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TITLE: Pyridostigmine Bromide, the Enteric Nervous System, and Functional Gastrointestinal Disorders in Gulf War Illness

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14. ABSTRACT Gulf War illness (GWI) is a chronic, multi-symptom disorder with no treatment. Exposure to anti-cholinergic drugs such as pyridostigmine bromide (PB) contributed to the development of GWI, but the mechanisms that connect the acute effects of PB with chronic dysfunction in multiple systems remain unclear. Gastrointestinal problems are frequent and debilitating chronic symptoms experienced by Gulf War veterans. The <u>overall objective</u> of this proposal is to understand how PB contributes to the development of functional gastrointestinal disorders in Gulf War illness. Given that the enteric nervous system (ENS) regulates gut functions, <u>we hypothesize that</u> that PB disrupts gut functions by creating persistent neuroinflammation within the ENS. The major activities in this reporting period include <i>in vivo</i> and <i>in vitro</i> studies to understand the acute effects of PB on the ENS, the neural control of gut functions, and the inflammatory response within the gut. Key outcomes from this reporting period include observations showing that exposure to PB creates acute and chronic changes to gut functions that include increased fecal pellet output, higher fecal fluid content, slower colonic transit, altered neuromuscular control, defective intestinal barrier function, and neurodegeneration in the ENS. Our results show that the acute exposure to PB significantly alters the anatomy and functions of the ENS. We propose that these changes contribute to the pathophysiology of GWI.					
15. SUBJECT TERMS Gulf War Illness, Pyridostigmine Bromide, Neuroinflammation, Gastrointestinal, Gut, Intestine, Enteric Nervous System, Autonomic					
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1. INTRODUCTION:

Gulf War Illness (GWI) is a chronic disorder characterized by a spectrum of six symptoms that include fatigue/sleep, pain, neurological/cognitive/mood, gastrointestinal (GI), respiratory and skin problems. Gut problems are over three times more common in Gulf War veterans and are a major source of low quality of life and poor health. Exposure to the anti-nerve gas drug pyridostigmine bromide (PB) is clearly linked with the development of GWI, but the exact mechanisms still remain unclear. The overall objective of this proposal is to understand how PB contributes to the development of functional gastrointestinal disorders in GWI. Our central hypothesis is that PB disrupts gut functions by creating inflammation within the branch of the nervous system that coordinates gut functions. Specifically, we hypothesize that persistent neuroinflammation is caused by chronic reactive gliosis. This study has two Specific Aims that link *in vitro* mechanistic studies with *in vivo* studies in mice to study how PB alters the integrative physiology of the enteric nervous system. Specific Aim 1 tests the hypothesis that reactive enteric gliosis driven by an acute exposure to PB causes enteric neurodegeneration and long-lasting abnormalities in gut function. Specific Aim 2 tests the hypothesis that decreasing reactive enteric gliosis with the anti-inflammatory drug, palmitoylethanolamide improves gut dysfunction driven by PB. Alterations in GI physiology influence multiple systems and directly impact behavior, metabolism and immunity. Thus, the importance of understanding the pathogenesis of persistent GI disturbances in Gulf War illness is two-fold: i) For improving the treatment of GI-specific problems and ii) For treating the broad, systemic nature of the illness as a whole.

2. **KEYWORDS:** enteric glia, enteric nervous system, gulf war illness, gastrointestinal disorders, pyridostigmine bromide, inflammation, intestinal barrier, gut, intestine, autonomic, peripheral nervous system

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

Major Task 1: Characterization of neuroinflammation caused by PB in the gut and brain.

Milestone(s) Achieved: Precise timeline of neuroinflammation caused by PB in the gut and brain and specific understanding of how PB affects the neural and glial systems that regulate gut functions.

- Overall, we have completed approximately 50% of this major task and our studies are progressing well. Specific details regarding the completion of subtasks within this major task are detailed below in “accomplishments”. We originally planned on completing this phase of the project at month 7, but additional troubleshooting with the animal model was required before we were able to begin experiments (see details below in section 5: “Changes/Problems”)

Major Task 2: Mechanistic study to define the role of glia in PB-induced neuroinflammation and gut dysfunction.

Milestone(s) Achieved: Understanding of how glial cells contribute to neuroinflammation and gut dysfunction caused by PB. Publication of at least one peer-reviewed paper.

