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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 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. In a nerve injury model in mice, we will determine whether the drugs will reduce pain sensation to mechanical stimulation and spontaneous pain. Then we will use imaging techniques to examine activities of the whole cerebral cortex in live animals. We 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. We expect that this study will contribute to better patient care and reduce the use of opioids and its lethal side-effects in soldiers and veterans.

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 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. 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 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.

2. Keywords

Neuropathic pain, cerebral cortex, NMDA receptor, in vivo calcium imagine, electrophysiology, patch clamp recording.

3. Accomplishments

What were the major goals of the project?

Specific Aim: To determine the effects of 1-Aminocyclopropanecarboxylic acid (ACPC), a NMDAR partial agonist, on reducing neuropathic pain and the underlying neurophysiological mechanism in a (tibial nerve injury) TNI model.

Major Task 1 : Acute effect of ACPC on pain

Subtask 1: Submit documents for IACUC and ACURO approval

Subtask 2: Injury or sham surgery, treatment, behavioral testing, and imaging in GCaMP6 mice

Major Task 2: Chronic effect of ACPC on pain

Subtask 1: Injury/sham surgery, treatment, behavioral testing, and imaging in GCaMP6 mice

Subtask 2: Data analysis

Major Task 3: In vitro electrophysiology after ACPC treatment

Subtask 1: Injury/sham surgery, treatment, and behavioral testing of WT animals

Subtask 2: Patch clamp recording of WT mice after TNI/sham surgery

Subtask 3: Data analysis

What was accomplished under these goals?

1) Major activities:

In three groups of mice (non-injured, injured, and injured+ACPC treatment), we measured both acute and chronic changes in mechanical hypersensitivity and used in vivo mesoscopic imaging to determine cortical activity in a TNI model in GCaMP6 transgenic mice and tested the effects of ACPC on these changes.

In three groups of mice (non-injured, injured, and injured+ACPC treatment), we also made patch clamp recording from brain slices and determined the effect of chronic treatment of ACPC for two weeks on neuronal excitability, excitatory and inhibitory synaptic transmissions, and neuronal intrinsic electric properties.

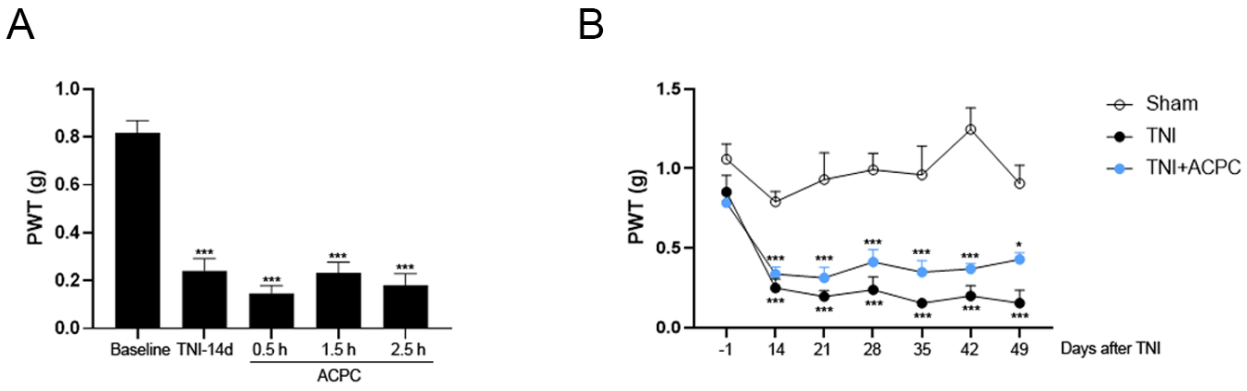
2) specific objectives:

To determine the acute and chronic effect of ACPC on reducing neuropathic pain;

