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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

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Uniformed Services University of the Health Sciences (USU)
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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. Specifically the device allows 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.

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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, 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, the ketamine metabolite 2R, 6R-hydroxynorketamine ((2R, 6R)-HNK) have been chosen as the test compound 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

RESEARCH CONDUCTED AT THE USU PERFORMANCE SITE.

1) Major Activities during this period.

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 6 Determine the optimal treatment regimen of (2R, 6R)-HNK for antinociception. **Go**

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. **Ongoing**
Major Task 9 Reversal of CFC induced hyperarousal and non-associative fear by sustained delivery of (2R, 6R)-HNK by MNA patch. **Ongoing**
Major Task 10 Validation of sensing device for EDA and HRV. **Ongoing**

2) Specific Objectives

Determine the efficacy of sustained (2R, 6R)-HNK release on outcomes of reduce mechanical and thermal hypersensitivity associated with rodent models of pain

Establish the ability of (2R,6R)-HNK's to attenuate incubation of fear memory, and the associated generalized anxiety and electrocardiogram profiles associated with the traumatic stressor.

3) Significant Results or Key Outcomes

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

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

Dose Response Curves of (2R,6R)-HNK reversal of mechanical hypersensitivity

Significant mechanical hypersensitivity associated with inflammatory pain was evident in female (t=10.07, df=25, P<0.001; Fig. 1A) and male rats (t=5.830, df=10 P<0.001; Fig. 1B) 24 hours post carrageenan injection, with animals exhibited responses threshold at ~40 g/f at baseline and ~20 g/f after induction of hypersensitivity.

As there was significant main effect of sex (F (1, 110) = 22.14, P<0.0001) driven by large standard deviations in female animals, dose response curves of females (Fig. 1C) and males (Fig. 1D) were evaluated separately. In females, a significant effect of (2R,6R)-HNK treatment (F (3.000, 49.37) =3.643, P<0.05) was identified at the 10 (p<0.05) and 30 mg/kg (p<0.05) doses, which increased the percent return to baseline values by approximately 10-15% relative to 0 mg/kg controls (Fig. 1C). (2R,6R)-HNK treatment in males (F(3, 37) = 0.4764, P<0.05)). 30 mg/kg and 56 mg/kg (2R-6R) HNK significantly increased the percent return to baseline (p<0.005 and p<0.05 respectively) (Fig 1D).

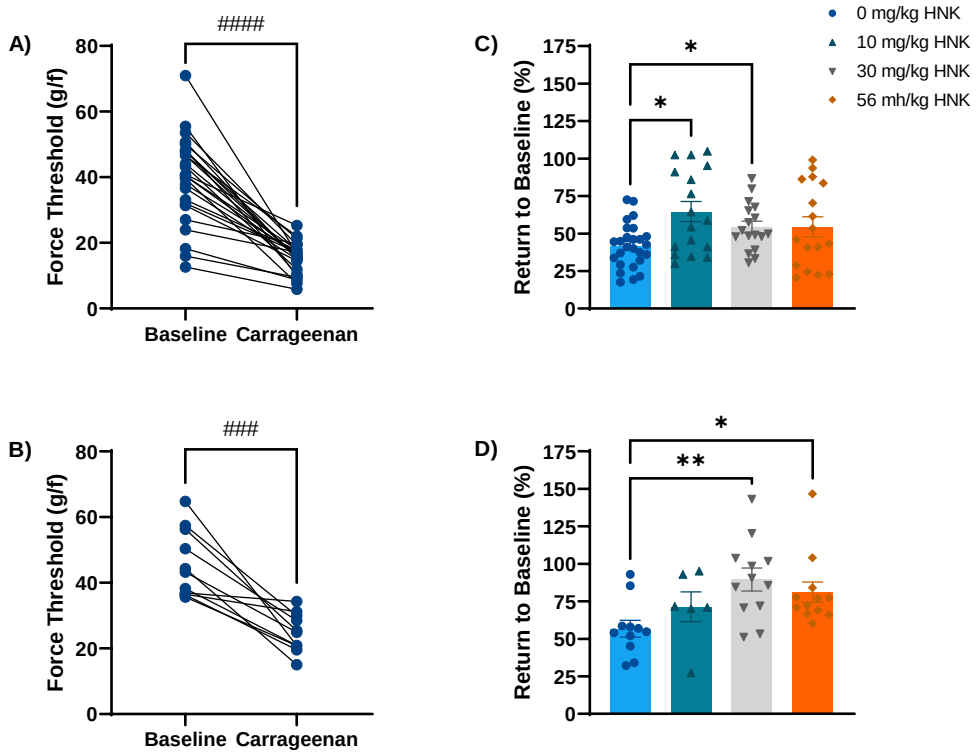

Figure 1. Evaluation of mechanical hypersensitivity with Electronic von Frey.
Ipsilateral measurements of the force threshold for withdrawal of hind paw at baseline and 24 hours after treatment of carrageenan induced inflammation in the 0 mg/kg HNK control group in **A** female and **B** male Sprague Dawley rats. ### and #### represents a significant carrageenan effect of P<0.0005 and 0.0001 respectively. The percentage return to baseline 24 hours post HNK treatment in carrageenan induced hypersensitive **C** female and male **D** rats. The symbols * and ** represent significant treatment effects of p<0.05 and p<0.01 respectively.

