AWARD NUMBER: W81XWH-21-2-0011

TITLE: Sustained Release of 2R,6R-Hydroxynorketamine by MacroPoSH Microneedle Patch for the Treatment of Post-Traumatic Stress Disorder and Pain

PRINCIPAL INVESTIGATOR: Irwin Lucki

CONTRACTING ORGANIZATION: Henry M Jackson Foundation for the Advancement of Military Medicine, Inc. (HJF), Bethesda, MD

REPORT DATE: August 2022

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2022	Annual	15Jul2021-14Jul2022

4. TITLE AND SUBTITLE

Sustained Release of 2R,6R-Hydroxynorketamine by MacroPoSH Microneedle Patch for the Treatment of Post-Traumatic Stress Disorder and Pain

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-21-2-0011

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Irwin Lucki, Caroline A. Browne
E-Mail: irwin.lucki@usuhs.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Henry M Jackson Foundation for the Advancement of Military Medicine, Inc. (HJF)
6720-A ROCKLEDGE DR STE 100
BETHESDA MD 20817-1891

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development 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

Sustained transdermal drug delivery by grooved microneedle arrays (MNA) can enhance patient compliance, reduce costs, and increase access to treatment. To date the major obstacle in developing such delivery devices is the drug loading capacity required to achieve clinically relevant doses. This proposal sets out a series of studies to develop and validate a novel wearable MNA Band-Aid like patch that can carry a payload sufficient to deliver an effective dose. The Partnering PI, Dr. Sonkusale's has developed this state of the art, low-cost technology in his laboratory. To demonstrate the advantages of MNA technology for drug delivery to treat disorders that have a high incidence in military service members and veterans, ketamine and its metabolite 2R, 6R-hydroxynorketamine ((2R, 6R)-HNK) have been chosen as the test compounds of interest. The MNA patches will be evaluated in rats on behavioral endpoints that are relevant to post traumatic stress disorder (PTSD) and chronic pain conditions. Targeting these endpoints to demonstrate the flexibility and functionality of the sustained delivery device will achieve the secondary goal of these studies. That is to provide preclinical evidence in support of developing (2R,6R)-HNK as a therapeutic for PTSD and chronic pain conditions.

Ultimately this device could be used to administered other prescription and non-prescription medications in general, and specifically allow for an alternative route of administration of (2R,6R)-HNK that can offer personalized treatment regimens for individuals suffering from intractable pain and PTSD. This technology and this medication could significantly improve combat casualty care treatment options and facilitate continued care of active-duty service members, veterans, and their families.

15. SUBJECT TERMS None listed.

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

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1. INTRODUCTION:

Sustained transdermal drug delivery by grooved microneedle arrays (MNA) can enhance patient compliance, reduce costs, and increase access to treatment. To date the major obstacle in developing such delivery devices is the drug loading capacity required to achieve clinically relevant doses. This proposal sets out a series of studies to develop and validate a novel wearable MNA Band-Aid like patch that can carry a payload sufficient to deliver an effective dose. The Partnering PI, Dr. Sonkusale's has developed this state of the art, low-cost technology in his laboratory. To demonstrate the advantages of MNA technology for drug delivery to treat disorders that have a high incidence in military service members and veterans, ketamine and its metabolite 2R, 6R-hydroxynorketamine ((2R, 6R)-HNK) have been chosen as the test compounds of interest. The MNA patches will be evaluated in rats on behavioral endpoints that are relevant to post traumatic stress disorder (PTSD) and chronic pain conditions. Targeting these endpoints to demonstrate the flexibility and functionality of the sustained delivery device will achieve the secondary goal of these studies. That is to provide preclinical evidence in support of developing (2R,6R)-HNK as a therapeutic for PTSD and chronic pain conditions.

