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TITLE: Ghrelin Signaling Regulates Microbiome-Gut-Brain Axis in Inflammatory Bowel Disease and Post-Traumatic Stress Disorder

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CONTRACTING ORGANIZATION: Texas A&M AgriLife Research, College Station, TX

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14. ABSTRACT

Gut microbiota is a critical regulator of host's metabolism, immune system and cognitive function. However, the microbiome-gut-brain axis has not been systematically studied in Inflammatory Bowel Disease (**IBD**), much less in Post-traumatic stress disorder (**PTSD**). A novel experimental "2-hit" model (**IBD-PTSD**) is established, where mice are subjected to dextran sulfate sodium (**DSS**)-induced ulcerative colitis and then conditioned fear (**CF**) memory test, to study the role of microbiome-gut-brain axis in these inflammatory pathologies. In this research period, we found that DSS-induced colitis lead to contextual memory deficit in female mice but not in male mice, even when colitis-associated disease symptoms such as diarrhea and rectal bleeding have subsided. The *in vivo* data suggest that hippocampus-dependent memory function was negatively impacted by an active episode of colitis, while the amygdala-dependent fear recall was relatively unaffected. Whether colitis-exposed mice show increased stress response will be further addressed in the following funding period. Downstream molecular, biochemical and microbiome analyses are currently underway to corroborate the *in vivo* data.

15. SUBJECT TERMS
Inflammatory Bowel Disease (IBD); Posttraumatic stress disorder (PTSD); Ulcerative colitis; Gut microbiome, Ghrelin

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1. INTRODUCTION:

Clinical and pre-clinical data pin-point inflammation as the main disease condition underlying inflammatory bowel disease (IBD) and Post-traumatic stress disorder (PTSD), which predisposes the subject to further inflammatory pathologies. The central hypothesis of the current research is that gut microbiota dysbiosis is the unifying factor contributing to pro-inflammatory pathologies underlying IBD and PTSD. Hence, agents that promote rebalancing of the microbiome could ameliorate disease symptoms. A novel experimental model of IBD (dextran sulfate sodium-induced ulcerative colitis) followed by Pavlovian fear conditioning and fear recall testing (referred to as IBD-PTSD paradigm) will be established and used test the hypothesis as well as the therapeutic potential of the gut hormone ghrelin.

2. KEYWORDS:

Inflammatory Bowel Disease (IBD); Posttraumatic stress disorder (PTSD); Gut microbiome, Ulcerative colitis; Ghrelin.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major goals:

- **Aim 1.** Define the dynamic and temporal changes in microbiota composition, metabolomics, inflammation and fear memory in experimental IBD-PTSD. (**Year 1**)
- **Aim 2.** Evaluate the ability of ghrelin to ameliorate experimental IBD-PTSD. (**Year 2**)

Updated Statement of Work for Year 1 (updates in the "Progress" column).

Specific Aim 1: *Define the dynamic and temporal changes in microbiota composition, metabolomics, inflammation and fear memory in experimental IBD-PTSD*	**Proposed Timeline**	**Progress**	**Site 1**
Major Task 1: *in vivo* experiment 1	Months	Calendar	
Local IRB/IACUC Approval	1-2	Approved on 01/17/2020	
ACURO Approval	2-3	Approved on 03/18/2020	
Subtask 1: Breeding of C57BL/6 mice for experimental group	3-4	Jun-Jul	Dr. Wu (n=40)
Subtask 2: Switch to open source diets and monitor body weight and body composition until mice reach 12 weeks of age.	4-6	Jul-Sep	Dr. Wu
Subtask 3: Perform animal experiment DSS-fear conditioning, sample collection	7-8	Oct-Dec	Dr. Wu
Milestone(s) Achieved: completion of sample and data collection from *in vivo* experiment 1.	8	Dec	

Major Task 2: molecular and biochemical characterization			
Subtask 1: fecal microbiome characterization	9-12	currently underway	Dr. Wu and genomics core facility
Subtask 2: fecal metabolome characterization	9-12	currently underway	Dr. Wu and metabolomics core facility
Subtask 3: multiplex analysis of serum cytokines and gut hormones, plasma acyl-ghrelin levels	9-12	currently underway	Dr. Wu
Subtask 4: Molecular characterization of colons: histology, qPCR	9-12	currently underway	Dr. Wu
Subtask 5: Molecular characterization of brain: histology, qPCR	9-12	currently underway	Dr. Wu
Milestone(s) Achieved: completion of sample processing and molecular characterization work. Completion of data analysis.	12	delayed	

What was accomplished under these goals?

