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PRINCIPAL INVESTIGATOR: Lee A. Shapiro

CONTRACTING ORGANIZATION: Texas A&M Health Science Center
College Station, TX 77845

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Lee A. Shapiro

E-Mail:lshapiro@medicine.tamhsc.edu

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14. ABSTRACT

The purpose of this research is to determine if vagus nerve stimulation (VNS) will be an effective therapeutic strategy for Gulf War Illness (GWI). GWI refers to a chronic complex of symptoms observed in afflicted personnel. GWI symptoms include cognitive impairments (memory and concentration problems), headaches, migraines, widespread pain, fatigue, gastrointestinal and respiratory issues, as well as other unexplained abnormalities that do not fit into classical medical diagnostic criteria. There are extensive clinical and experimental data showing that VNS treatment exerts beneficial effects in many of the aforementioned symptom domains associated with GWI. We are in the process of testing the efficacy of VNS treatment on behavioral, cognitive, inflammatory, neuroinflammatory and neuroanatomical outcome measures. We have had our manuscript on altered nociceptive sensitivity accepted for publication. We have also demonstrated changes in hippocampal astrocytes, hippocampal neurogenesis, and changes to cognitive function that rely on intact hippocampal neurogenesis.

15. SUBJECT TERMS

Permethrin, pyridostigmine bromide, inflammation, neuroinflammation, astrocyte activation, cholinergic anti-inflammatory pathway, astrocytes, neurogenesis

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1. **INTRODUCTION:**

The purpose of this research is to determine if vagus nerve stimulation (VNS) will be an effective therapeutic strategy for Gulf War Illness (GWI). GWI refers to a chronic complex of symptoms observed in afflicted personnel. GWI symptoms include cognitive impairments (memory and concentration problems), headaches, migraines, widespread pain, fatigue, gastrointestinal and respiratory issues, as well as other unexplained abnormalities that do not fit into classical medical diagnostic criteria. There are extensive clinical and experimental data showing that VNS treatment exerts beneficial effects in many of the aforementioned symptom domains associated with GWI. Therefore, using an established animal model of GWI, we will test the efficacy of vagus nerve stimulation, initiated at a time-point analogous to >20 years after the initial exposure to GWI compounds, on cognitive, behavioral, inflammatory, neuroinflammatory, and neuroanatomical outcomes.

2. **KEYWORDS:** Permethrin, pyridostigmine bromide, inflammation, neuroinflammation, astrocyte activation, cholinergic anti-inflammatory pathway

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

The major goals of the project as stated in the approved SOW were to perform the analysis on all mice.

Using year 2 as an example, the experimental groups are listed below:

Specific Aim 1, Year 2. List of groups and mice per group	N
Group 1: Naïve mice	9
Group 2: GWI controls (exposed to chemicals only, no further manipulations	9
Group 3: Vehicle treated controls (exposed to saline + diluting agent (DMSO), no further manipulations)	9
Group 4: GWI animals (exposed to chemicals) implanted with VNS stimulator, stimulators not turned on	9
Group 5: GWI animals (exposed to chemicals) implanted with VNS stimulator and stimulators turned on	9

Specific Aim 2, Year 2. List of groups and mice per group	N
Group 1: Naïve mice	9
Group 2: GWI controls (exposed to chemicals only, no further manipulations	9
Group 3: Vehicle treated controls (exposed to saline + diluting agent (DMSO), no further manipulations)	9
Group 4: GWI animals (exposed to chemicals) implanted with VNS stimulator, stimulators not turned on	9
Group 5: GWI animals (exposed to chemicals) implanted with VNS stimulator and stimulators turned on	9

In each case, the mice are exposed to GWI chemicals at experimental days 1-10. Then, the animals receive standard care, for ~ 220 days, after which they are tested on the Von Frey pain test pre-test. 3-5 days after this behavior testing, mice in the vagus nerve stimulator implantation groups undergo this procedure. Then, after a

2- to 5-day recovery time, the VNS stimulators are turned on for either 2 weeks (Aim 1), or 4 weeks (Aim 2). After the completion of the stimulation paradigm, the following behavioral tests occur:

Task order	Task type	Task Duration
1	Von Frey pain threshold post-test	1 day followed by 3 days of rest
2	Open field test	1 day followed by 3 days of rest
3	Object location test	1 day followed by 3 days of rest
4	Novel object recognition test	1 day followed by 3 days of rest
5	Morris water maze test	7 day learning + 1 day probe test and visible platform test

After these behavioral tasks, mice are sacrificed for anatomical and biological analysis.

What was accomplished under these goals?

