AWARD NUMBER: W81XWH-16-1-0658

TITLE: Omega-3 Polyunsaturated Fatty Acid Status, Microglial Activation, Stress Resilience, and Cognitive Performance

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CONTRACTING ORGANIZATION: Oregon Health & Science University

REPORT DATE: May 31st 2019

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
MAY 2019	Annual	1 May 2018 – 30 Apr 2019

4. TITLE AND SUBTITLE
Omega-3 Polyunsaturated Fatty Acid Status, Microglial Activation, Stress Resilience, and Cognitive Performance

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0658

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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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
It is widely reported across mammalian species that deficiency in the dietary intake of omega-3 polyunsaturated fatty acids (n-3 PUFA) negatively impacts cognitive performance and mood. A plethora of literature also implicates n-3 PUFA deficiency in disorders such as ADHD, PTSD, major depressive and bipolar disorders, and schizophrenia. Defining potential neuronal mechanisms that link n-3 PUFA levels to cognitive and behavioral deficits has important implications given that the trend of the modern diet has been toward reduced n-3 PUFA intake. Here, we propose human and rodent experiments to evaluate whether the anti-inflammation/pro-resolution effects of n-3 PUFA deficiency contribute to the adverse effects on cognitive performance and affect. In addition, these experiments focus on the expression of dietary n-3 PUFA deficiency in late adolescence/young adulthood—a developmentally critical period during which an individual is vulnerable to mood, psychotic and addictive disorders. We will use a positron emission tomography (PET) imaging strategy in humans as a marker of activated microglia in individuals with low and high plasma n-3 PUFA. In parallel animal studies, we will directly measure microglia activation in an animal model of n-3 PUFA deficiency and determine whether supplementation during early adulthood reverses this effect in correlation with behavior.

15. SUBJECT TERMS
Omega-3 fatty acids, microglia, brain inflammation

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	11	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

Background: Dietary deficiency in omega-3 polyunsaturated fatty acid (n-3 PUFA) is a common feature of the modern diet. Across mammalian species, deficiency in the intake of this essential fatty acid negatively impacts the ability to withstand stress and cognitive performance. Accordingly, recent studies in healthy civilian and military populations indicate a strong relationship between red blood cell (RBC) n-3 PUFA levels and a wide range of brain related problems including impaired cognitive performance, and increased anxiety, impulsivity and suicide. Precise brain mechanisms that underlie the behavioral detriments of n-3 PUFA deficiency and whether they can be reversed by supplementation are largely unknown. The overarching goal of this proposed work is to inform of us about specific brain mechanisms by which dietary n-3 PUFA deficiency and supplementation affects brain and behavior. The mechanistic focus will be on immune responses around neurons in brain regions that are critical for stress reactivity and cognitive performance.

Purpose (Aim2): To determine whether an animal model of n-3 PUFA deficiency is associated with brain microglia activation and whether supplementation during early adulthood reverses this effect in correlation with behavior. In an experimental animal model that mimics current western dietary n-3 PUFA deficiency, we have observed behavioral determents that suggest impaired cognitive performance and anxiety. We hypothesize that this dietary deficiency leads to an immunological insult in the brain and propose to use microglia activation as a method of quantification of this insult. Microglia are the residents of macrophage cells and are the first line of immune defense in the brain. Animals will undergo behavioral characterization before the post-mortem microglial measures. Upon establishing that there is microglia activation in brain regions of interest, we will test whether supplementation during early adulthood reverses this insult in correlation with behavior.

Scope: Establish that brain inflammation is a potential mechanism that underlies behavioral impairments in n-3 PUFA deficient diet, and quantify the impact of supplementation on reversing the inflammatory response and restoring the behavioral impairment. This has the potential to inform the clinical testing of oral and parenteral n-3 PUFA formulations as a treatment for the multitude of conditions where neuroinflammation is a focus, ranging from traumatic brain injury and multiple sclerosis to mood disorders and PTSD.

2. KEYWORDS

Omega-3 fatty acids, microglia, brain inflammation

3. ACCOMPLISHMENTS

What were the major goals of the project?