- We are currently completing the studies from Major Task 1 and compiling those results in preparation for publication. We plan on beginning, and completing studies under Major Task 2 in the coming year.

Major Task 3: Effects of reversing gliosis with PEA on gut function and neuroinflammation.

Milestone(s) Achieved: Evaluation of a potential therapeutic approach to reduce systemic neuroinflammation and restore healthy gut function in a mouse model of GWI. Publication of results in at least one peer-reviewed paper.

- These studies have not yet begun, but we plan to begin, them within the next funding period.

- **What was accomplished under these goals?**

- 1) **Major Activities:** The major activities in this reporting period include *in vivo* and *in vitro* studies to understand the acute effects of PB on the enteric nervous system, the neural control of gut functions, and

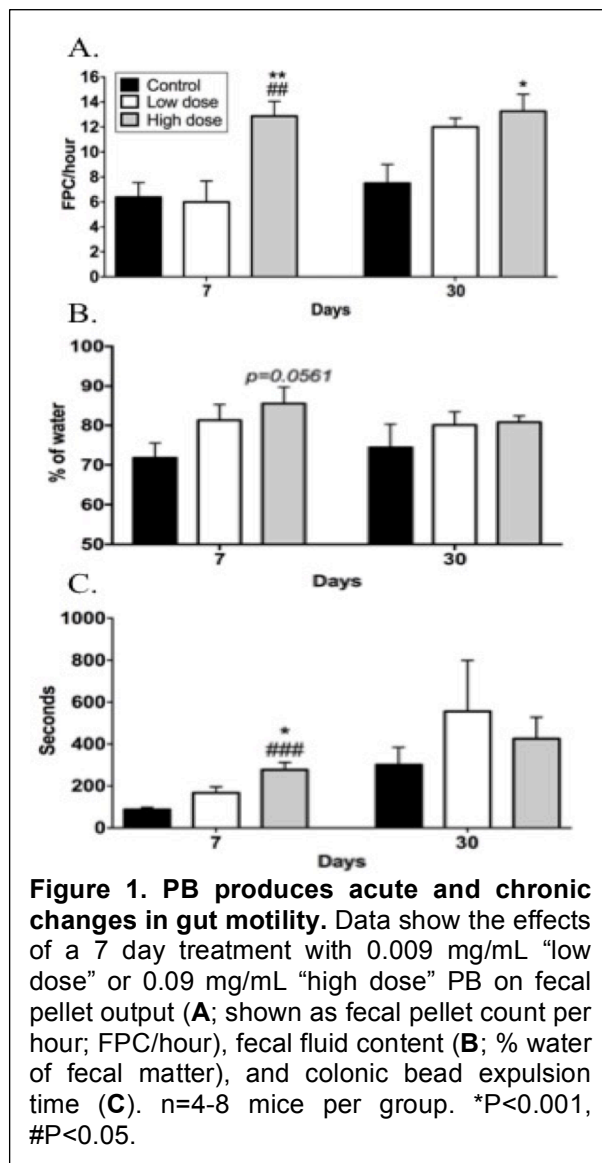
the inflammatory response within the gut and brain. Additionally, major activities in this period included essential preliminary studies to refine the animal model and drug-dosing regime.

- 2) **Specific Objectives:** Specific objectives in this reporting period were to: *i)* Assess the effects of PB on gut motility and intestinal disease *in vivo*, *ii)* Determine how exposure to PB impacts the neural control of gut functions with *ex vivo* assays, *iii)* Use functional imaging of glia and neurons in live tissue from mice exposed to PB to understand how PB affects cellular activity, *iv)* Determine the time course of neuroinflammation driven by PB in the gut and the brain.

3) **Significant Results/Key Outcomes:**

- i)* We made good progress towards the completion of our goals during the first funding period. Our first goal was to assess potential acute and chronic effects of PB on gut motility. To this end, we assessed gut motility in mice immediately following a 7-day PB treatment that models exposure to the drug in the Gulf War, and at several weeks following the drug treatment (30 days). However, prior to conducting these experiments, we undertook essential preliminary experiments to refine the dose and administration route of the drug PB as suggested by both institutional and ACURO reviews of our animal protocols. Oral administration of the drug is the most relevant route, but it was not clear if oral gavage or administration via drinking water was more appropriate. We did not observe significantly different effects between the two administration routes and did not observe avoidance when we administered the drug in drinking water (data not shown) so we decided to proceed with dosing via drinking water to avoid any possible complications with gavage. Likewise, institutional and ACURO animal care committees recommended testing two doses of the drug PB; one calculated based on body weight and one based on body surface area, which may be a more accurate way to extrapolate human dosing to animal models. Therefore, we proceeded with our study using two clinically relevant doses of PB. The first, 0.009 mg/mL PB in drinking water ("low dose"), is based on body weight measurements and the second, 0.09 mg/mL PB in drinking water ("high dose"), is based on body surface area measurements.