1) **Major activities**: We organized the studies such that we purchase 10 mice at a time, and within every 10 mice ordered, 2 mice are randomly assigned to each of the 5 groups in Aim 1 or 2. In total, by the end of year 2, we have ordered all 220 mice listed in the SOW, and we completed all behavioral studies on all mice within year 3.

   Of these, 132 mice have been injected with the Gulf War chemicals, 44 have been injected with DMSO (vehicle controls), and 44 were in the naïve group.

   We have removed a total of 47 mice from the experiment. Of these 47 mice, the breakdown of removal is as follows: Naïve (6.4%), DMSO (8.5%), GWI (46.8%), and GWI mice implanted with the VNS stimulators (38.3%). The mice have been removed for: mortality, fighting or other wounds that could not be adequately treated without compromising the variables, surgical implantation failure.

   In total, all 22 of the 22 groups of mice have completed all of the tasks, including behavioral analysis, specified in the experimental design.

   As reported after year 1 and 2, using outside funds, we confirmed the efficacy of our implementation of the GWI model, using a group of 4 GWI mice, 4 DMSO mice and 2 Naïve mice. 1 GWI mouse and 1 DMSO mouse did not survive to be tested behaviorally (fighting wounds). Despite being under-powered, we performed behavioral testing between 3 and 5 months after the induction of the GWI chemicals (or DMSO). These time points were selected because Dr. Crawford, the originator of this model, has previously demonstrated behavioral/cognitive impairments at these time points. In performing these preliminary experiments, it also enabled us the opportunity to completely work out our behavioral protocols, on collaboration with Dr. Shetty. Our results confirm the previous studies from Dr. Crawford, showing trends (object location task and pattern separation task) or significant impairments (open field, Von Frey pain test), in our behavioral tasks, and confirmed our ability to implement the GWI model, as well as the behavioral testing. We have also performed additional cognitive testing and observed a deficit in GWI mice on the pattern separation task. Notably, performance on this task is related to adult hippocampal neurogenesis, and we have also observed alterations to neurogenesis in the GWI mice compared to naïve age- and litter-matched controls.

   In Year 3, we completed the analysis of the Von Frey nociceptive assay. We have now had our manuscript accepted for publication in the journal, “Neurotoxicology”.

2) **Specific Objectives**: Above and beyond the group of mice that we paid for using our other lab funds, all of the mice used as part of the grant have met the specific objectives as specified in the statement of work.

3) **Significant Results**: Notably, we have observed an altered Von Frey mechanical nociception test in the GWI mice. We observed that at 10 weeks after the GWI induction, the mice had hypersensitive nociceptive sensitivity (Fig 1). At 40 weeks after GWI induction (Fig. 1), the GWI mice are hyposensitive in the Von Frey test, and this hyposensitivity is reversed in both the 2 and 4 week vagus nerve stimulation groups (Fig 1). These findings allow for several interpretations. The first is that GWI chemicals increase the pain sensitivity within the first 3 months after induction of the GWI model. The second, is that it appears as though all animals in all groups had a reduced sensitivity in the second Von Frey test. This suggests that the mice may have become habituated to the stimulus. At the latest time points tested, the GWI mice appear to be more hyposensitive than the other groups. This increased sensitization could reflect endogenous analgesic and or anti-inflammatory mechanisms that are released in the GWI mice in response to chronic pain. Finally, the 2 week and 4 week VNS stimulated mice appear to be hypersensitive to the pain. This could reflect a reduction in inflammation, and also possibly inter-related with endogenous pain mechanisms, or could also indicate that the VNS re-introduces nociceptive hypersensitivity.

The data below were initially presented in poster format at the 2017 Society for Neuroscience Conference..