Major goals of the project (aim 2, animal study)

The major tasks listed in the approved SOW (6/2017) with listed milestones and target dates within the first 12 months are included below:

Major Task 1: Finalize and submit ACURO application

Major Task 2: Initiation and maintenance of colonies of first and second generation n-3 deficient animals (Timeline target date 2-30 months)

Major Task 3: behavioral testing in deficient animals before and after supplementation, and compared to adequate animals in the same age range. There are four subject groups in this Major Task: (1) animals on adequate diet, (2) animals on deficient diet that remain on that diet, (3) animals on deficient diet that shift to, and remain on, an adequate diet "long-term" beginning after weaning, (4) animals on deficient diet that shift to adequate diet "short-term," one week before behavior testing. Behavioral testing for target date 6-12 months included open field, elevated plus maze, and delayed alternation in two of the proposed 4 groups.

Major task 4: anti-Iba1 immunohistological staining to estimate microglial number and activation. Procedures include perfusion and tissue prep after termination of behavior testing, target date 3-26 months followed by histological assessment and analyese target date 24-36 months.

What was accomplished under these goals?

Major Task 1: ACURO approval in place. Millstone achieved

Major task 2: This task has been accomplished. We have successfully established first and second generation of n-3 PUFA deficient and adequate colonies of rats in the laboratory. These animals are being used for studies in major task 3 and 4. As part of this task, this year we also started a third colony where n-3 PUFA deficient animals (age- and litter-matched animals) are placed on a DHA+EPA supplemented diet to establish if supplementation is sufficient the impact of n-3 PUFA deficiency.

Major Task 3: This task is 50% complete. Behavioral testing for stress and anxiety has been completed using an automated quantification SMART video tracking system (Harvard Apparatus) which reduces potential subjective influence of manual rating by investigator for the three group of n-3 PUFA adequate "A", deficient "D", and deficient diet that shift to, and remain on, an adequate diet beginning after weaning "S" and the component of the analyzed data for the elevated plus maze (EPM) is shown on figure 1 below.

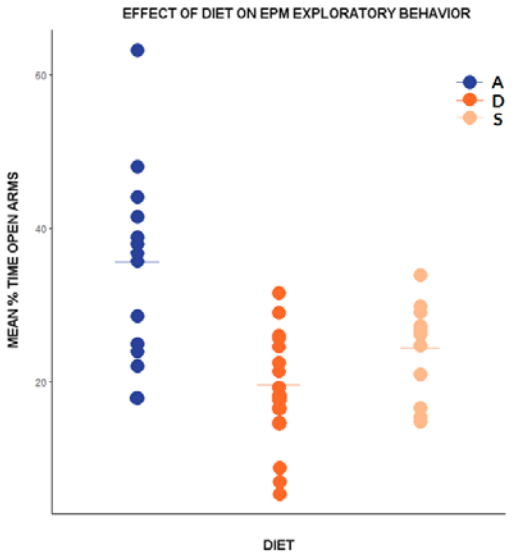


Figure 1. Effect of diet on a test of anxiety. The y axis shows percent of time spent in open sections of an elevated plus maze in animal on n-3 PUFA adequate (A) and deficient (D) and supplemented deficient (S) diets. Less time spent in an open arm is indicative of increased fear and anxiety. We find that deficient diet increases anxiety that was only partially reversed by supplementation ($p=0.214$), while adequate diet animals spent significantly more time in open arms than deficient diet animals ($p<0.000$).

The data in figure 1 combines both male and female subjects. As the study is progressing and we are collecting data from higher subjects, we have begun to have the statistical power to analyze the impact of diet on different sexes.

