AWARD NUMBER: W81XWH-18-1-0390

TITLE: Protein Kinase C Epsilon Inhibitors to Treat Pain

PRINCIPAL INVESTIGATOR: Jon Levine, MD, PhD

CONTRACTING ORGANIZATION: The Regents of the University of California, San Francisco

REPORT DATE: September 2020

TYPE OF REPORT: Annual Progress Report (02 year)

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
SEPT 2020	Annual	01 September 2019 – 31 August 2020

4. TITLE AND SUBTITLE

Protein Kinase C. Epsilon Inhibitors to Treat Pain

5a. CONTRACT NUMBER
W81XWH-18-1-0390

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Jon D. Levine, M.D., Ph.D.

E-Mail: jon.levine@ucsf.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Regents of the University of California at San Francisco
513 Parnassus Avenue
San Francisco, CA 94117

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

During the second year of this grant the team at UCSF has the following accomplishments:
We have evaluated a novel PKCε inhibitor designed by the Dr. Messing (PI), CIDD-0150612. We demonstrated that CIDD-0150612 could prevent the pain/hyperalgesia induced by ψεRACK, a selective PKCε agonist, as well as that produced by the classical pronociceptive inflammatory mediator, prostaglandin E_2. In a separate set of experiments, we tested the more clinically relevant protocol, namely the reversal of pain/hyperalgesia by CIDD-0150612. We found that when administered in the setting of established pain/hyperalgesia, CIDD-0150612 was able to reversibly inhibit pain/hyperalgesia. Compared to our initial lead compound, CIDD-0150043, CIDD-0150612 appears to be at least as potent at inhibiting PKCε associated hyperalgesia. In addition, compared to CIDD-0150043, our earlier lead PKCε inhibitor, CIDD-0150612 is substantially more selective for PKCε, with respect to ROCK, the major off target effect with negative impact on its ultimate clinical usefulness (PKCε/ROCK of 74.9 vs. PKCε/ROCK of 42.6).

15. SUBJECT TERMS
Protein kinase C epsilon, non-opioid analgesic, highly selective inhibitor

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	18	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

We propose to develop potent and selective inhibitors of the enzyme PKCε as therapeutics that can be administered orally or intravenously for the treatment of acute and chronic pain. This application addresses the FY17 PRMRP Topic Area "Non-Opioid Pain Management".

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Non-opioid analgesics, protein kinase Cε, medicinal chemistry, preclinical pain models.

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 2: Identify novel PKCε inhibitor scaffolds and series through high-throughput screening (HTS) and molecular modeling.
Major Task 2: PKCε-induced hyperalgesia screen.

Subtask 1: Test analogs for inhibition of PKCε-induced acute mechanical hyperalgesia in rats.

- Team: Levine lab at UCSF: Jon Levine, Prof. Paul Green, PhD, and technician Niloufar Mansooralavi
- Test single concentrations of compounds for inhibition of mechanical hyperalgesia induced by local intradermal injection of the selective PKCε agonist ΨεRACK.
- Perform dose-response experiments on the most effective analogs.