In this reporting period (03/01/2020-02/28/2021), research work has been significantly delayed and disrupted because of the COVID-19 pandemic. The research activities had been limited to essential only (e.g. animal welfare) in ~March-May 2020; there were no new breeding or colony expansion allowed, and no new orders of reagents unless they are for maintaining essential activities (e.g. animal diets). In addition, no research personnel had been hired, the PI had performed most of the research activities, with assistance from undergraduate students under the PI's direction.

The following research activities had been carried out after "shelter-in-place" were lifted and research activities were resumed (observing social distancing and safety guidelines):

- Completed a cohort of *in vivo* experiment to establish the IBD-PTSD model.
    - Preliminary data showed that 9 days were not sufficient for complete recovery from an active episode of DSS-induced colitis. Although both male and female mice had recovered from diarrhea (fecal consistency score < 3) and rectal bleeding (bleeding score <2) from Day 10 onwards, there were still mild symptoms present, including soft and darkened stool, and reduced body weight (Appendices, **Figure 1**).

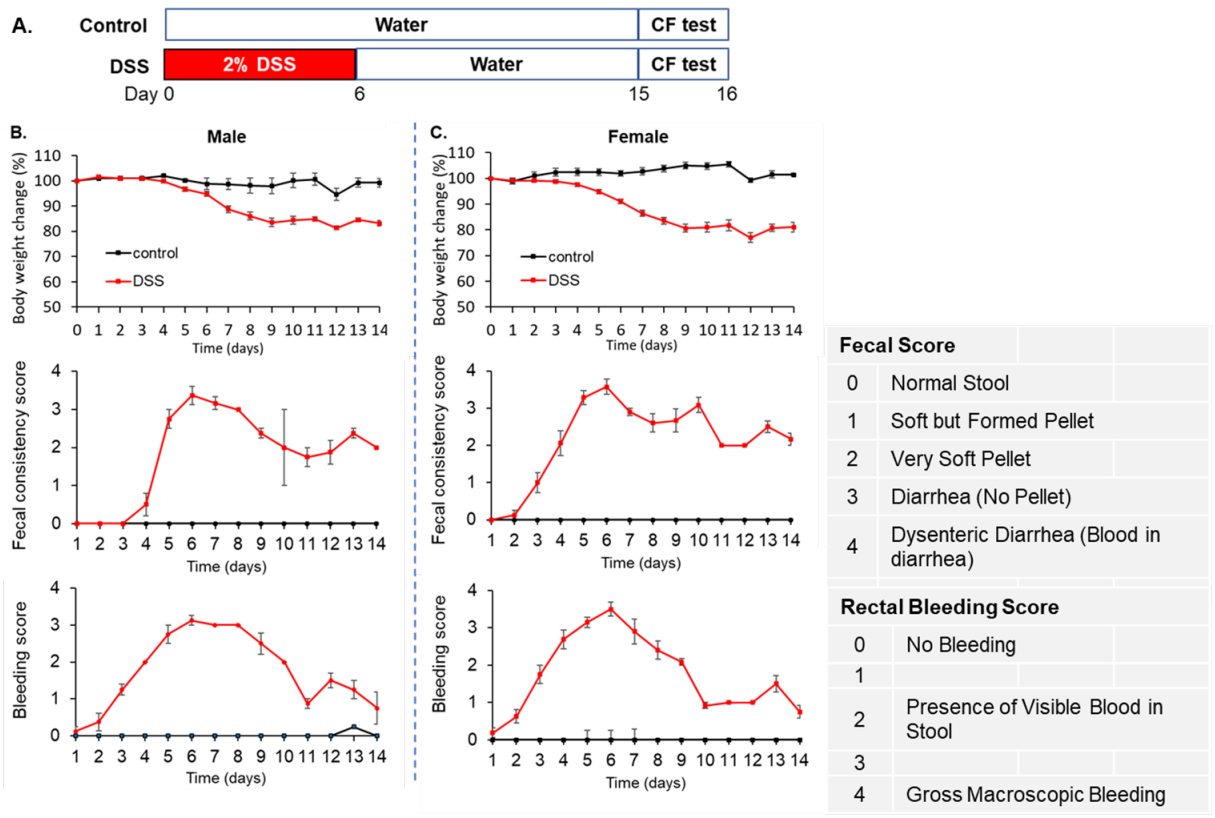


Figure 1. Pilot experimental IBD-PTSD model in C57BL/6 mice. (**A**) Schematic diagram of experimental IBD-PTSD model. Mice were given normal (control) or 2% DSS in drinking water for 6 days (Day 0-6), then switched to normal drinking water and allowed to recover, mimicking clinical remission. On Day 15, mice were subjected to auditory fear conditioning, and on Day 16, context and auditory fear recall tests were performed. Disease activities, including body weight, fecal consistency and rectal pathologies were monitored everyday; data from male mice are shown in (**B**) and female mice in (**C**).