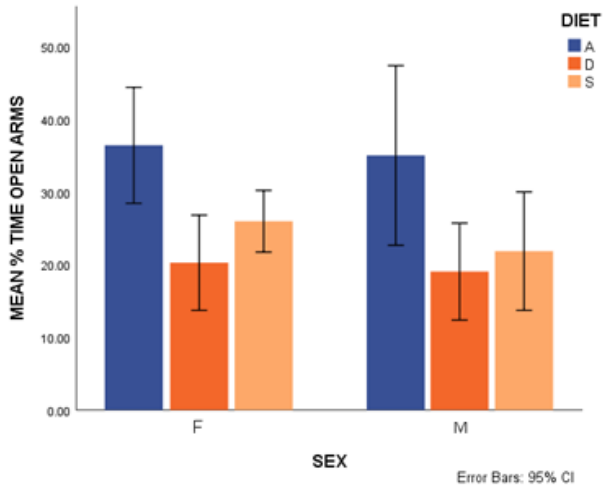


Figure 2. Interaction of diet and sex on anxiety. Data shown on figure 1 is divided by sex. The impact of n3 PUFA deficiency and supplementation is comparable in both sexes.

Major task 3:

This task is ongoing and tissue being successfully processed. As described in last years' progress, we shifted the method used for microglia analysis from immunopreoxidase staining and manual processing to fluorescent immunohistochemistry for automated microglia quantification and morphometry analysis. This method allows us to quantify microglia activation as shown in figure (below).

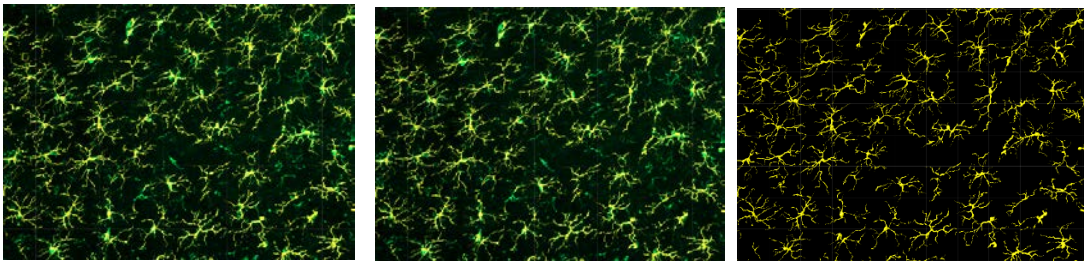
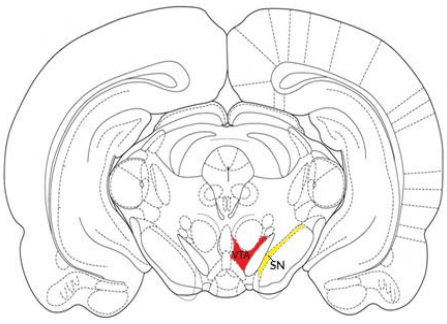


Figure 2: Immunofluorescent images of microglia (green) and the automated 3D model of dendrite morphology (yellow) generated using IMARIS Filament Tracer software.

To measure microglia activation in dopamine contain regions of ventral tegmental area (VTA) and substania nigra (SN), we used a Chicken-anti-TH primary (Abcam, ab76442) combined with Goat-anti-chicken IgY H&L Alexa Fluor 594 (Abcam, ab150176) to allows visualization of TH-containing neurons on another red channel. Dissected regions of VTA (red) and SN (yellow) are depicted on the right. Cell nuclei are also visualized on the blue channel with a DAPI stain (Vector Laboratories, H-1200). Staining cell nuclei allows quantification of all cells in an image, as well as determination of the percent of cells in a region that express Iba1 or TH.