2. KEYWORDS:

Microneedle array, sustained drug delivery, (2R, 6R)-HNK, pain, post traumatic stress disorder

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Under Specific Aim 1 - Fabrication, characterization, and ex vivo validation of (2R-6R)-HNK transdermal pharmacokinetics of grooved microneedle array (MNA).
Major Task 1 Fabrication of grooved microneedle arrays (MNA)
Major Task 2 Active electronic control of drug delivery and ex-vivo validation
Major Task 3 Combine sensing device for PTSD biomarkers
Under Specific Aim 2 - Confirm the antinociceptive and antiallodynic effects of sustained (2R, 6R)-HNK MNA infusions in a rodent model of chronic pain.
Major Task 4 Obtain IACUC approval
Major Task 5 Characterization of blood and brain concentrations of (2R, 6R)-HNK in male and female rats post MNA infusions.
Major Task 6 Determine the optimal treatment regimen of (2R, 6R)-HNK for antinociception.
Major Task 7 Confirm that MNA delivery of (2R, 6R)-HNK attenuates allodynia, thermal hypersensitivity and gait impairment induced by CCI of the sciatic nerve.
Under Specific Aim 3: Determine the impact of repeated (2R, 6R)-HNK MNA patch infusions on fear memory retention and generalized fear.
Major Task 8 Attenuation of contextual and generalized fear following sustained delivery of (2R, 6R)-HNK by MNA patch
Major Task 9 Reversal of CFC induced hyperarousal and non-associative fear by sustained delivery of (2R, 6R)-HNK by MNA patch
Major Task 10 Validation of sensing device for EDA and HRV

What was accomplished under these goals?

RESEARCH CONDUCTED AT THE USU PERFORMANCE SITE.

1) Major Activities.

Under Specific Aim 2 - Confirm the antinociceptive and antiallodynic effects of sustained (2R, 6R)-HNK MNA infusions in a rodent model of chronic pain.

Major Task 4: Obtain IACUC/ACURO approval

Major Task 6: Determine the optimal treatment regimen of (2R, 6R)-HNK for hypersensitivity

2) Specific Objectives

Major Task 4: Obtain IACUC/ACURO approval.

Major Task 6: Optimize and pharmacologically validated models of hypersensitivity.

3) Significant Results or Key Outcomes

Major Task 4: IACUC/ACURO approval granted.

Major Task 6: Hypersensitivity models have been optimized and pharmacologically validated.

Approach

Due to technical difficulties with the thermal place preference assay, we opted to utilize two alternative outcomes measures that are already approved in the IACUC/ACURO protocols, mechanical hypersensitivity using von Frey filaments and thermal hypersensitivity using a hot/cold plate. The following models have been established and characterized using the above endpoints; oxaliplatin-induced neuropathy and λ-carrageenan induced inflammatory pain.

As there was a delay in specific aim 1 optimizations of the microneedle array (MNA) delivery device, the impact of subcutaneously delivered (2R, 6R)-HNK was used to establish the potential of the drug in these models. This route of administration is more comparable to transdermal delivery ithan conventional intraperitoneal administration and was chosen to move the project forward in terms of the secondary objective of the program, to develop the preclinical assessment of (2R, 6R)-HNK as a novel therapeutic for the indications of pain and PTSD.

Methods

1) Animals: Male and female Sprague Dawley rats (Charles River Laboratories, Cary, NC), approximately 350-400 g, were housed two per cage, maintained on a standard 12-hour light/dark cycle (lights on at 06:00), and provided with food and water *ad libitum*. The animals were assigned to treatment groups according to paw withdrawal threshold measurements at baseline for

carrageenan experiments or following the fifth injection of oxaliplatin to ensure equal representation of hypersensitivity in each group. All experiments were carried out in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals and with approval from the Uniformed Services University of the Health Sciences Institutional Animal Care and Use Committee.

