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TITLE: Pharmacological Enhancement of Cortical Activity for Controlling Chronic Pain

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CONTRACTING ORGANIZATION: Indiana University School of Medicine

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Chronic pain is a major problem of public health in both civilians and military personnel. Patients with neuropathic pain often experience severe pain that is often refractory to current treatment options. The chronic pain not only severely compromises the quality-of-life, employment, and recovery, but also leads to opioid addiction and life-threatening drug over-dose and abuse. Because the lesions or injuries that lead to neuropathic pain initially cause loss of nervous sensory input to the brain, a recent hypothesis proposes that a compensatory reaction and plasticity (termed homeostatic plasticity) is responsible for developing and maintaining the pain. If this is true, then stimulating brain activity to replenish the lost sensory input should help the body to recovery the lost activity and control the pain. The goal of this research proposal is to develop a new treatment option by enhancing the activity of nerve cells in the related areas of the brain. Specifically, we will test a compound that are known to mildly enhance neuronal activity in the brain (but without over-activating neurons to cause seizures). In a well-established nerve injury model in mice that usually causes neuropathic pain, we will first determine whether the drugs will reduce pain sensation to mechanical and thermal stimulation and spontaneous pain. Then we will use imaging techniques to examine activities of individual neurons as well as the whole cerebral cortex in live animals, which will tell us whether and how the drugs affect brain activity. We may also record electrical activity from single brain neurons to understand how the drugs affect single neurons and the input they receive from synapses. Findings from this study have the potential to open new avenues for pain treatment and to limit the amount of medication that is needed for effective pain relief. Because pain is often a major factor limiting the quality of life of neurological patients in soldiers and veterans, we expect that this study will contribute to better patient care and reduce the use of opioids and its lethal side-effects.

15. SUBJECT TERMS

NONE LISTED

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1. Introduction

Chronic pain is a major problem of public health in both civilians and military personnel. Patients with neuropathic pain often experience severe pain that is often refractory to current treatment options. The chronic pain not only severely compromises the quality-of-life, employment, and recovery, but also leads to opioid addiction and life-threatening drug over-dose and abuse. The goal of this research proposal is to develop a new treatment option by enhancing the activity of nerve cells in the related areas of the brain. Specifically, we will test a compound that are known to mildly enhance neuronal activity in the brain (but without over-activating neurons to cause seizures). In a well-established nerve injury model in mice that usually causes neuropathic pain, we will first determine whether the drugs will reduce pain sensation to mechanical and thermal stimulation and spontaneous pain. Then we will use imaging techniques to examine activities of individual neurons as well as the whole cerebral cortex in live animals, which will tell us whether and how the drugs affect brain activity. We may also record electrical activity from single brain neurons to understand how the drugs affect single neurons and the input they receive from synapses.

2. Keywords

Animal study

Calcium imaging

Cerebral cortex

D-cycloserine (DCS)

GCaMP6

Homeostatic plasticity

Mesoscopic imaging

Neuropathic pain

Two-photon microscopy

Treatment

3. Accomplishments

What were the major goals of the project?

Specific Aim: To determine the effects of NMDAR partial agonists on reducing neuropathic pain and the underlying neurophysiological mechanism in a TNI model.	**Timeline**
Major Task 1: Acute effect on pain	Months
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Subtask 1: Injury/sham surgery, treatment, and behavioral testing of WT animals (n=30)	11-15
Subtask 2: Patch clamp recording of WT mice after TNI/sham surgery (n=30)	12-16
Subtask 3: Data analysis	12-16
Milestone 2: Prepare manuscript for publication	17-18

What was accomplished under these goals?

1) Major activities: We made big cranial windows on eighteen mice and right tibial nerve injures (TNI) were made two weeks after. Ten mice with good cranial window were used for in vivo two-photon and mesoscopic imaging of activity, they were assigned to 3 groups including sham, TNI plus saline, and TNI plus 1-aminocyclopropanecarboxylic acid (ACPC)(200mg/kg, i.p. daily).

