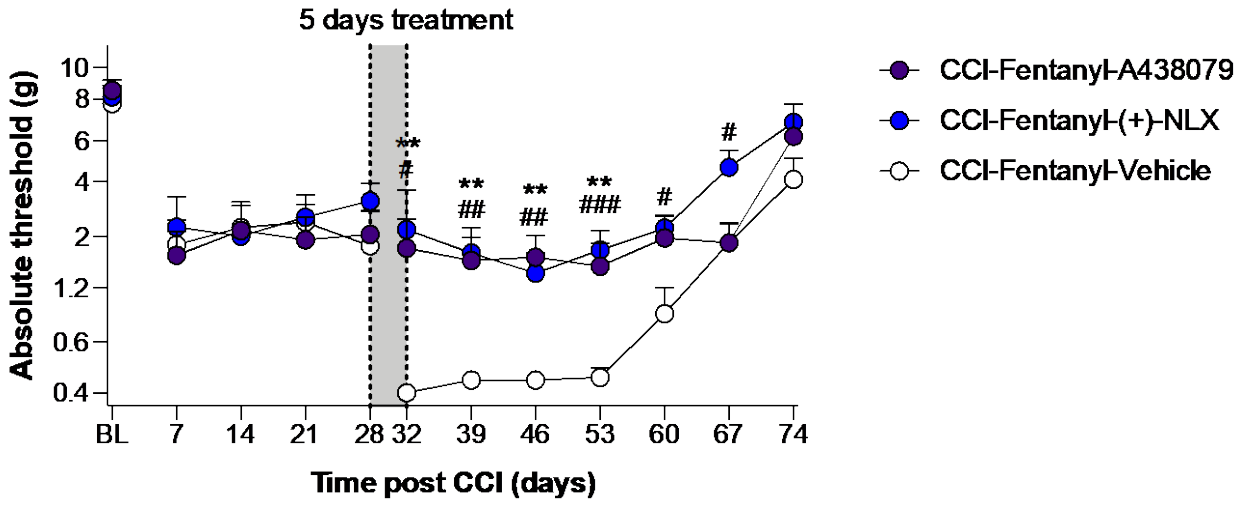

Figure 2. CCI surgery was performed in male Sprague Dawley rats. Twenty-seven days later, rats were implanted with intrathecal osmotic minipumps to administer vehicle, (+)-naloxone (60 ug/h) or A438079 (30 ng/h). Subcutaneous osmotic minipumps were also implanted to administer fentanyl for 5 days (0.01 mg/kg/h). Von Frey testing was performed at baseline, prior to pump implantation, and at regular intervals after treatment concluded. N=6/group. Treatment x time: $F_{12, 90} = 2.73$; $P = 0.004$; treatment: $F_{2.69, 40.33} = 35.32$; $P < 0.001$; time: $F_{2, 15} = 14.91$; $P < 0.001$. A438079 vs. vehicle: $^{**}P < 0.01$; (+)-NLX vs. vehicle: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

2. Test if non-opioids cause exaggerated CCI-allodynia. Tasks 10, 11, Aims 3B,C

After the research closure (see below), we have resumed the studies. The experiments for Tasks 10 and 11 are in progress.

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

As noted in the previous report, animal and laboratory research was suspended by MD Anderson due to COVID-19 from March 22 to June 15. All lab personnel were working from home during this period. Experimental work was restarted in shifts from June 15 to September 4. Regular work hours resumed on September 7. The shutdown and slow re-opening have delayed progress on this project. The experiments for Tasks 10 and 11 are in progress.

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 manuscript is in press in the Journal of Neuroscience Research:

Green-Fulgham SM, Ball JB, Maier SF, Rice KC, Watkins LR and Grace PM. Suppression of voluntary wheel running by unilateral chronic constriction injury: Enduring therapeutic effects of a brief course of treatment with morphine combined with TLR4 or P2X7 antagonists. *J Neurosci Res*. 2020 (*in press*).

This work was supported under this grant, and reports our findings on the effects of CCI, morphine, and TLR4 and P2X7R antagonists on voluntary wheel running behavior.

Dr. Grace gave an invited webinar for the Pain Research Forum (May 18), entitled "Opioids and Pain Persistence: A Role for Neuroimmune Mechanisms", presenting work supported by this grant. The webinar had record attendance for the monthly PRF series, with 870 people around the world tuning in through Zoom and the Facebook livestream.

https://www.painresearchforum.org/forums/webinar/139647-prf-seminar-–opioids-and-pain-persistence-role-neuroimmune-mechanisms

The following article was published.
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. 160; 2634-40.

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.

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: 5% 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: Jiahe Li, Ph.D.
Project Role: Postdoctoral Researcher
Researcher Identifier (e.g. ORCID ID): none
Nearest person month worked: 5% 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: 15% effort for this quarter
Contribution to Project: Conducted experiments and analyzed data

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.