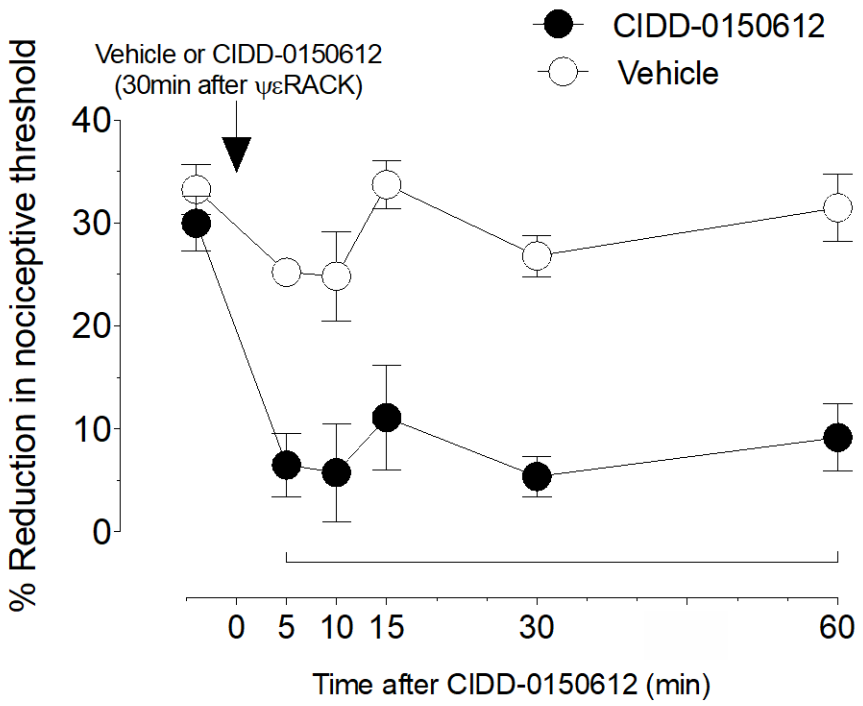
Animals used: Sprague Dawley rats (Charles River) N=312.

In Progress (completed for second lead compound)

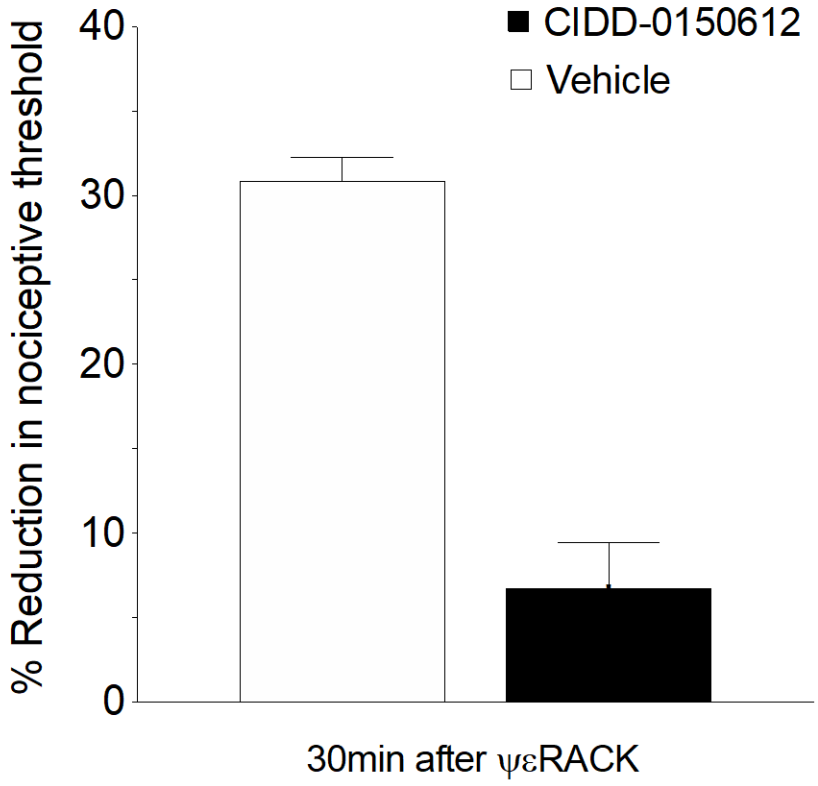
What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

During the second year of this grant the team at UCSF has the following accomplishments:
We have evaluated a novel PKCε inhibitor designed by the Dr. Messing (PI), CIDD-0150612. We demonstrated that CIDD-0150612 could prevent the pain/hyperalgesia induced by ψεRACK, a selective PKCε agonist, as well as that produced by the classical pronociceptive inflammatory mediator, prostaglandin E_2. In a separate set of experiments, we tested the more clinically relevant protocol, namely the reversal of pain/hyperalgesia by CIDD-0150612. We found that when administered in the setting of established pain/hyperalgesia, CIDD-0150612 was able to reversibly inhibit pain/hyperalgesia. Compared to our initial lead compound, CIDD-0150043, CIDD-0150612 appears to be at least as potent at inhibiting PKCε associated hyperalgesia. In addition, compared to CIDD-0150043, our earlier lead PKCε inhibitor, CIDD-0150612 is substantially more selective for PKCε, with respect to ROCK, the major off target effect with negative impact on its ultimate clinical usefulness (PKCε/ROCK of 74.9 vs. PKCε/ROCK of 42.6).