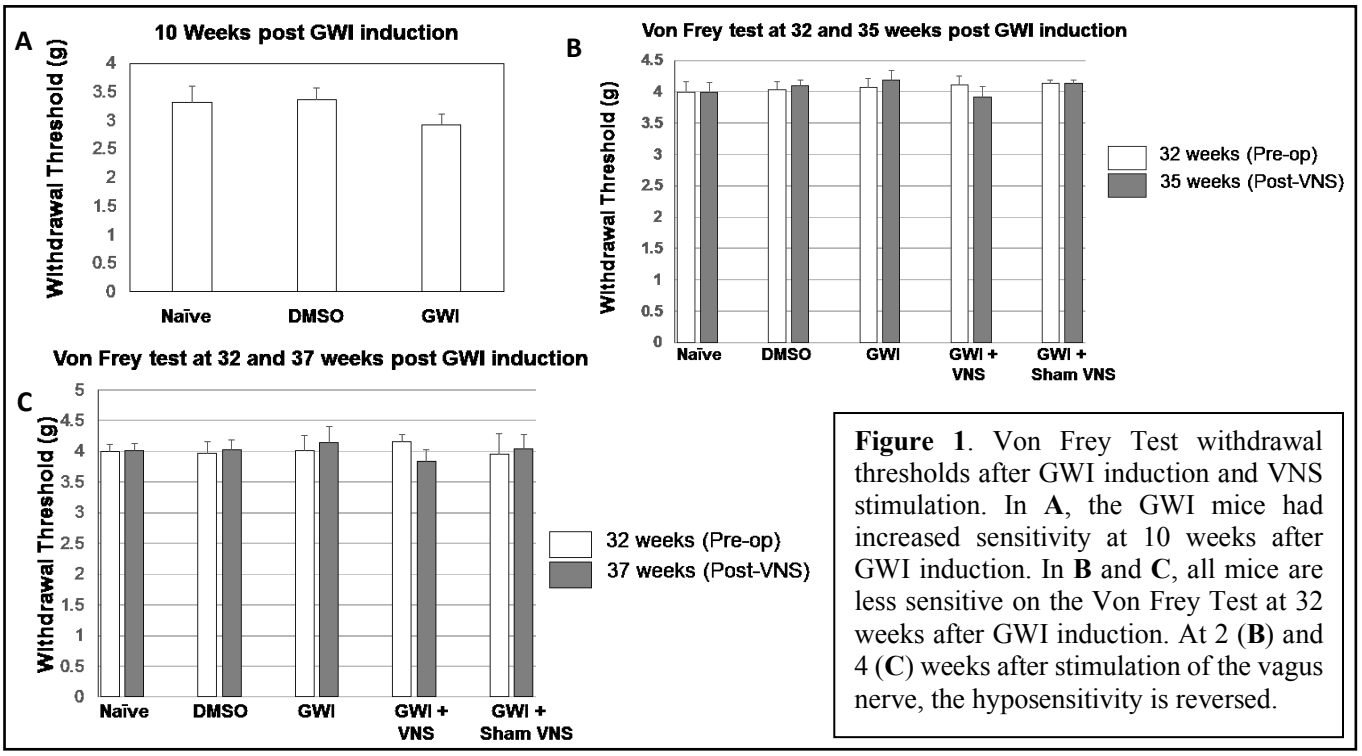


Figure 1. Von Frey Test withdrawal thresholds after GWI induction and VNS stimulation. In **A**, the GWI mice had increased sensitivity at 10 weeks after GWI induction. In **B** and **C**, all mice are less sensitive on the Von Frey Test at 32 weeks after GWI induction. At 2 (**B**) and 4 (**C**) weeks after stimulation of the vagus nerve, the hyposensitivity is reversed.

After presenting these results and discussing these findings with experts in the field, we next prepared a manuscript based on these results. This manuscript was accepted for publication in September, 2018, and we are currently in the process of editing the proofs.

In addition to the pain sensitivity data, we have also obtained preliminary results on one of our neuroanatomical variables. These results relate to our assessment of astrocytes in the hippocampus. As can be seen in **Fig. 2**, in GWI mice, there appears to be a significant increase in the number of radial glial processes that course through the dentate gyrus granule cell layer. The sham VNS had minimal effect on these astrocytic processes, but the vagus nerve stimulation mice appear more similar to the naïve mice. The data below were initially presented in the 2016-2017 progress report.

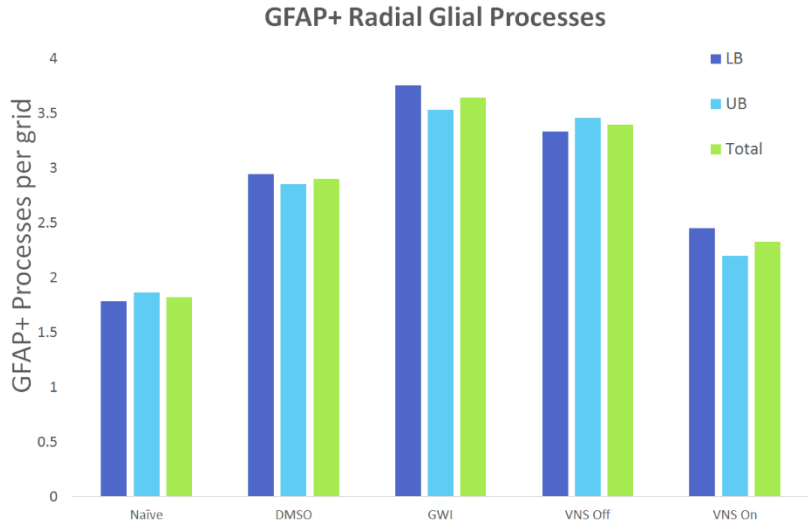


Figure 2. Graph of the mean number of radial glial processes passing through the suprapyramidal (UB) and infrapyramidal (LB) blades of the dentate gyrus granule cell layer. Note that GWI injection paradigm causes an increase in the number of radial glial processes, and vagus nerve stimulation (VNS on) partially restores the number of radial glial processes toward the level of naïve mice.

