AWARD NUMBER: W81XWH-17-1-0268

TITLE: Investigating the role of creatine in oligodendrocyte regeneration during CNS remyelination

PRINCIPAL INVESTIGATOR: Jeffrey K. Huang

CONTRACTING ORGANIZATION: Georgetown University

REPORT DATE: July 2020

TYPE OF REPORT: Annual Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
July 2020	Annual	15Jun2019 - 14JUN2020

4. TITLE AND SUBTITLE

Investigating the role of creatine in oligodendrocyte regeneration during CNS remyelination

5a. CONTRACT NUMBER
W81XWH- 17-1-0268

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Jeffrey K. Huang

E-Mail: jh1659@georgetown.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Georgetown University Washington, DC 20057

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: Chronic oligodendrocyte and myelin loss contributes of axonal dysfunction and neurodegeneration in multiple sclerosis (MS). Although oligodendrocyte precursor cells (OPCs) are abundant in the CNS, and are able to regenerate myelin in the early stages of MS, it remains unknown why remyelination fails in the chronic stage of MS. One possibility, which remains to be investigated, is that regenerated oligodendrocytes, despite differentiating from OPCs, fail to survive in MS lesions. It is known that oligodendrocytes appear abnormal and die in MS lesions. Therefore strategies to enhance survival of newly regenerated oligodendrocytes in MS would improve their ability to remyelinate axons. We have found that creatine, a compound involved in cell survival and energetic metabolism, promotes oligodendrocyte survival in culture. When experimental demyelination was performed on mice lacking the expression of Gamt, the enzyme responsible for creatine synthesis, we found that most of the newly regenerated oligodendrocytes died instead of restoring myelin. Remarkably, when creatine was injected directly into the demyelinated tissue, we found that the number of regenerated oligodendrocytes and the extent of myelin labeling in lesions increased significantly. These unexpected and intriguing observations suggest that brain-synthesized creatine plays a crucial role in stimulating remyelination by enhancing regenerated oligodendrocyte survival. Therefore, the goal of this project is to investigate the protective and proregenerative effect of creatine on regenerated oligodendrocyte survival and remyelination in mice. To achieve our goals, we will examine a genetically modified mouse mutant that does not express Gamt in oligodendrocytes, and assess its ability to maintain survival of regenerated oligodendrocytes and myelin after demyelination. We will also determine if altered creatine levels and reduced regenerated oligodendrocyte survival occurs in aged mice, since advanced age has been suggested as a contributor to remyelination failure and disease progression in MS.

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**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	8	**19b. TELEPHONE NUMBER** *(include area code)*

Grant: W81XWH-17-1-0268
Title: Investigating the Role of Creatine in Oligodendrocyte Regeneration during CNS Remyelination
Principal Investigator: Jeffrey K. Huang
CDMRP 3rd year report

1. INTRODUCTION

The goal of this study is to test the hypothesis that rOL protection through creatine improves CNS remyelination. To this end, we will determine i) if creatine synthesis in rOLs is required for rOL survival and remyelination, ii) if diminished creatine levels and rOL integrity contribute to remyelination impairment associated with aging, and iii) if the systemic administration of creatine and cyclocreatine (a blood brain barrier permeable creatine analog) can improve CNS remyelination. We will also determine iv) what transcripts and pathways in rOLs are affected with creatine deficiency.

2. KEYWORDS:

Creatine, guanidinoacetate methyltransferase (GAMT), remyelination, oligodendrocytes, transgenic mice, experimental demyelination, aging.

3. ACCOMPLISHMENTS

The major goals of this reporting period was to examine creatine vs. cyclocreatine administration on mice (Specific Aim 2, Major Task 2). Our last reporting period of Oct 2019, and unfortunately our lab was shut down in March due to the pandemic, and remains closed still on the date of the 3rd year report deadline. Therefore, we report here about 4 months of work since the last reporting period. Major activities described below:

Specific Aim 2: Analysis of creatine biosynthesis and rOL viability in aged mice.

Major Task 2: Analyze effect of systemic creatine/cyclocreatine administration on rOL survival and remyelination in aged mice. In the prior year, have found that mice fed under normal diet exhibit creatine in the brain even in mice lacking GAMT expression. Since this observation, we have put all of our mice under creatine low/deficient diet to examine the effect of GAMT loss of function or systemic creatine administration on remyelination. Moreover, previously we have also found that mice lacking GAMT expression under creatine deficient diet displayed a significant reduction in oligodendrocytes in the corpus callosum. Therefore, in this reporting period, instead of performing lysolecithin induced demyelination, we performed cuprizone demyelination to induce demyelination in the mouse corpus callosum on mice under normal vs. creatine deficient diet (**Fig. 1A**). These mice are fed 0.2% cuprizone for 5 weeks to induce demyelination. To determine the effect of creatin/cyclocreatine on remyelination, mice after 5 weeks of cuprizone intoxication were fed with 1) normal diet, 2) creatine deficient diet, 3) 2% creatine, and 4) 2% cyclocreatine for 2 weeks during the period of myelin repair.