- Preliminary data showed that in the remission period after an active episode of colitis, hippocampus-dependent contextual fear memory was significantly decreased in the colitis-exposed female mice (**Figure 2C**, top panel) but not in male mice (**Figure 2B**, top panel). On the other hand, auditory fear memory (amygdala-dependent) were not significantly different between control and DSS-exposed mice, in both male and female mice (**Figure 2B & 2C**, bottom panels). These data suggest that a prior episode of gut inflammation did not impact the amygdala-dependent fear memory function, but affected hippocampus-dependent memory function in female mice. Downstream molecular analyses are underway to assess neuroinflammation markers and cytokine expression levels in these brain regions.
- Fecal, serum and tissue samples have been collected. Serum corticosterone levels will be measured as indicators of stress response. Other subtasks for Major Task 2 are currently underway (See updated Statement of Work, “Progress” column).

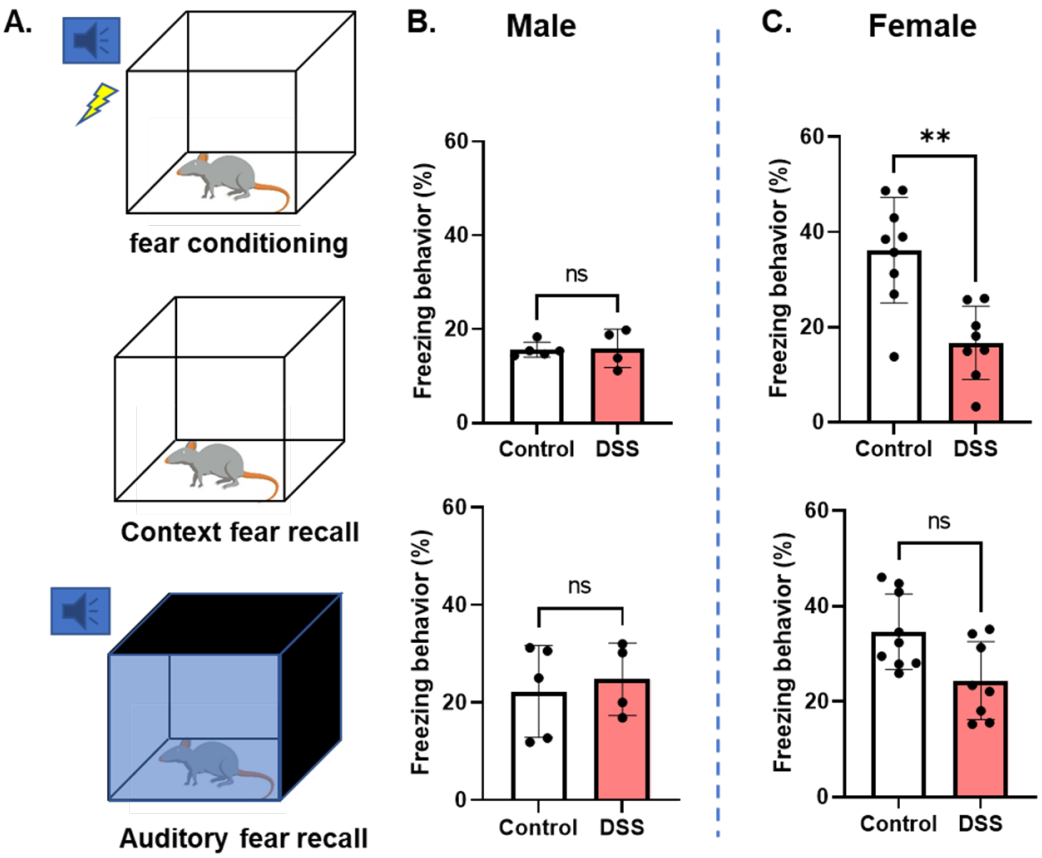


Figure 2. Female mice showed significant decrease in contextual memory after an active episode of colitis. (**A**) Schematic diagram of conditioned fear memory test. After an active episode of colitis, on Day 15, mice were subjected to auditory fear conditioning, and on Day 16, context and auditory fear recall tests were performed. Data from male mice are shown in (**B**) and female mice in (**C**).