2) Drugs: (2R, 6R)-HNK was obtained from the National Center for Advancing Translational Sciences (NCATS; Bethesda, MD), synthesized as previously described (Morris et al., 2017). Doses were chosen based on our previous work for morphine and (2R, 6R)-HNK (Yost et al., 2022). Intraplantar injection with low viscosity λ-carrageenan (TCI America, C28711G) induced localized inflammatory pain. Morphine hydrochloride (10 mg/kg; Spectrum Chemicals; New Brunswick, NJ) was used as a reference control to reverse neuropathy induced by administration of oxaliplatin (Sigma Aldrich, Y0000271) induced neuropathy. Physiological saline (0.9% NaCl; Quality Biological, Gaithersburg, MD) was used as the vehicle for all compounds. Doses were calculated according to the molecular weight of the base and were administered via the intraperitoneal route at 10 ml/kg volume, except for λ-carrageenan, which is detailed below.

3) Localized paw inflammation: λ-Carrageenan was delivered by intraplantar injection, subcutaneous administration of 100 µl of a 1% λ– carrageenan solution via 26g needle into the plantar surface of the animal's left hind paw. This resulted in paw swelling and increased sensitivity to mechanical pressure within 2 hours of injection. This inflammatory induced mechanosensitivity persists for at least 48 hours.

4) Oxaliplatin Induced Neuropathy: To induce this model, animals were administered oxaliplatin (4 mg/kg) on two consecutive days every week (Monday and Tuesday) for two weeks, following by a final fifth injection at the start of the third week. Animals were then screened for thermal and mechanical hypersensitivity relative to their initial baseline results on week one to confirm the onset of neuropathy.

5) Mechanical sensitivity: An electronic von Frey aesthesiometer (Stoelting Co.; Wood Dale, IL; product number 57814) was used to automatically measure the threshold to mechanical stimuli in response to mid-plantar stimulation with a filament. The instrument includes an electronic unit, touch stimulator and software to compare applied force with desired target force. The results are reported as a paw withdrawal threshold in response to increasing force (gF). Briefly, the animals were placed in small plastic enclosures atop a wire mesh platform. The filament was gently applied at a steady force to the plantar surface of the animal's hind paw until a response is elicited, either a rapid withdrawal or lick of the affected paw. The response was measured four times, with intervals of 5 minutes between each trial. Baseline measurements prior to model onset were obtained at least one day before induction of the localized inflammation and three weeks prior to oxaliplatin experiments.

6) Thermal sensitivity: Antinociception was assessed by measuring paw withdrawal latencies on a Thermal Analgesia Meter, also referred to as a cold/hot plate (Ugo Basile, Stoelting Co.; Wood Dale, IL) set to either 5°C (cold) or 50°C (hot). The apparatus consists of the plate with touch screen display to set the fixed or ramping temperature, the plate is surrounded by a plastic restraining cylinder and cover. After placement on the plate, the latency for the rat to either jump or lick a hind paw was measured. The animal was immediately removed from the plate upon response, cut off times at which the test is automatically ended for cold stimuli was 180 s, for hot stimuli the cut off was 60 s.

Results

Oxaliplatin Induced Neuropathy: Figure 1 depicts the effects of the reference control on a model of oxaliplatin induced neuropathy. At baseline, paw withdrawal thresholds are approximately 50-70 gF. In Figure 1A, Morphine effectively returns oxaliplatin mechanical hypersensitivity to baseline levels 0.5 and 1 h following administration. Similarly in Figure 1B, morphine restores the latency to respond to a noxious thermal stimulus to pre-neuropathy baselines, with most animals reaching the 180 s cut off time. However, the effects are short lived, with animals exhibiting a return of mechanical and thermal hypersensitivity within 4 h of morphine treatment. The ability of (2R, 6R)-HNK to attenuate these effects will be screened in the next cohort of animals to establish the optimal subcutaneous dose.