A


CIDD-0150612
Vehicle
Vehicle or CIDD-0150612
(30min after ψεRACK)
% Reduction in nociceptive threshold
40
30
20
10
0
0
5
10
15
30
60
Time after CIDD-0150612 (min)

B


CIDD-0150612
Vehicle
% Reduction in nociceptive threshold
40
30
20
10
0
30min after ψεRACK

A. Male rats were treated intradermally with ψεRACK (1 μg/5 μL). Thirty minutes after ψεRACK, mechanical nociceptive threshold was evaluated to confirm the presence of hyperalgesia induced by ψεRACK. Immediately after determining the presence of hyperalgesia, vehicle (1% DMSO + saline, 5 μL) or the PKCε inhibitor (CIDD-0150612, 1 μg/5 μL), was injected in the same site, on the dorsum of the hind paw. Mechanical nociceptive threshold was evaluated 5, 10, 15, 30 and 60 min after intradermal vehicle or PKCμ inhibitor. In this prevention protocol ψεRACK-induced hyperalgesia was markedly attenuated by CIDD-0150612 ($F(_{1,10})$=55.30, ***p=0.0008; when ψεRACK-induced hyperalgesia was compared between vehicle and CIDD-0150162 groups; two-way repeated-measures ANOVA followed by Holm-Šídák multiple comparison test). .
n=6 paws, per group.
B. Male rats were treated intradermally with vehicle (DMSO + saline, 5 μL) or the PKCε inhibitor (CIDD-0150612, 1 μg/5 μL) following 10 min later, by ψεRACK (1 μg/5 μL) injected at the same site, on the dorsum of the hind paw. When mechanical nociceptive threshold was evaluated 30 min after intradermal ψεRACK CIDD-0150612 markedly reversed ψεRACK-induced hyperalgesia, in this reversal protocol (t_{10}=7.995, ****p=0.0001, when ψεRACK-induced hyperalgesia was compared between vehicle and CIDD-0150612 groups, unpaired Student's t-test). n=6 paws, per group.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Members of the team regularly attend the pain research seminars at UCSF.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Our results have been provided to Dr. Messing and his research team in Austin Texas, who will use this data to design the next generation of PKCε inhibitors to include both increased selectivity for PKCε with respect to ROCK, as well as other important properties of drugs (e.g., oral availability, prolonged half-life and lower toxicity.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will continue to study new compounds developed by Dr. Messing at The University of Texas, Austin as well as validate preclinical models of pain syndromes against which these compounds can be tested, including additional models of inflammatory, neuropathic and chronic widespread (generalized) pain syndromes.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the

reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

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

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	Jon Levine, MD, PhD
Project Role:	Principal Investigator
Researcher Identifier:	0000-0003-0681-5545
Nearest person month worked:	1
Contribution to Project:	Responsible for the overall design and execution of the experiments, as well as writing and publishing the results.
Name:	Paul Green, PhD
Project Role:	Co-Investigator
Researcher Identifier:	0000-001-76486826
Nearest person month worked:	1
Contribution to Project:	Design the nociception assay experiments, administer test compounds and oversee the behavioral studies. Also, liaise with University of Texas, Austin team with regards to transfer of agents and dissemination of behavioral data.
Name:	Niloufar Mansooralavi
Project Role:	Staff Research Associate
Researcher Identifier:	None
Nearest person month worked:	2
Contribution to Project:	Experiments in performing behavioral assays, general laboratory protocols, including anesthesia, drug administration and euthanasia. Responsible for preparation of solutions, maintaining equipment and ordering rats and other day-to-day laboratory operations.