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?

The *in vivo* data suggest that cognitive function was negatively impacted by an active episode of colitis; whether colitis-exposed mice show increased stress response will be further addressed in the following reporting period. Downstream molecular, biochemical and microbiome analyses are currently underway to corroborate the *in vivo* data. Working relationships with the core facilities at Texas A&M University, including the Molecular Genomics Core and the Integrated Metabolomics Analysis Core have been established, to ensure successful completion of the project.

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:

Experimental timeline is delayed, research plan as laid out in the original statement of work will likely extend 12 months to Feb-2023, due to the COVID-19 pandemic.

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

Due to the COVID-19 pandemic, research work has been disrupted and delayed. These delays have significantly impacted on the timeline of the animal experiments and subsequent downstream analyses.

Changes that had a significant impact on expenditures

Due to the COVID-19 pandemic, research work has been disrupted and delayed. These delays have significantly impacted on the timeline of the animal experiments and subsequent downstream analyses, as well as hiring staff. Consequently, these delays have resulted in more than 25% unobligated balance to be carried over to the next reporting period.

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**

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Chia Shan Wu*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*ORCID ID: 0000-0002-6034-939X*
Nearest person month worked:	*5*
Contribution to Project:	*Dr. Wu has performed all work described in this reporting period.*
Funding Support:	*Dr. Wu is also supported by AG061726A (R21), National Institute on Aging.*

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

COLLABORATIVE AWARDS:

QUAD CHARTS:

attachments.

9. APPENDICES:

Quad chart and award chart are attached to the report.

W81XWH-20-1-0127: Ghrelin Signaling Regulates Microbiome-Gut-Brain Axis in Inflammatory Bowel Disease and Post-Traumatic Stress Disorder

PI: Chia Shan Wu, Texas A&M AgriLife Research, Texas

Budget: $301,528.00

Topic Area: Inflammatory Bowel Diseases

Mechanism: W81XWH-19-PRMRP-DA

Research Area(s): SCS Coding

Award Status: Period of Performance
01 March 2020 – 28 February 2022

Study Goals:

Accumulating evidence indicate that gut microbiota dysbiosis promote systemic inflammation, which may be the unifying factor contributing to the inflammatory pathologies in inflammatory bowel disease (IBD), which in turn induces neuroinflammation and cognitive dysfunction. In addition, meta-analysis of clinical data suggests that patients with posttraumatic stress disorder (PTSD) exhibit an increased state of inflammation and are at greater risk of developing IBD; however, whether IBD patients are at greater risk of developing PTSD, and whether gut microbiota dysbiosis associated with IBD contribute to the inflammatory pathology in PTSD remain undetermined. Ghrelin, a 28 a.a. peptide hormone produced mainly by the stomach and gut, has been suggested to exert anti-inflammatory effects. Consistently, ghrelin deficiency leads to increased susceptibility to diet-induced adipose tissue inflammation, exacerbated fasting-induced muscle atrophy, and a pro-inflammatory shift in gut microbiota composition. The central hypothesis is that ghrelin contributes to gut barrier function and promotes microbiota symbiosis, thereby protecting the animal to inflammatory pathologies associated with IBD and subsequent PTSD. A novel experimental model of IBD (dextran sulfate sodium-induced ulcerative colitis) followed by Pavlovian fear conditioning and fear recall testing (referred to as IBD-PTSD paradigm) will be established and used test the hypothesis.

Specific Aims:

- Aim 1. Define the dynamic and temporal changes in microbiota composition, metabolomics, inflammation and fear memory in experimental IBD-PTSD.
- Aim 2. Evaluate the ability of ghrelin to ameliorate experimental IBD-PTSD.