To determine the acute effect of treatment with (ACPC) on cortical activity, we made in vivo two photon and mesoscopic imaging in 2 weeks after injury. ACPC was injected intraperitoneally and evoked cortical activities were repeatedly imaged every 30 minutes until three and half hours.

For chronic effect, we did calcium imaging from the same group of mice on the second day following Von Frey pain assessments for up to 7 weeks. Spontaneous activity was imaged for 3 minutes, a period sufficient for calculating cortical connectivity. Then the hind paw with TNI , for each animal, was stimulated with single electrical pulse at different intensities to determine a threshold for evoked imaging activities in the sensory cortex, and then used the double-threshold intensity to stimulate when recording. Von Frey tests and imaging (spontaneous and evoked) were performed once a week from 2 weeks to 7 weeks after injury.

2) Specific objectives: To determine the acute and chronic effect of ACPC on neuropathic pain and cortical activity in a TNI model.

3) Significant results and conclusion: We found that injection of ACPC caused a decrease in the amplitude of evoked calcium responses from 0.5 hour to 3.5 hours after the injection (n=4).

We found that there was no significant change in activity level in sham group during a 7 weeks' imaging period. In the TNI group, there was a significant increase in the excitability of both neuron and neural network after two weeks after injured. While treatment with ACPC reduced the increased activity from 2 weeks after ACPC treatment. There was a significant difference between TNI and ACPC groups. Von Frey

pain assessment of paw withdrawal threshold showed unchanged pain threshold in sham group, dramatic decrease in the TNI group that lasted for at least 7 weeks. With ACPC treatment, the pain threshold was higher than the TNI control group. There were significant differences between each pair of groups, suggesting that ACPC treatment ameliorated TNI induced neuropathic pain.

4) Other achievements: Nothing to Report.

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?

a. We will use in vivo two-photon and mesoscopic activity imaging and behavioral testing in more animals with TNI to test the acute and chronic effects of ACPC on neuropathic pain.

b. We will work on developing compute program for automatic detection and analysis of imaging data

c. We will conduct electrophysiological recordings to determine the effect of ACPC on neurons.

4. Impact

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

a. We will develop a computer program that use artificial intelligence and imaging analysis technology to automatically analyze neuronal activity from GCaMP6 imaging data, which will greatly increase the speed of analysis and same time and will be usable to people in the neuroscience field.

b. The current data support our hypothesis that targeting NMDA with a partial agonist is effective in controlling neuropathic pain, which, if confirmed in next experiments, will provide a novel strategy for the treatment of neuropathic pain and guide the development of new drugs.

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

We are working on developing a computer program for automatic detection of active neurons from calcium imaging data. Although this was not included in our grant proposal, it will greatly increase the speed of data analysis and will benefit our project and the neuroscience field.

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

The project is delayed and will continue to be delayed due to the COVID-19 pandemic.

Changes that had a significant impact on expenditures

Keeping salary payments and other expenditures during period of the shutdown of our university due to COVID-19 pandemic will likely reduce fund available for the project. However, we will try to catch up project progress with the extension of project period.

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

N/A

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

Nothing to Report

7. Participants & Other Collaborating Organizations

7.1. What individuals have worked on the project?

Name	Xiaoming Jin	Yadav Adhikari	Allison Moore
Project role	PI	Graduate Student	Graduate Student
Researcher Identifier	0000-0002-8671-8640		
Nearest person month worked	3	6	3
Contribution to Project	Overseeing project, research designing, troubleshooting, preparing animal protocol.	Making cranial windows, in vivo imaging experiment and data analysis	Making cranial windows, in vivo imaging experiment and data analysis
Funding Support			

7.2. 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

7.3. What other organizations were involved as partners?

Nothing to Report

8. Special Reporting Requirements

Nothing to Report

9. Appendices

None.