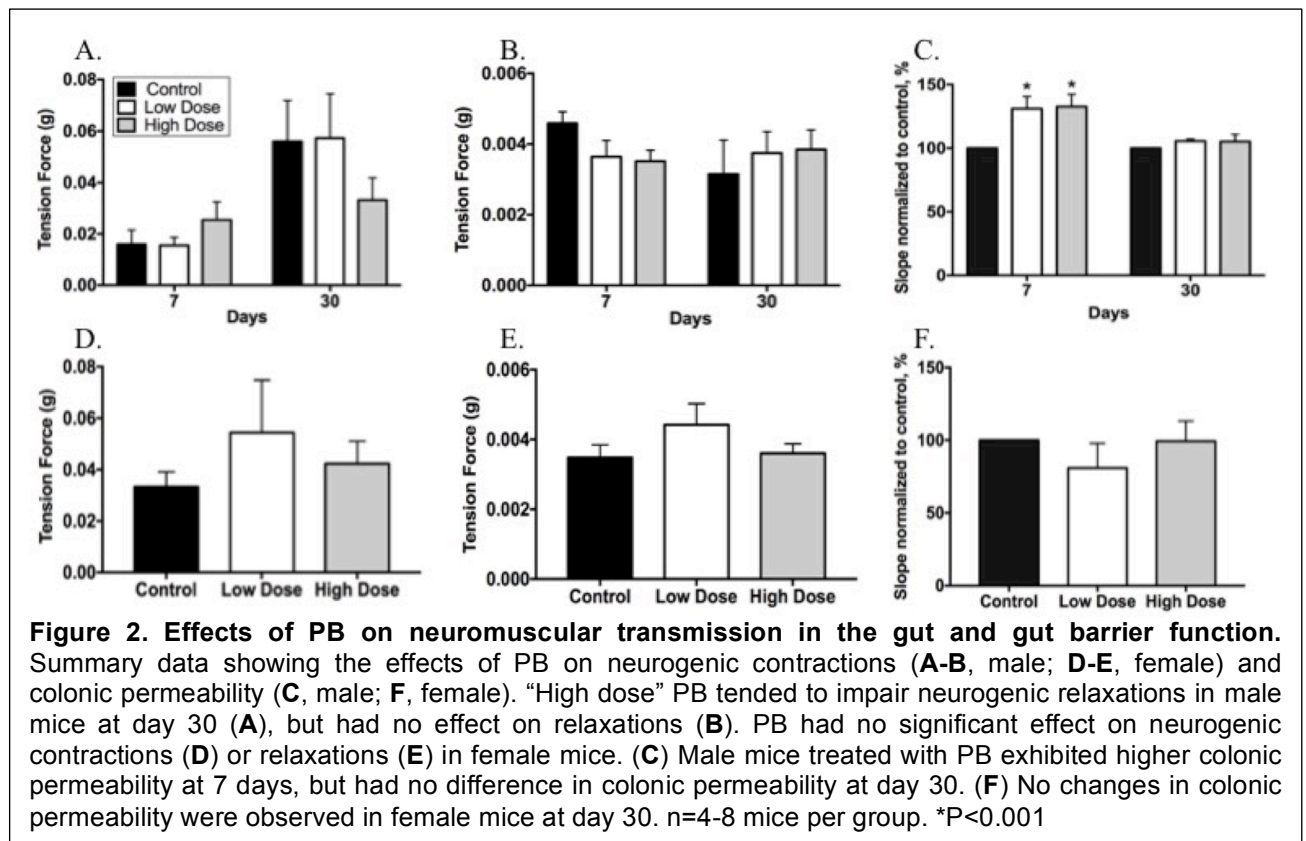
Our motility measurements show that PB significantly affects gut motility during the drug treatment period and that these changes persist for several weeks following exposure to the drug. We began our experiments by assessing key measurements of gut motility *in vivo* that included assessing the fecal pellet output, the water content of their fecal matter, and colonic motility by assessing the time taken to expel a small glass bead inserted into the colon. We performed fecal pellet counts by measuring the number of pellets produced per hour, and collected measurements on the first day of treatment and then every three days afterward until tissue was harvested on day 7 or 30 for further analysis. Our results show that male mice exposed to 0.09 mg/mL PB exhibit increased fecal pellet output on the last day of PB treatment (High dose day 7: 102% day 7 control, 114% day 7 low dose, $p < 0.01$; High-PB day 30: 77% day 30 control, $p < 0.05$) (**Figure 1A**) and that these mice continue to exhibit increased fecal