We have so far examined the effect of creatine deficient diet on remyelination compared to normal diet. We found that mice under creatine deficient diet lost significant weight compared to mice on normal diet at the end of the experiment (**Fig. 1B**). Moreover, mice under creatine deficient diet also displayed fewer oligodendrocyte lineage cells, including NKX2.2+ OPCs and CC1+ mature oligodendrocytes (**Fig. 1C-G**). We next compared the effect of 2% creatine diet with mice under creatine deficient diet, and found that creatine enriched diet resulted in greater density of mature oligodendrocytes compared to those under creatine deficient diet. The level of mature oligodendrocytes is comparable to that in mice under normal diet (**Fig. 2**). Our analysis of mice treated with cyclocreatine and creatine have been put on hold because of the mandatory lab shut down. We have the mouse tissues ready and will perform the immunostaining analysis and quantification when we can return to the lab. In addition, we plan to repeat this experiment on aged mice to determine if creatine/cyclocreatine affects remyelination efficiency.

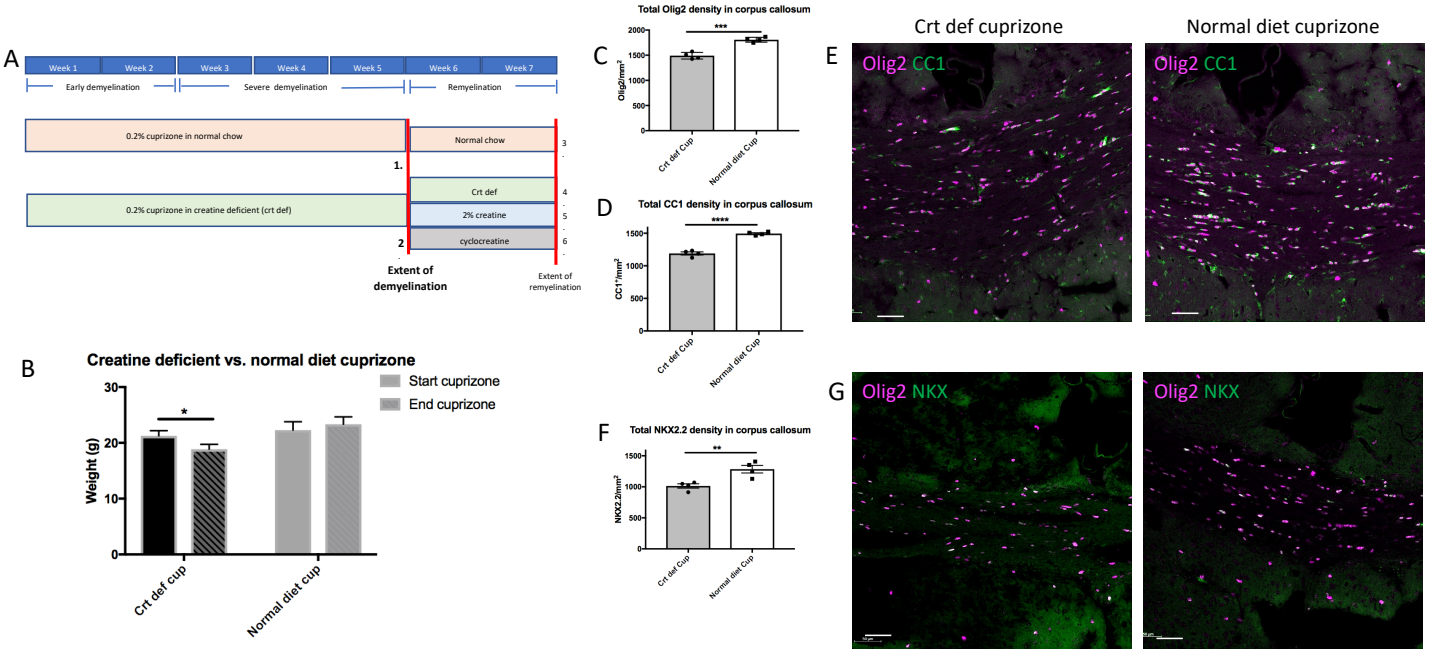

Fig 1. Creatine deficiency during cuprizone insult leads to more weight loss and significantly fewer mature oligodendrocytes (OL) and oligodendrocyte precursor cells (OPC) compared to normal diet cuprizone. A) Diagram of cuprizone treatment to understand the extend of demyelination under creatine deficiency (group 2.) compared to normal diet (group 1.). B) Creatine deficiency leads to significant weight loss over the course of 5 weeks of cuprizone treatment. C) Creatine deficiency cuprizone leads to significant reduction in total OL (top) and D) mature OL (bottom) with E) visualization of immunohistochemistry on right. F) Creatine deficiency cuprizone leads to significant reduction in OPC compared to normal diet cuprizone. G) Visualization of immunohistochemistry on right. N=4; Unpaired t-test; scale bar is 50 microns.