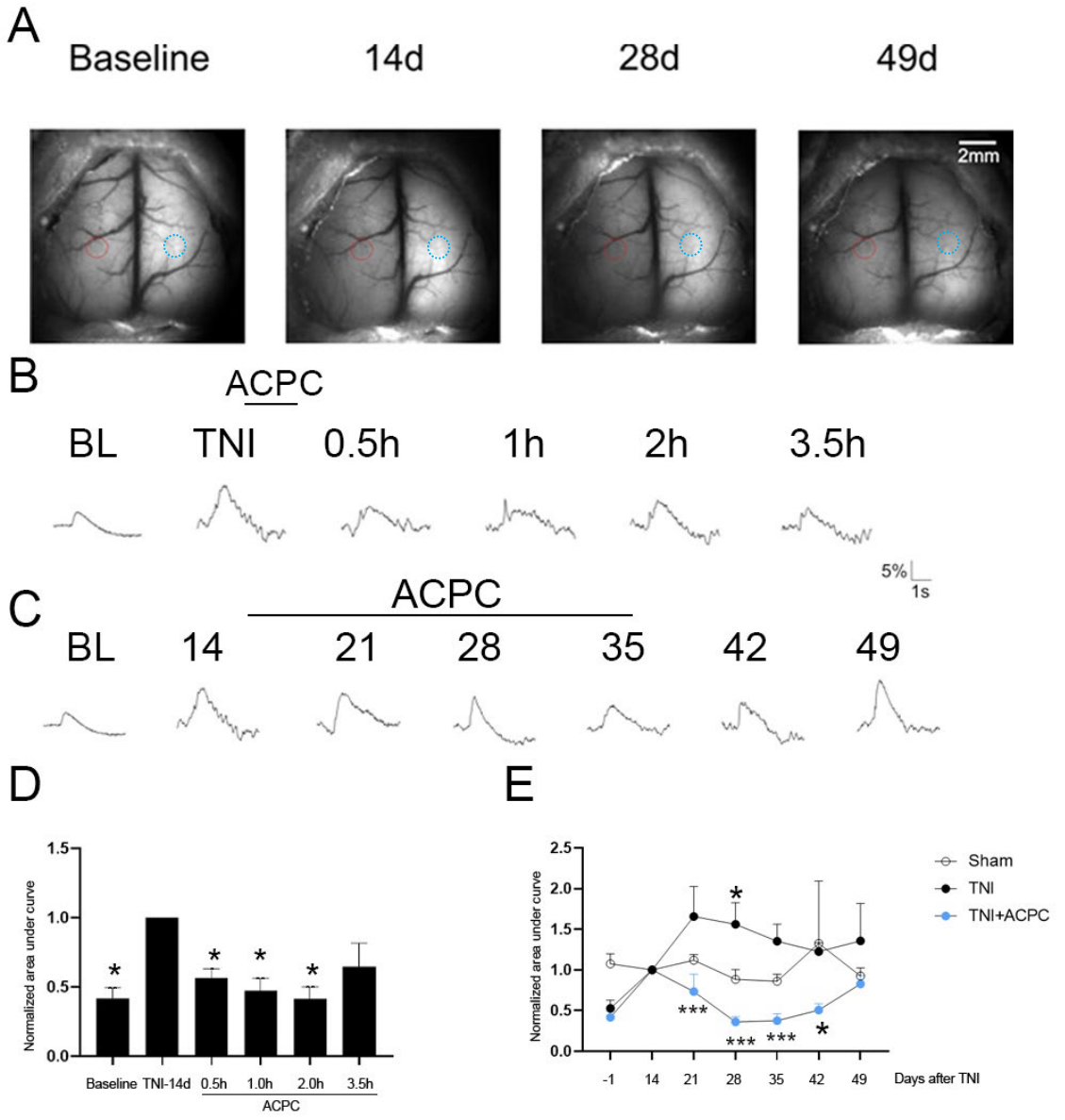
To determine the acute and chronic effects of ACPC on reducing cortical hyperexcitability;

To determine how ACPC reducing neuronal hyperexcitability.

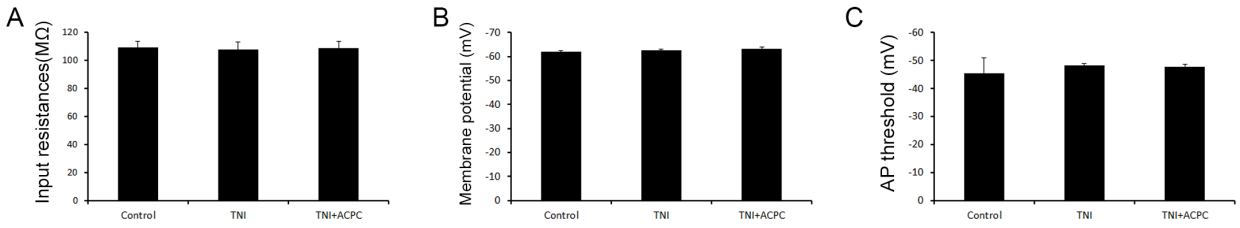
3) significant results or key outcomes:



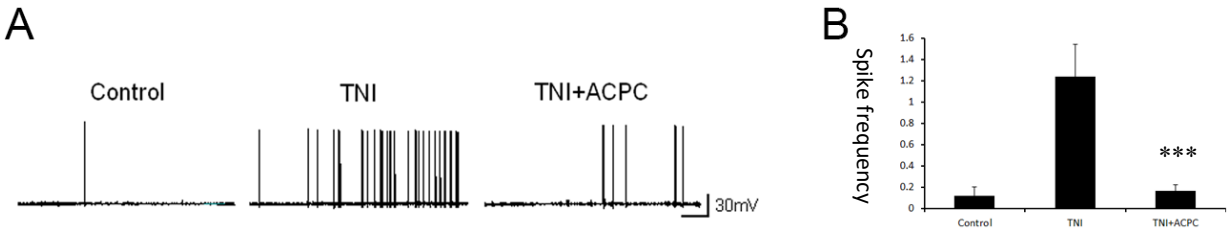
a. Tibial nerve injury caused mechanical hypersensitivity in the injured animals, acute ACPC treatment in 3.5 hours did not reduce this hypersensitivity (A). In contrast, chronic ACPC treatment for 3 weeks caused a clear trend of reduction in hypersensitivity (B above). Although the current result did not reach statistic significance, we will increase the sample size of the experiment to better demonstrate the effect. n=7-10 mice/group.



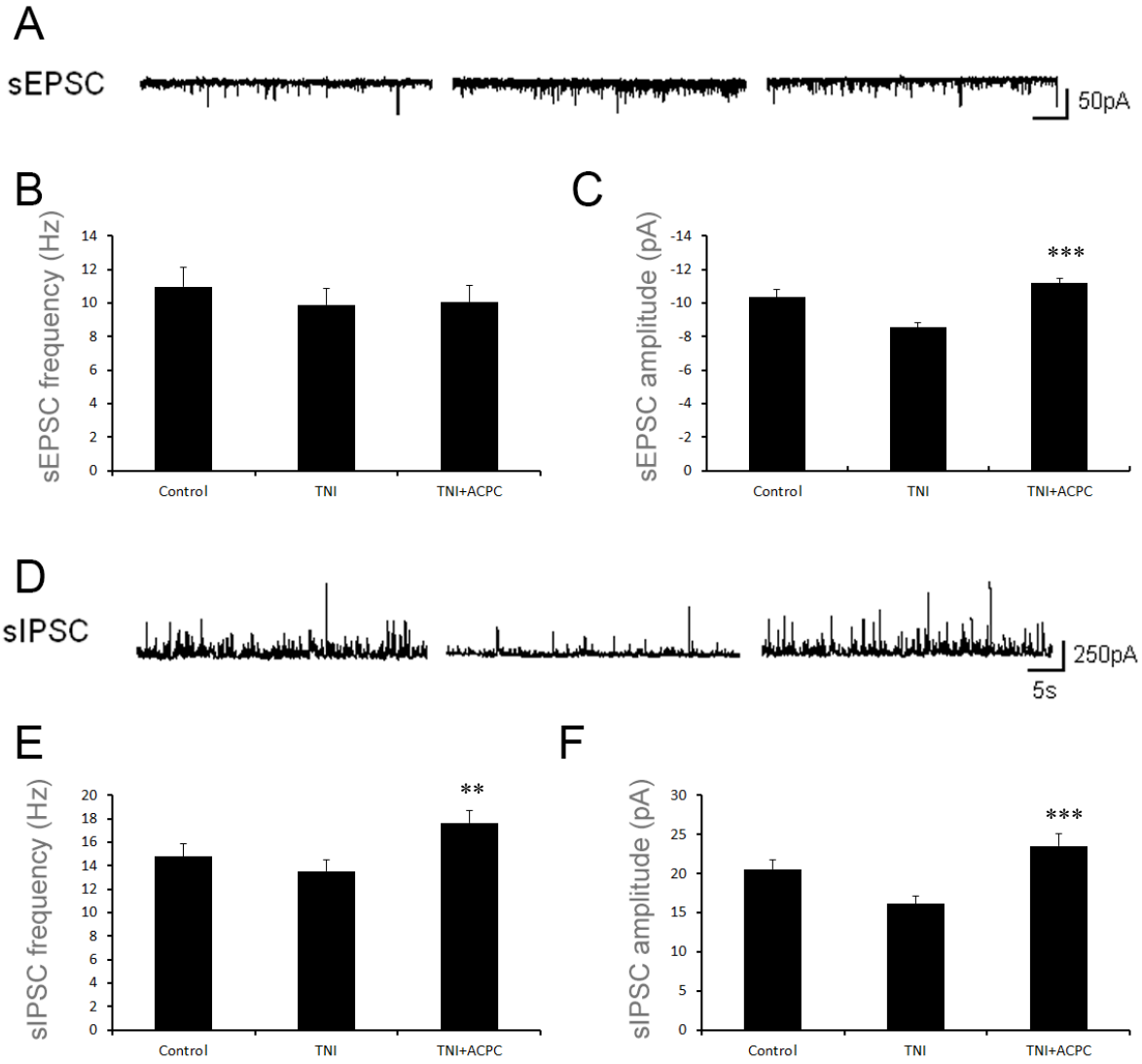
b. In the figure above, in vivo mesoscopic calcium imaging was used to repeatedly image both cortical hemispheres at different times after TNI in GCaMP6 transgenic mice (A). Cortical sensory evoked responses were measured from contralateral primary somatosensory cortex (blue circles). Both acute treatment (B and D) and chronic treatment for 3 weeks (C and E) of ACPC induced statistically significant decreases in evoked calcium signals, suggesting reduced cortical hyperexcitability in neuropathic pain mice (*: $p<0.05$ and ***: $p<0.001$, one-way ANOVA). n=7mice/group