pellet output at day 30, 23 days following exposure to the drug (**Figure 1A**). Calculating the fluid content of fecal matter revealed exposure to the 0.09 mg/mL “high dose” increased fecal fluid content after the 7-day drug treatment (19%, $p=0.0561$) (**Figure 1B**), but that this was a transient effect that was not observed at 30 days. Specifically assessing colonic motility by measuring the time required to expel a small glass bead inserted into the colon (colon bead expulsion assay) showed that mice exposed to the “high dose” PB exhibited decreased colonic motility acutely (High dose day 7: 218% longer than day 7 control, $p<0.001$, and 66% longer than day 7 low dose, $p<0.05$), but we did not observe significantly different differences between groups at the 30 day time point (**Figure 1C**). Although the data shown above are from male mice, we also conducted parallel studies in female mice. Interestingly, we did not observe significant effects of PB on our measures of gut motility in female mice (data not show). Together, these results indicate that exposure to PB in the route, concentration, and timeframe that troops were exposed to during the Gulf War creates acute and chronic changes to gut functions.

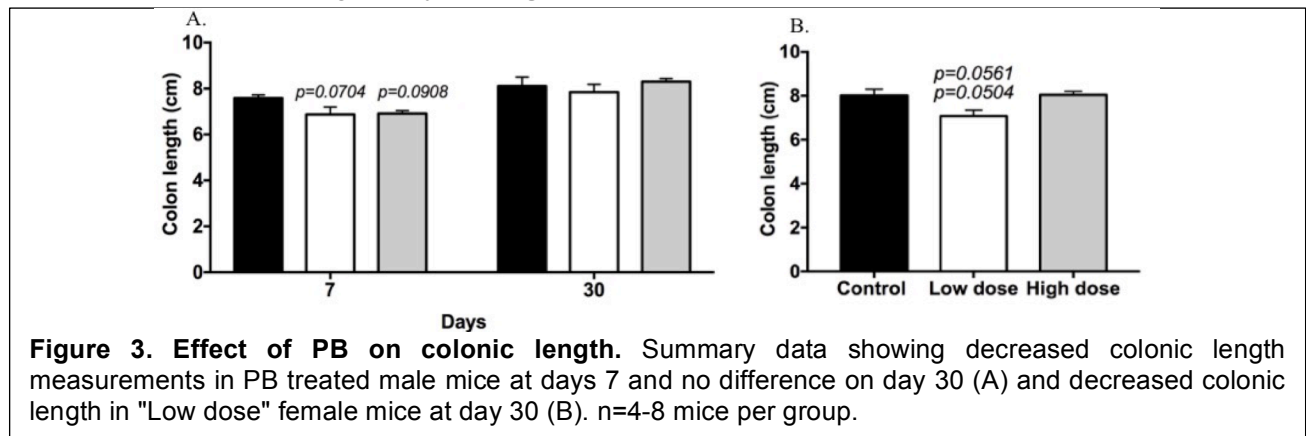
- ii) Our results above suggest that exposure to PB alone is sufficient to alter key gut functions during the 7 day drug treatment and that persistent effects of PB on motility are still present one month following drug treatment. Therefore, we conducted more refined *ex vivo* studies to specifically address the effects of PB on the neural control of gut functions. At the time of harvest, we assess specific parameters of neuromuscular function of the colon with isometric muscle tension recordings and assessed colonic permeability using Ussing chambers. Isometric contractions were record from segments of distal colon under 2 g passive tension with a force transducer. Electrical field stimulation (1-30 Hz) was applied through platinum concentric electrodes to evoke neurogenic contractions and relaxations. Our data from these experiments show that male mice exposed to the “high dose” of PB tended to have impaired contractile force at 30 days (**Figure 2A**). We did not observe any significant effect of PB treatment on neurogenic relaxations in male mice (**Figure 2B**). In female mice, the “low dose” PB treatment tended to increase neurogenic contractions and relaxations at day 30, but these changes did not reach statistical significance (**Figure 2D-E**).