Name:	Suncerray Hudson, BA, MA
Project Role:	Project Coordinator
Researcher Identifier:	None
Nearest person month worked:	1
Contribution to Project:	Project database (quality control) officer to oversee the data management program for the laboratory. Responsible for coordinating with all investigators and technician in the laboratory to ensure that data are appropriately entered and available on the lab databases.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

There has been a change in active support.
There is no senior/key personnel changes.

Jon Levine, PI

Previously active grant has closed

Title: NOCICEPTOR MECHANISMS IN THE TRANSITION FROM ACUTE CHRONIC PAIN
Time Commitment: 20% [2.4 calendar months]
Agency: NIH/NINDS **Project Number:** NS084545
Grants Officer: Michael L Oshinsky
Performance Period: 04/01/2014 – 05/31/2019
Funding Level: $342,139/yr Direct
Description & Specific Aims: The goal of this project is to evaluate whether cytoplasmic polyadenylation element binding protein (CPEB), a regulator of protein translation in axons that has been implicated in neuroplasticity, orchestrate the effects of protein kinase Cε (PKCε) on a variety of downstream proteins including P2X2/3, Na+-K+ ATPase, Hsp90 and calcium-calmodulin II (CaMKII) that has been implicated in neuroplasticity in high threshold Aplysia sensory neurons.

Previously pending grant now active

Title: HYALURONAN SIGNING TO NOCICEPTORS IN INFLAMMATORY PAIN
Time Commitment: 25% [3.0 calendar months]
Agency: NIH/NIAMS **Project Number:** AR075334
Grants Officer: Andrew Jones
Performance Period: 04/05/2019 - 01/31/2024
Funding Level: $364,646/yr direct
Description & Specific Aims:
This study will identify basic mechanisms underlying the role of ECM in pain and analgesia, which has important implications for basic as well as clinical pain research as it will provide the basis for rational development of new classes of HA-based analgesics and expand the scope of clinical conditions for which HA-based therapies may be effective. Our specific aims will test our central hypothesis, and elucidate the mechanisms by which HA affects nociceptor function.

Paul Green, Co-PI

Previously active grant has closed

Title: NOCICEPTOR MECHANISMS IN THE TRANSITION FROM ACUTE CHRONIC PAIN
Time Commitment: .4934% [5.92 calendar months]
Agency: NIH/NINDS **Project Number:** NS084545
Grants Officer: Michael L Oshinsky
Performance Period: 04/01/2014 – 05/31/2019
Funding Level: $342,139/yr Direct
Description & Specific Aims: The goal of this project is to evaluate whether cytoplasmic polyadenylation element binding protein (CPEB), a regulator of protein translation in axons that has been implicated in neuroplasticity, orchestrate the effects of protein kinase Cε (PKCε) on a variety of downstream proteins including P2X2/3, Na+-K+ ATPase, Hsp90 and calcium-calmodulin II (CaMKII) that has been implicated in neuroplasticity in high threshold Aplysia sensory neurons.

Previously pendingg grant now active

Title: HYALURONAN SIGNING TO NOCICEPTORS IN INFLAMMATORY PAIN
Time Commitment: 15% [1.8 calendar months]
Agency: NIH/NIAMS Project Number: AR075334
Grants Officer: Andrew Jones
Performance Period: 04/05/2019 - 01/31/2024
Funding Level: $364,646/yr direct
Description & Specific Aims:
This study will identify basic mechanisms underlying the role of ECM in pain and analgesia, which has important implications for basic as well as clinical pain research as it will provide the basis for rational development of new classes of HA-based analgesics and expand the scope of clinical conditions for which HA-based therapies may be effective. Our specific aims will test our central hypothesis, and elucidate the mechanisms by which HA affects nociceptor function.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (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: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Nothing to Report