c. Chronic ACPC treatment did not change neuronal intrinsic properties in cortical layer V pyramidal neurons, including input resistance (A), resting membrane potential (B), and action potential threshold (C).



d. In the figure above, chronic ACPC treatment for 3 weeks significantly reduced the enhanced spontaneous firing of cortical layer V pyramidal neurons of the injured animals (A and B)(***: p<0.001, when compared between TNI and TNI+ACPC groups, one-way ANOVA), suggesting a reduced excitability of these neurons.



e. In the figure above, chronic ACPC treatment for 3 weeks significantly caused a significant increase in the amplitude of the spontaneous excitatory synaptic currents (sEPSCs) (A, B, and C), but it significantly increased both frequency and amplitude of the spontaneous inhibitory synaptic currents (sIPSCs) (D, E, and F) (p<0.01 and p<0.001, one-way ANOVA), The results suggest that enhancing synaptic inhibition is a major mechanism of ACPC.

4) other achievements.

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

Nothing to Report

How were the results disseminated to communities of interest?

The results were presented to the neuroscience research community in the Indiana University School of Medicine.

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

Nothing to Report

4. Impact

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

The results from this project support the feasibility of using partial agonists of NMDA receptors for the control of neuropathic pain. This is a novel strategy for the treatment of neuropathic pain, because conventional treatment is to inhibit or suppress neural activity while our strategy support the effectiveness of enhancing activity for pain control. As predicted by homeostatic plasticity hypothesis, the results may open the opportunity to control neuropathic pain by developing drugs to stimulate neuronal activity.

What was the impact on other disciplines?

The activity enhancement strategy may be effective and applicable to treat other neurological diseases featuring hyperexcitability, such as different types of acquired epilepsy.

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

Nothing to Report

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

Because the CODIV-19 pandemic has significantly delayed the progress of this project, we requested and was approved for a no-cost extension for half a year for the completion of the project.

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

The project was completed at the end of extension period.

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

N/A

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

Publications, conference papers, and presentations

Nothing to Report

Journal publications

Chai Z, Ma C, Jin X. Cortical stimulation for treatment of neurological disorders of hyperexcitability: a role of homeostatic plasticity. Neural Regen Res. 2019 Jan;14(1):34-38. doi: 10.4103/1673-5374.243696.

Manuscript is in preparation to report the results.

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

Nothing to Report

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	*Xiaoming Jin*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-8671-8640*
Nearest person month worked:	*1*
Contribution to Project:	*Overseeing project, research designing, troubleshooting, preparing animal protocol.*
Name:	*Yadav Adhikari*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*7.5*
Contribution to Project:	*Prepare mouse cranial window, in vivo activity imaging, mouse behavior, and data analysis*
Name:	*Allison Moore*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*7.5*
Contribution to Project:	*Prepare mouse cranial window, in vivo activity imaging, and data analysis*
Name:	*Wenhui Xiong*
Project Role:	*Assistant Scientist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*5*
Contribution to Project:	*Prepare TNI model, ACPC treatment, electrophysiological recording, mesoscopic imaging, and data analysis*
Name:	*Minghai Shao*
Project Role:	*Research technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*4*
Contribution to Project:	*Animal maintenance, genotyping, and mouse surgery.*

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

8. Special Reporting Requirements

Nothing to Report

9. Appendices

Nothing to Report