Interestingly, we observed significantly increased colonic permeability in male mice exposed to either dose of PB at the end of the 7-day drug treatment. We assessed colonic barrier function in

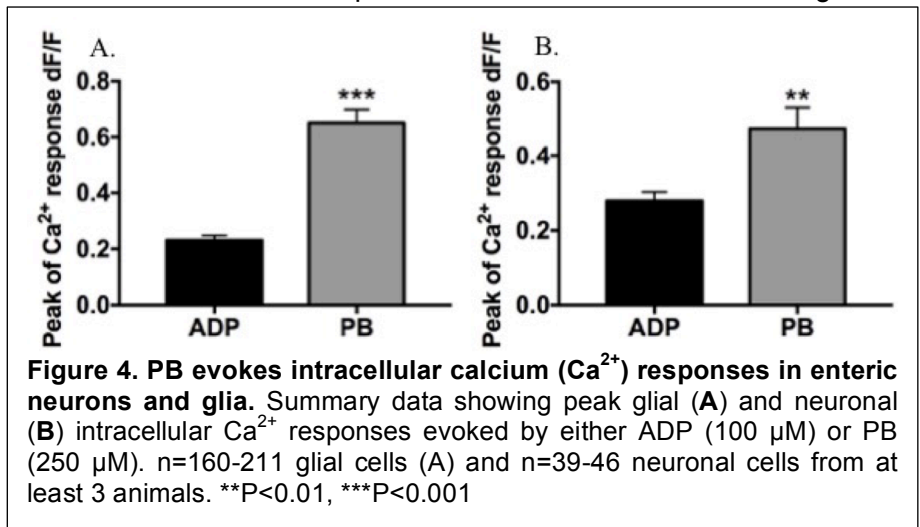


these experiments by measuring the flux of the cell-impermeant dye fluorescein-5-(and-6)-sulfonate in Ussing chambers. Samples of buffer were collected from the serosal chamber before dye addition and every following 20 min (100 uL duplicates were collected and buffer was replenished). Fluorescence intensity was measured and gut wall permeability was assessed from the slope of the values of the last 4 time points. PB significantly increased dye flux at day 7 regardless of dose (31.0% 0.009 mg/mL, 32.7% 0.09 mg/mL, $p < 0.05$ both) (**Figure 2C**). However, this effect was transient and was not observed at the 30-day time point. We also did not observe any significant differences in dye flux in female mice at day 30 (**Figure 2F**). Importantly, increased intestinal permeability is a key risk factor for common diseases such as irritable bowel syndrome and inflammatory bowel disease. Therefore, our results suggest that impaired intestinal barrier function during PB treatment could permit bacterial translocation and contribute to systemic inflammation.

At the time of harvest, we measured colonic length because this is an important indicator of ongoing inflammation and fibrotic processes in the gut. Importantly, colonic shortening is observed in animal models of impaired barrier function such as the dextran sodium sulfate (DSS) model of colitis. Our data from these measurements show that exposure to PB tended to reduce colonic length acutely (at the end of the 7 day treatment) in male mice, but we observed no differences in colonic length at day 30 in male mice (**Figure 3A**). Interestingly, female mice exposed to the “low dose” of PB tended to have colonic shortening at day 30 (**Figure 3B**).



- iii) The above studies in whole animals and organs show that PB alters key gastrointestinal functions. Based on these data, we hypothesized that these changes reflect abnormal cellular activity within the enteric nervous system because the enteric nervous system is responsible for coordinating intrinsic gut reflexes. We tested this hypothesis by assessing the effects of PB on the activity of enteric neurons and glia using calcium imaging. We conducted our Ca^{2+} imaging studies in live whole mount preparations of myenteric plexus isolated from the mouse colon that we loaded with the Ca^{2+} indicator dye Fluo-4. Our data from these experiments show that PB evokes significant activity within enteric glial cells (**Figure 4**). Surprisingly, glial Ca^{2+} responses evoked by PB were significantly larger (181.0%, $p < 0.001$) than those evoked by adenosine phosphate (ADP), a potent glial agonist that drives Ca^{2+} responses through the activation of P2Y1 receptors (**Figure 4**). These results support the hypothesis that PB



significantly alters the activity of the enteric nervous system and suggest that these changes could contribute to altered gut functions.