Key Accomplishments and Outcomes:

Publications: none to date

Patents: none to date

Funding Obtained: none to date

Ghrelin Signaling Regulates Microbiome-Gut-Brain Axis in Inflammatory Bowel Disease and Post-Traumatic Stress Disorder
PR192467
W81XWH2010127

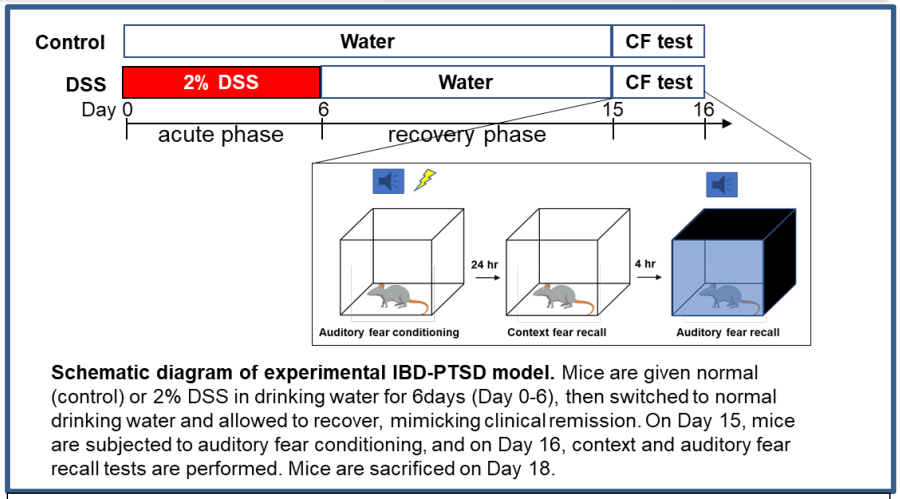
PI: Chia Shan Wu **Org:** Texas A&M AgriLife Research **Award Amount:** $301,528.00

Study/Product Aim(s)

- Aim 1. Define the dynamic and temporal changes in microbiota composition, metabolomics, inflammation and fear memory in experimental IBD-PTSD.
- Aim 2. Evaluate the ability of ghrelin to ameliorate experimental IBD-PTSD.

Approach

Gut microbiota is a critical regulator of host's metabolism, immune system and cognitive function. However, the microbiome-gut-brain axis has not been systematically studied in Inflammatory Bowel Disease (**IBD**), much less in Post-traumatic stress disorder (**PTSD**). A novel experimental "2-hit" model (**IBD-PTSD**) will be established, where mice are subjected to dextran sulfate sodium (**DSS**)-induced ulcerative colitis and then conditioned fear (**CF**) memory test, to study the role of microbiome-gut-brain axis in these inflammatory pathologies.



Schematic diagram of experimental IBD-PTSD model. Mice are given normal (control) or 2% DSS in drinking water for 6days (Day 0-6), then switched to normal drinking water and allowed to recover, mimicking clinical remission. On Day 15, mice are subjected to auditory fear conditioning, and on Day 16, context and auditory fear recall tests are performed. Mice are sacrificed on Day 18.

Accomplishment: DSS-induced colitis led to contextual memory deficit in female mice but not in male mice, even when colitis-associated disease symptoms such as diarrhea and rectal bleeding have subsided.

Timeline and Cost

Activities CY	20	21	22	23
In vivo IBD-PTSD model (Aim 1)				
Molecular analyses (Aim 1)				
Ghrelin treatment (Aim 2)				
Molecular analyses (Aim 2)				
Estimated Budget ($K)	**$147K**	**$154K**	**$000**	**$000**

Updated: 03/17/2021

Goals/Milestones

CY20 Goal – IBD-PTSD model
☑ In vivo DSS-induced colitis followed by conditioned fear tests.
☐ Molecular analyses: microbiome, metabolome, inflammation.
Delayed due to COVID-19.

CY21 Goals – Test therapeutic potential of ghrelin
☐ In vivo evaluation of ghrelin's effects in IBD-PTSD.
☐ Molecular analyses: microbiome, metabolome, inflammation.

CY22 Goal – Completion of molecular analyses
☐ Molecular analyses: microbiome, metabolome, inflammation.

Comments/Challenges/Issues/Concerns

- Timeline is delayed, experimental plan will likely extend 12 months to Feb-2023.
- Spending is off by more than one quarter, due to COVID-19 pandemic.

Budget Expenditure to Date
Projected Expenditure: $147,260.
Actual Expenditure: $63,812.