Oxaliplatin produced long-lasting hypersensitivity that was reversed by (2R,6R)-HNK.

Mechanical Von Frey was tested at 1, 4 and 6 weeks post-oxaliplatin injections. Oxaliplatin attenuated the scores for threshold sensitivity at 1 week (p=0.0149), 4 weeks (p= 0.0273) and at 6

weeks (p=0.0008) (Figure 2A).

With (2R,6R)-HNK administration, animals were tested at 4 and 24 hrs and compared to their 4 week baselines. Two-way ANOVA showed a significant time x condition ($F(3, 41) = 4.996$, $p=0.0048$) (Figure 2B). Tukey's multiple comparison test showed the oxaliplatin-treated animals had a significantly greater withdrawal threshold compared to the saline-treated animals at 4 hours ($p<0.0001$) and 24 hours ($p<0.0001$) following a (2R,6R)-HNK injection. Control animals given (2R,6R)-HNK also showed an increase in withdrawal threshold 24 hours after treatment ($p=0.0004$) (Figure 2B).

At 8 weeks post the final oxaliplatin treatment, the hot plate test was analyzed. Results showed that the oxaliplatin-treated animals at baseline had a significantly reduced latency compared to the vehicle-treated animals ($p=0.0256$) (Figure 2C). The animals were then treated with 30mg/kg of (2R,6R)-HNK and their latencies were tested 4 and 24 hours post-injection. There was found to be a significant main effect of oxaliplatin-treatment ($F(3, 41) = 14.63$, $p<0.0001$) (Figure 2D).

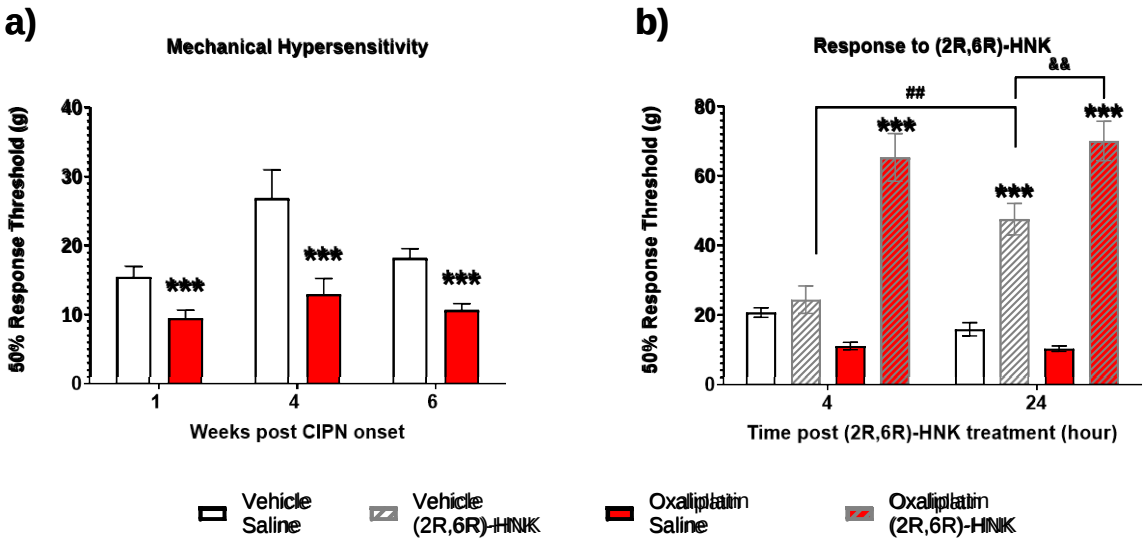

Figure 2. Oxaliplatin increased hypersensitivity with (2R,6R)-HNK reversal. Mechanical hypersensitivity was confirmed 1 ($p=0.0149$), 4 ($p= 0.0273$), and 6 weeks ($p=0.0008$) post the final oxaliplatin injection (Fig. 2A). (2R,6R)-HNK reversed the hypersensitivity after systemic injection in the oxaliplatin-treated animals at 4 ($p<0.0001$) and 24 hours ($p<0.0001$) and in control animals treated with (2R,6R)-HNK at 24 hours ($p=0.0004$) (Fig. 2B). Thermal hypersensitivity (not shown) was confirmed in testing done 8 weeks following the final oxaliplatin injection (Fig. 2C). (2R,6R)-HNK treatment showed a significant reversal of thermal hypersensitivity ($F(3, 41) =14.63$, $p< 0.0001$) (Fig. 2D).