- iv) One explanation for the effects of PB on gut functions is that exposure to PB disrupts the coordination of gut functions by driving neuroinflammation in the enteric nervous system. We tested this hypothesis by assessing key indicators of enteric neuroinflammation such as enteric gliosis, neurodegeneration, and changes in neurochemical coding. We did not observe differences in glial fibrillary acidic protein (GFAP) expression that would suggest reactive gliosis in day 7 animals (data not shown). However, we observed a significant loss (18.3% loss, $p < 0.05$) of myenteric neurons (Hu immunoreactive) in male mice exposed to the “high dose” of PB at day 7 (**Figure 5A**). Male mice exposed to the “low dose” of PB showed a trend toward an increase in the proportion of inhibitory neurons expressing the enzyme neuronal nitric oxide synthase (nNOS) at day 7 (35%, $p = 0.0602$) (**Figure 5B**). Together, these results show that the 7 day treatment with PB drives significant changes in the enteric nervous system that include a loss of neurons and a reorganization of the neurochemical coding pattern of the surviving neurons. These are important observations that could explain a portion of the persistent effects of PB on gut functions.

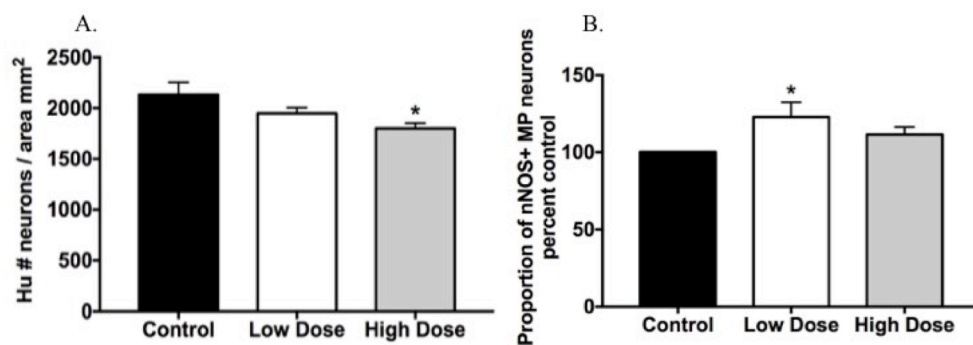


Figure 5. PB drives neurodegeneration and alters neurochemical coding in the enteric nervous system. Summary data showing the effects of PB on the survival (A) and proportion of neurons expressing nNOS (B) in the colonic myenteric plexus of male mice after a 7 day PB treatment. $n = 4$ animal per group. * $P < 0.05$

Summary: Our experiments in this first funding period provide critical information regarding the effects of PB on gut motility, gut permeability, and specific effects on the enteric nervous system. These new data provide novel insight into mechanisms that could contribute to the development of Gulf War Illness in humans. Our continuing studies aim to identify how these changes in the gastrointestinal tract contribute to neuroinflammation in the brain and to identify key mechanisms and factors that contribute to the systemic effects of PB. We anticipate that the outcome of our studies will be the identification of mechanisms that could be targeted by therapeutics to improve the quality of life of Gulf War veterans.

4) Other achievements:

As noted above in “Significant Results/Key Outcomes” section *i*, we also performed essential experiments to refine both the dosing and route of drug administration in our animal model. These experiments were suggested following the review of our protocols by both institutional and ACURO animal care committees. These studies were important first steps to ensure that our animal model accurately reflects the human exposure route and dose experienced by Gulf War veterans. Our successful completion of these preliminary studies provides additional confidence in our animal model in our studies going forward and was an essential addition before proceeding with longer time point studies.