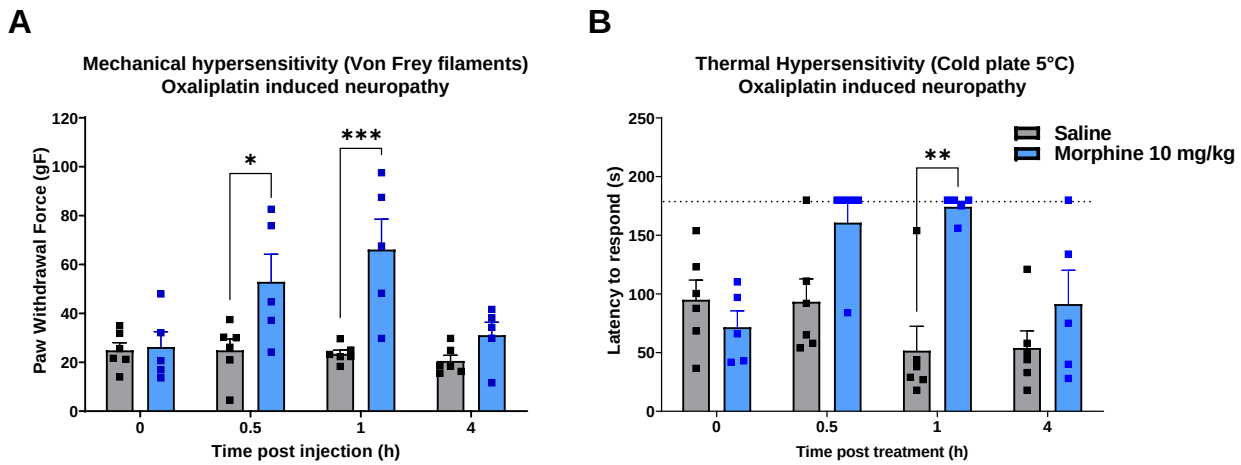

Figure 1 – Oxaliplatin Induced Neuropathy. 1A depicts the ability of morphine (10 mg/kg) to reverse the mechanical hypersensitivity to light touch associated with peripheral neuropathy (Treatment x Time interaction: ($F(3, 27) = 4.618$, $P=0.0098$)). Similarly in **1B,** morphine (10 mg/kg) normalized responding to a thermal stimulus (cold plate set to 5°C) to pre oxaliplatin levels of responding, with the cut off at 180 s, (Time X Treatment interaction: $F(3, 27) = 5.952$, $P=0.0030$). The symbol *, ** and *** represent a p value <0.05, <0.01 and <0.001 respectively, relative to the saline treated subjects.

Carrageenan induced inflammatory pain: Based on our previous work in mice, we have established that the optimal dose of (2R, 6R)-HNK is 10 – 30 mg/kg in this inflammatory pain model for male and female mice administered intraperitoneally. Accordingly, a pilot study was conducted to ascertain the ability of subcutaneously administered (2R, 6R)-HNK 10 mg/kg to attenuate carrageenan induced mechanical hypersensitivity. On the test days, measurements were taken two hours following carrageenan (time -30 in figure 2). Immediately following this measurement animals were treated with (2R, 6R)-HNK and retested at 1, 4 and 24 h. Figure 2 depicts the higher paw withdrawal threshold in rats treated with (26, 6R)-HNK at 24 h post treatment, suggestive of the potential for the drug to reverse mechanical hypersensitivity. These data support the hypothesis that (2R, 6R)-HNK can produce analgesia. Once the patches are made available, we will conduct the dose response curve to establish the optimal dose and duration of action out to 72 h.