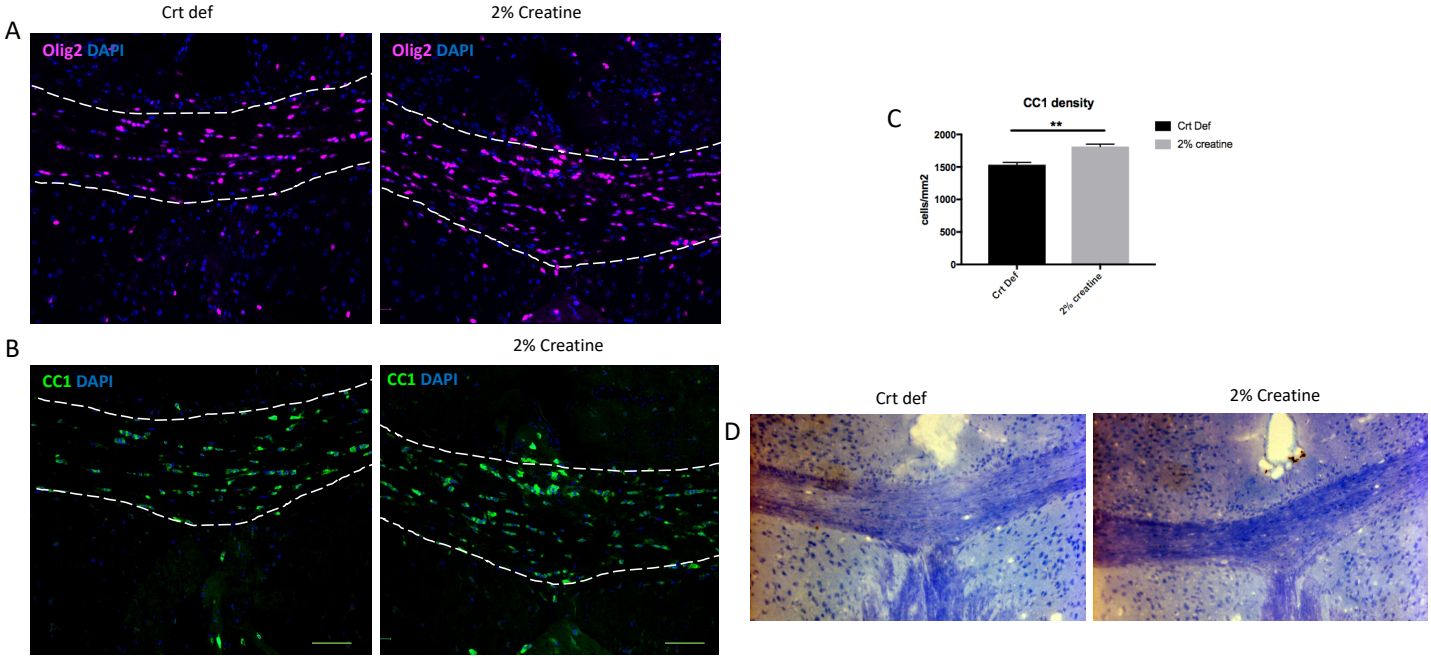

Fig 2. Two percent creatine in recovery diet following cuprizone leads to increased mature oligodendrocytes (OL) and enhanced myelination. A) Immunohistochemistry of increased total OL in animals treated with 2% creatine. B) Immunohistochemistry of increased mature OL in animals treated with 2% creatine. C) Quantification of significantly increased mature OL in 2% creatine diet. D) Luxol blue staining shows enhanced remyelination in 2% creatine treatment group. N=4; Unpaired t-test; scale bar is 50 microns.

4. IMPACT: Nothing to Report

5. CHANGES/PROBLEMS: We have decided to perform cuprizone demyelination in the corpus callosum instead of lysolecithin demyelination in the mouse spinal cord. This strategy would be more consistent with Other than this issue, this is no significant changes in the project or direction.

6. PRODUCTS: None to report

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Name:	*Lauren Rosko*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	12
Contribution to Project:	*Ms. Rosko has performed work the cuprizone experiment*

GOALS FOR EXTENSION PERIOD:

1. To complete analysis of cyclocreatine diet on mouse remyelination in young and aged mice.
2. Perform RNA-sequencing analysis of brain and demyelinated tissues of of Gamt-/- and WT mice (Aim 3).
3. Publication of our analysis of GAMT conditional knockout mice, and creatine and cyclocreatine administration in mice.