- **What opportunities for training and professional development has the project provided?**
 - Dr. Siomara Hernandez-Rivera, Ph.D. is the postdoctoral research associate assigned to this project. Dr. Hernandez-Rivera is an underrepresented minority woman who comes from a disadvantaged background and working on this project has given her the opportunity to gain essential technical and professional skills that will allow her to become a successful scientist. In addition, working on this project has allowed Dr. Hernandez-Rivera to broaden her knowledge base and explore a new area of research. Dr. Hernandez-Rivera has learned many new techniques to accomplish the experimental goals in this project. For example, Dr. Hernandez-Rivera has received training in live cell imaging and is now proficient at calcium imaging experiments in the enteric nervous system. In addition, Dr. Hernandez-Rivera has received technical training in mouse models of intestinal inflammation, ex vivo and in vivo measures of motility and barrier function, and immunohistochemical studies of the enteric nervous system. While working on this project Dr. Hernandez-Rivera is receiving one-on-one mentorship from Dr. Gulbransen (PI) in technical, conceptual, and career development areas. Dr. Hernandez-Rivera and Dr. Gulbransen have a scheduled weekly meeting to discuss broad project and professional development goals and Dr. Gulbransen and Dr. Hernandez-Rivera have daily conversations regarding more detailed aspects of her research. Dr. Hernandez-Rivera has been integrated within a core group of researchers at Michigan State University who focus on gut research (the “MSU gut group”) and her interactions within this group have allowed her to network and broaden her knowledge base. These will be important skills as she develops into an independent researcher. Dr. Hernandez-Rivera presents her work regularly at the Gulbransen lab’s weekly lab meeting and receives feedback on her work and presentation skills. She is currently receiving mentorship on presenting her data in written format and Dr. Gulbransen is mentoring her in manuscript preparation. Dr. Hernandez-Rivera will present her research at the Great Lakes Glia meeting (October 16, 2017) and also plans to attend one larger meeting (Experimental Biology or Digestive Disease Week) to present her work.
- **How were the results disseminated to communities of interest?**
 - We have spent the first year compiling data and anticipate being able to publish this data in a manuscript soon. In addition, results from this project will be disseminated to the science community in a presentation at the Great Lake Glia Meeting this October 15-17, 2017 and we are planning to present the results from this project at Experimental Biology 2018.
- **What do you plan to do during the next reporting period to accomplish the goals?**
 - In the next funding period, we plan to complete the studies described above and compile this data into a manuscript for publication. We are currently assessing brain neuroinflammation with a multiplex neuroinflammation array and plan to include this data in our manuscript to understand the correlation between gut and brain inflammation after exposure to PB. We plan to begin our studies of the long-term effects of PB by assessing groups at 5 months post exposure and will perform in vivo and in vitro assays to understand the long-term effects of PB on gut function and inflammation in the gut and brain. We also plan to continue our cellular imaging studies to understand the mechanisms associated with the increase in glial activity and neurodegeneration following exposure to PB. We will also measure cellular activity in neurons and glia using calcium imaging in tissues from mice at 1-month and 5-month post-PB exposure. Finally, we plan to start experiments from major task 3 that will test the beneficial effects of the anti-inflammatory drug palmitoylethanolamide (PEA).

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
 - Our results suggest novel mechanisms of disease progression driven by acute drug exposure in the intestine. These findings could significantly impact the current usage of similar drugs and also the current theories of Gulf War Illness disease pathogenesis.
- **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?**
 - Our current results suggest novel mechanisms of disease pathogenesis in Gulf War Illness. These findings could uncover new therapeutic targets that could improve the quality of life of veterans suffering from Gulf War Illness.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
 - One major change in the approach was the addition of a second drug concentration (as described above). This change was in response to recommendations made during the initial review (and approval) of our animal use protocols and we have incorporated this second dose (calculated based on body surface area to more accurately model human concentrations in mice) into our study. We also modified some of the assays that we are conducting to study gut functions in isolated organ preparations. First, we decided to test neuromuscular function using isometric muscle tension recordings in organ baths rather than recording colonic migrating motor complexes (CMMCs). The reason for this change was because the isometric muscle tension recordings allow us to specifically assess both excitatory and inhibitory parameters of neuromuscular transmission and allow us to more accurately tease apart mechanisms that may contribute to dysmotility. Second, we have added an Ussing chamber assay that allows us to assess gut barrier function. Gut barrier function is an important aspect of disease susceptibility and changes in barrier function could permit the passage of bacteria and contribute to systemic inflammation. Given the importance of this mechanism, we are including this measurement in our study.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
 - The initiation of the project was slightly delayed due to the time required to hire the postdoc. She was able to begin one month after the start of funding and then received training in the techniques required to complete the project. As noted above, important points were raised during the review of our animal protocols that prompted us to conduct initial studies to refine the drug dosage and administration route prior to proceeding with our study. Oral administration of PB is the most clinically relevant route, but it was unclear if mice would avoid drinking water containing PB or if they would experience adverse effects from drinking water containing PB that would cause them to be removed from the study. We addressed this issue by running a pilot study where we administered PB via drinking water in two doses, one based on body weight and one based on body surface area. We compared this with the administration of PB via daily oral gavage as an alternative method. We found that mice did not avoid drinking water with PB, did not experience significant adverse effects, and that the administration via water produced more accurate results than with daily gavage. This method is also less invasive and avoids potential complications of the gavage itself.