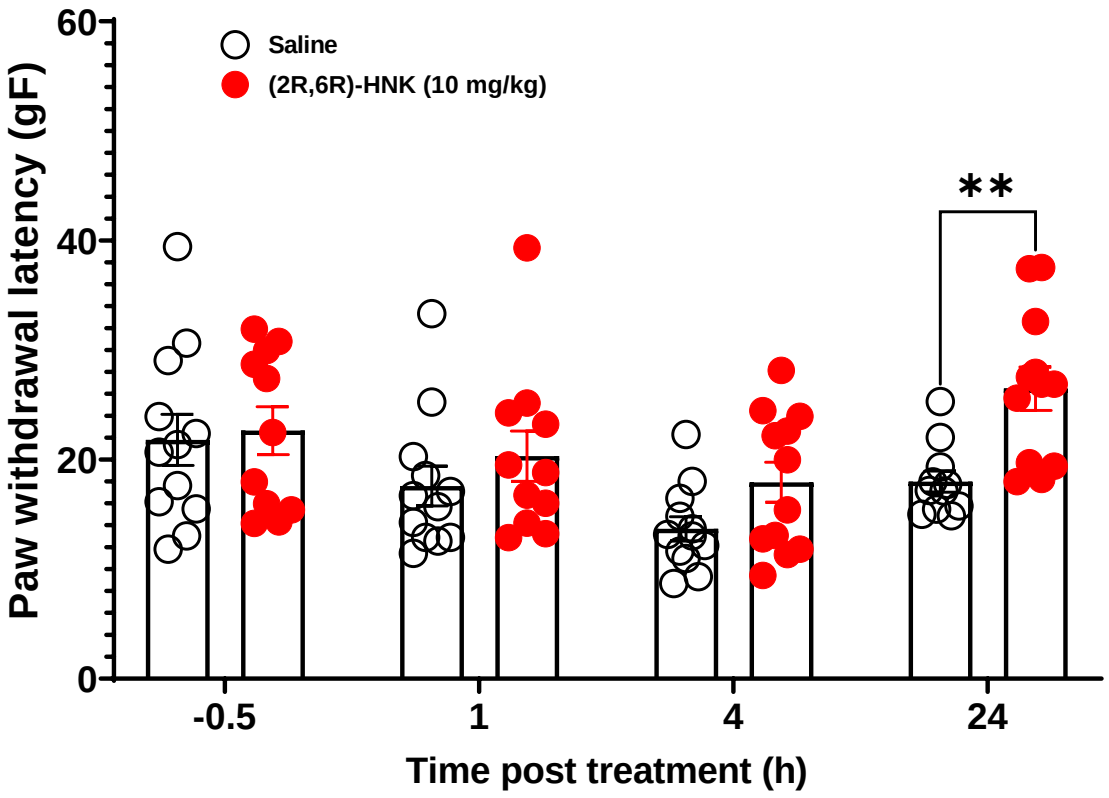

Figure 2 – Carrageenan induced inflammatory pain. Following induction of hypersensitivity, rats were treated with (2R, 6R)-HNK 10 mg/kg and tested 1, 4 and 24 h post treatment. (Main effect of treatment $F(1, 22) = 5.026$, $p = 0.0354$). The symbol ** represent a p value <0.01 relative to the saline treated group.

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

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

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

1) Once the patches are made available the ability of (2R, 6R)-HNK to attenuate mechanical and thermal hypersensitivity following oxaliplatin and carrageenan administration will be evaluated with optimal doses identified and duration of action established with reference to the controls morphine, duloxetine and (2R,6R)-HNK's parent drug ketamine. 2) Complete incubation of fear model validation 3) Complete characterization of non-associative fear (elevated zero maze) and hyperarousal (acoustic startle and prepulse inhibition) following incubation of fear. 4) Validation of HRV as a biomarker of stress in fear conditioned exposed animals (ECGenie) for comparison with novel device proposed under specific aim 3.

4. IMPACT:

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

Nothing to report.

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

There have been delays in hiring members into the positions at both sites. As such there has been a delay in receiving the patches from the partnering PI.