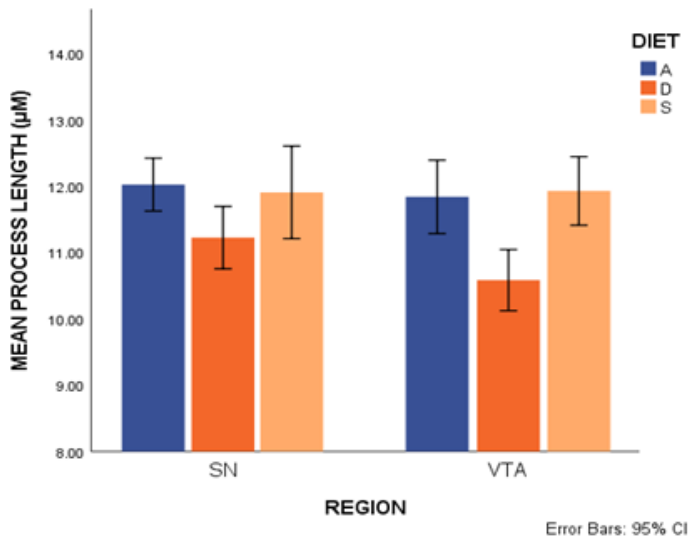


Figure 4. Effect of diet on microglia activation in dopamine containing regions. The y axis shows mean length of microglia processes animal on n-3 PUFA adequate (A) and deficient (D) and supplemented deficient (S). Data was obtained from SN and VTA. Processes in VTA were significantly shorter in deficient animals compared to adequate animals (p=0.001) and supplemented animals (p=0.001).

The analyzed data so far indicates that n3 PUFA deficiency significantly affects microglia activation in some dopamine containing regions, and that some these effects may be ameliorated by supplementation. Data connection is continuing to assess microglia activation in other brain regions.

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

Postdoctoral training of personnel involved in the project

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. Continue Major Task 2 to provide sufficient subjects complete behavioral data collection for deficient and adequate diet cohorts

2. Complete Major Task 3

3. Complete Major Task 4

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

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

None. The changes we made last year to automate behavioral data collection and quantification of microglia activation in specific brain regions were successful. We are rapidly collecting data and do not see any obstacles in completing the proposed work in the next 12 month.

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

Nothing to report

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

Nothing to report

Significant changes in use or care of vertebrate animals.

Not applicable

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Nothing to report

  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**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Bita Moghaddam, PhD
Project Role: Partnering PI
Nearest person month worked: 1 calendar month
Contribution to Project: Dr. Moghaddam supervised the project, including completion of protocols, overseeing all aspects of animal testing and data analysis.

Tara Chowdhury, PhD
Project Role: Postdoctoral Researcher
Nearest person month worked: 1 calendar months
Contribution to Project: Dr. Chowhury performed the behavioral testing and initial stages of microglia measures. As procedure became automated, her role was replaced by Nicole Kahn

Kathryn Wallin-Miller, PhD
Project Role: Postdoctoral Researcher
Nearest person month worked: 6 calendar months
Contribution to Project: Dr. Wallin-Miller has established the new method of microglia assessment and was responsible for post-processing analysis of the tissue for Major Task 3.

Nicole Kahn
Project Role: Research Assistant
Nearest person month worked: 6 calendar months
Contribution to Project: Ms Kahn was responsible for all breeding (Task 2) and assisting with behavior testing and analysis (Tasks 3)

Madeleine Allen
Project Role: Research Assistant
Nearest person month worked: 6 calendar months

Contribution to Project: Ms. Allen was responsible for initial stages of tissue processing.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No changes in senior/key personnel

Changes in active support for PI Bita Moghaddam:

NEW

None

Ended (closed)

R56 MH084906 - 06A1 (Moghaddam) 07/01/17 – 06/31/19 (NCE) 2.76 calendar
NIMH/NIH
"Inhibitory Control of Prefrontal Cortex"
Anxiety is a debilitating symptom of most psychiatric disorders including PTSD, major depression, and addiction. The proposed studies aim at understanding the neuronal basis of anxiety and its impact on goal-directed behavior.
Role: PI

OVERLAP

There is no overlap.

Other organizations involved as partners:

University of Pittsburgh
Pittsburgh, Pennsylvania
Partner's Contribution to the project:
Collaboration

This award involved a Partnering Award at the University of Pittsburgh, Partnering PI: Dr. Rajesh Narendran. Dr. Narendran will submit an independent progress report per the instructions for collaborative awards. There are no additional organizations involved as partners.

8. SPECIAL REPORTING REQUIREMENTS

Partnering PI report will be filed separately for Aim 1 (human study) by Dr. Rajesh Narendran, University of Pittsburgh, Pittsburgh, PA

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.
N/A

9. APPENDICES:

Nothing to report