Therefore, we concluded that administration via drinking water was the most relevant and effective model to study the effects of PB in vivo. These initial tests were important to ensure the overall success of our study. We are now confident that we will be able to proceed with the longer-term study without complications based on the animal model.

- **Changes that had a significant impact on expenditures**

- Including two dosages of PB (as requested by institutional and ACURO animal care committees) increases the number of animals that we will use in our study. This will impact animal costs and we will attempt to cover these costs under the existing budget. Given that our most significant effects are associated with male mice dosed with 0.09 mg/mL PB, we may proceed to study this group in more detail and exclude the 0.009 mg/mL group and/or female animals from longer-term or mechanistic studies. However, as including these groups would give additional insight into disease susceptibility, we would like to maintain these groups.

- **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**

- NA

- **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**

- Great Lake Glia Meeting, Traverse City, MI October 15-17, 2017

- **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:	Brian D. Gulbransen, Ph.D.
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	orcid.org/0000-0003-1145-3227
Nearest person month worked:	1.2
Contribution to Project:	Oversees all aspects of the project. Provides critical review of data and coordinates the efforts of staff. Provides mentorship for the postdoctoral trainee (Dr. Hernandez-Rivera) and oversees the work of the technician (Dr. Fried). Contributes to writing manuscripts, composing figures, and the presentation of results.
Funding Support:	RO1DK108798-01 (Manfredsson, Galligan, Gulbransen) 10/01/16-09/30/21 NIH \$1,918,750 <i>Alpha-synuclein aggregation disrupts motility, synaptic transmission, and calcium signaling in the myenteric plexus of the rat colon.</i> Major goals: To study how alpha-synuclein aggregation in enteric neurons and/or glia specifically affects the neural control of gut functions. PI: Manfredsson Role: Co-investigator (30% effort) GW150178 W81XWH1610631 (Gulbransen) 09/15/2016-09/14/2019 DoD Gulf War Illness New Investigator Research Award \$627,497 Pyridostigmine bromide, the enteric nervous system and functional gastrointestinal disorders in Gulf War illness. Major goals: To study how the anti-nerve gas drug pyridostigmine bromide contributes to functional gastrointestinal disorders in Gulf War illness through effects on the enteric nervous system. Role: PI RO1 DK103723-01 (Gulbransen) 07/01/15-06/30/20 NIH/NIDDK \$1,961,760 <i>Role of enteric glia in the death of neurons during gut inflammation</i> The goal of this project is to understand the role of enteric glial cells in the regulation of enteric neuropathy. Our central hypothesis is that purinergic activation of enteric glial cells differentially regulates neuron survival

	depending on glial activation by ADP or adenosine. Role: PI Senior Research Award 327058 (Gulbransen) 07/01/15-06/30/18 Crohn's and Colitis Foundation of America (CCFA) \$347,490 <i>Role of enteric glial cells in the initiation of neuron death in Crohn's disease</i> The goal of this project is to test the central hypothesis that reactive enteric glial cells promote lethal P2X7R activation in neighboring neurons to cause neuron death in Crohn's disease. Role: PI
Name:	Siomara Hernandez-Rivera, Ph.D.
Project Role:	Postdoc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Dr. Hernandez-Rivera has performed all the in vivo and in vitro experiments mention before in the section 3: accomplishment.
Funding Support:	None (supported by this grant)
Name:	David Fried
Project Role:	Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Mr. Fried provides technical support for all ex vivo measurements of gut motility including assisting with experiments and data analysis.
Funding Support:	None (supported by this grant and RO1 DK103723)

- **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:** NA
- **QUAD CHARTS:** NA

9. APPENDICES:

- Nothing to report