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

There have been delays in hiring staff at both sites. Consequently the optimization and validation of the patches in vitro and ex vivo by the partnering PI and his team have been delayed. This has not however impacted the overall project goals as the partnering PI has progressed development on the microfluidics devices and anticipates that the first set of patches for in vivo testing will be shipped to the USU site within the next three months. With the team in place and the speed at which staff have performed duties thus far, the objectives for FY22 and FY23 will be achieved rapidly.

Due to technical difficulties with the thermal place preference assay, we opted to utilize two alternative outcomes measures that are already approved in the IACUC/ACURO protocols, mechanical hypersensitivity using von Frey filaments and thermal hypersensitivity using a hot/cold plate. The following models have been established and characterized using the above endpoints; oxaliplatin-induced neuropathy and λ-carrageenan induced inflammatory pain.

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Changes that had a significant impact on expenditures

Delays in hiring. The first staff member hired on the project joined the group in Dec 2021 and the second member jointed that lab in May 2022. This has impacted overall expenditure.

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

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.

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)**

  Nothing to report.

- **Technologies or techniques**

  Nothing to report.

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

Nothing to report.

- **Other Products**

Initial data are supportive of the analgesic potential of (2R, 6R)-HNK. This is the first demonstration of its ability to alleviate mechanical hypersensitivity in rats.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Irwin Lucki*
Project Role:	*Initiating PI, USU*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-8801-7840*
Nearest person month worked:	*2*
Contribution to Project:	*Dr. Lucki liaised with HJF, the USU IACUC, ACURO and CDMRP to obtain approval for the studies to commence. He has conducted monthly meetings with staff to assess progress on the project development, performed the administrative tasks associated with this research effort and hired staff members to complete the objectives.*
Funding Support:	*Federal employee*

Name:	*Caroline A Browne*
Project Role:	*Co-I, USU*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-7463-7870*
Nearest person month worked:	*5*
Contribution to Project:	*Dr. Browne has assisted with all written reports, IACUC/ACURO approvals etc. She is responsible for daily supervision and training of staff members and is also involved in ensuring the completion of all planned experimental aspects of the research program.*
Funding Support:	*HJF employee in support of the Department of Pharmacology, USU. Funding derived from Transforming Technology for the Warfighter initiative and this grant.*

Name:	*Kaitlin Castell*
Project Role:	*Research Associate, USU*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*2*
Contribution to Project:	*Ms Castell has commenced experiments to optimize the incubation of fear model in rats and assessed the optimal s.c. dose of (2R, 6R)-HNK to alleviate fear memory recall for comparison with the MNA delivery drug. She is actively conducted dose response curves for optimal (2R, 6R-HNK analgesia under two conditions, oxaliplatin neuropathy with Ms Campanile. Ms Castell is also establishing molecular assays to quantify the impact of sub cutaneous versus MNA delivered (2R, 6R)-HNK on glutamatergic neurotransmission within relevant brain regions.*
Funding Support:	*HJF employee in support of the Department of Pharmacology, USU. Funding derived from this grant.*

Name:	*Maria Campanile*
Project Role:	*Research Assistant, USU*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*8*
Contribution to Project:	*Ms Campanile has optimized the oxaliplatin neuropathy and carrageenan induced inflammation models and is commencing studies with sciatic nerve injury for future assessments of (2R, 6R)-HNK's analgesic potential. She also supports Ms Castell's efforts in performing the fear conditioning paradigm and the molecular evaluation of drug and stress outcomes.*
Funding Support:	*HJF employee in support of the Department of Pharmacology, USU. Funding derived from this grant.*

Has there been a change in the the active support

The following grant was completed in 08/21. Kappa Receptor Antagonists as Rapid Acting Antidepressants, a RO1 administered by the National Institutes of Mental Health , Rockville, MD.

What other organizations were involved as partners?

Organization: Henry M Jackson Foundation (HJF)
Location: 6720A Rockledge Drive, Bethesda, Maryland 20817
Contribution: Contracting organization., administer the grant and employ staff members in support of the project.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

Attached

9. APPENDICES:

None