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

Nothing to Report

How were the results disseminated to communities of interest?

Oral Presentations.
Reversal of Mechanical and Thermal Hypersensitivity in Rat Models of Inflammatory Pain and Peripheral Neuropathy by (2R, 6R)-Hydroxynorketamine, Caroline A. Browne PhD, Maria A. Campanile BSc, Justin O. Pampalone BSc, Sean Collier BSc, Kaitlin R. Castell, MHS, Irwin Lucki, PhD MHSRS August 15, 2023.
Tunable transdermal delivery of ketamine for treatment of post-traumatic stress disorder Hasika Suresh, Eli Curry, Kinan Rabbat Caroline Browne, Irwin Lucki and Sameer Sonkusale. MHSRS August 14, 2023.

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 dose response curves for reduction of fear memory recall, non-associative fear (elevated zero maze) and hyperarousal (acoustic startle and prepulse inhibition) following incubation of fear.
3) Optimize the dose response curve for the reversal of stress associated ECG profiles.

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

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

The co-I at USU is leaving USU/HJF but will remain associated with the program in her new position. Weekly meetings and appropriate access to the data in shared drives have been instituted to ensure continuity of methods, data assessments and manuscript preparation to avoid any potential delays in achieving the objectives.

Changes that had a significant impact on expenditures

More staff were working on the program in FY2023.

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:	*9.33*
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: *Maria Campanile*
Project Role: *Research Assistant, USU*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *11*
Contribution to Project: *Ms Campanile has optimized the incubation of fear model in rat. The optimal s.c. dose of (2R, 6R)-HNK to alleviate fear memory recall for comparison with the MNA delivery drug is ongoing. MS Campanile has completed the dose response curves for optimal (2R, 6R-HNK analgesia under two conditions, oxaliplatin neuropathy and carrageenan induced inflammation. She is now commencing studies with sciatic nerve injury model for MNA studies.*
Funding Support: *HJF employee in support of the Department of Pharmacology, USU. Funding derived from this grant.*

Name: *Kaitlin Castell*
Project Role: *Research Associate, USU*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *11*
Contribution to Project: *Ms Castell is supporting the work of Ms Campanile establishing molecular assays to quantify the impact of sub cutaneous versus MNA delivered (2R, 6R)-HNK on glutamatergic neurotransmission within relevant brain regions. She also assists Ms Campanile in pain model assessments.*
Funding Support: *HJF employee in support of the Department of Pharmacology, USU. Funding derived from this grant*

Name: *Justin Pampalone*
Project Role: *Research Assistant, USU*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *7*
Contribution to Project: *Mr Pampalone has worked with Ms Campanile to complete the dose finding experiments in the carrageenan induced inflammation and oxaliplatin neuropathy models. He 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 active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

The following grant was awarded to the PI as PI

- (2R,6R)-hydroxynorketamine, a putative therapeutic for comorbid post-traumatic stress disorder and alcohol use disorder, supported by the CDMRP - Pharmacotherapies for Alcohol and Substance Use Disorder Alliance (PASA), with the performance period of 01/12/2022 – 31/07/2024 -

The following grants were awarded to the Co-I as PI

- Evaluating the potential of (2R, 6R)-hydroxynorketamine as a novel treatment for rheumatoid arthritis, supported by a FY23 Intramural Short-Term Discovery Award, Uniformed Services University, Bethesda, MD 20814, with the performance period 10/30/2022 - 09/30/2023 -

- Preclinical technology validation – Prolonged Delivery of Intranasal Ketamine, supported by the Transforming Technology for the Warfighter Program, Uniformed Services University, Bethesda, MD 20814, with the performance period 12/01/2022 -11/30/2023 -

The following grants were awarded to the Co-I as a Co-I

- Neuroprotection for extreme military high altitude operations through hypoxic preconditioning supported by the USU Intramural High Priority Awards, The Uniformed Services University of the Health Sciences Office of Research, 4301 Jones Bridge Road, Bethesda, MD, 20814, with the performance period 01/11/2022 – 31/10/26 -
- Acute and long-term impact of stressors on the stress axis and cognitive performance, supported by the Office of Naval Research, with the performance period 01/07/2023 – 30/06/2026 -

What organizations were involved as partners?

Organization: Henry M Jackson Foundation for the Advancement of Military Medicine, Inc. (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