As we had not yet performed statistical analysis of these data, error bars were not included in the graphs. Nevertheless, the data appear to suggest either an increase in the number of astrocytes and/or their processes. In a number of previous experiments, we have shown that these astrocytes, as well as their processes are intimately related to the adult born neurons in this region. Moreover, changes in the number or morphology of these astrocytes has been associated with changes to the number, location and morphology of the adult born neurons. Thus, in addition to our quantification of the adult born neurons, we will also assess the relationship of these astrocytes with the adult born neurons. In addition, we will be performing a more comprehensive analysis of the number and morphology of the astrocytes.

During year 3, we have now presented these data as a poster, and in a second poster, we presented data on the analysis of quantification of the number of GFAP+ astrocytes in the hippocampus. The method used for these cell counts is shown below:

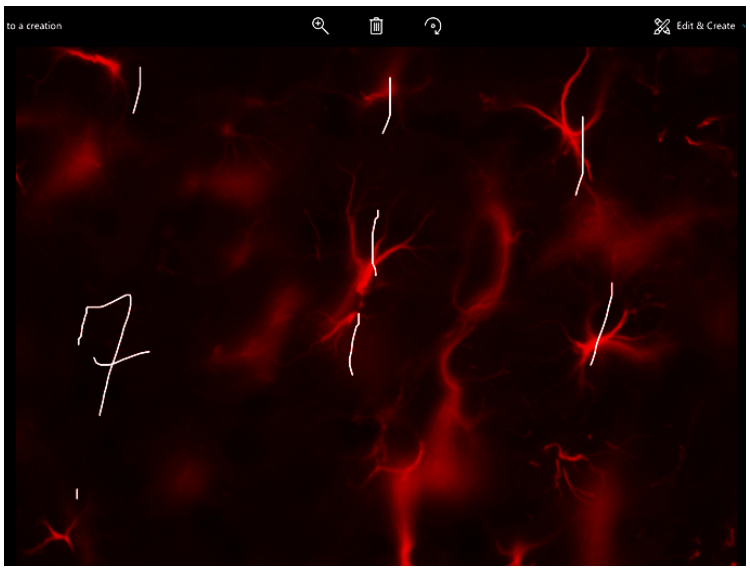


Figure 3. Example of counting scheme used to quantify the astrocytes in a given region of GWI mice. Images are captured by a rater blind to the condition of the mice. A second rater, also blinded to the condition of the mice then opens up the photo and counts those astrocytes that meet the criteria for counting. The total number of astrocytes is then totaled per slice. For each region of interest, the average number of astrocytes per slice are then calculated.

In addition to two posters presented on the astrocytes, we also presented a third poster on the adult born neurons in the dentate gyrus of the GWI mice. As these mice are almost 1 year old at the time of analysis, the baseline levels of neurogenesis in the dentate gyrus is quite low. Thus, we used the following methods to quantify these cells:

1. Images of DCX-labeled immature neurons in the hippocampal dentate gyrus were captured by a rater blind to the condition of the mice.
2. Other raters, also blind to the condition of the mice, then counted the number of cells in the infrapyramidal and suprapyramidal blades of the dentate gyrus
3. Raters only counted those neurons with >60% of the membrane borders of their perikaryon in the plane of focus.

A sample image taken from a mouse from the GWI study is shown below:

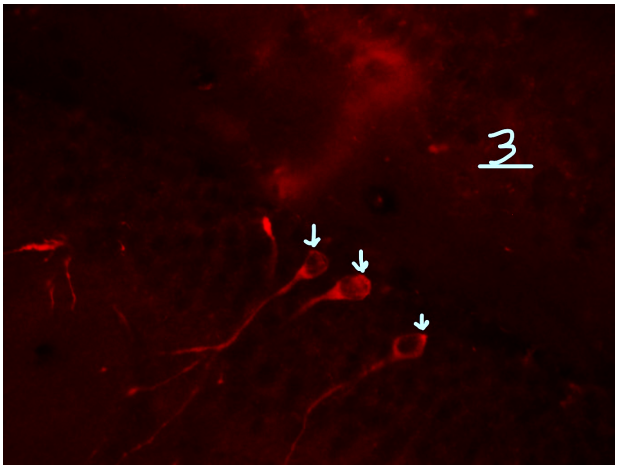


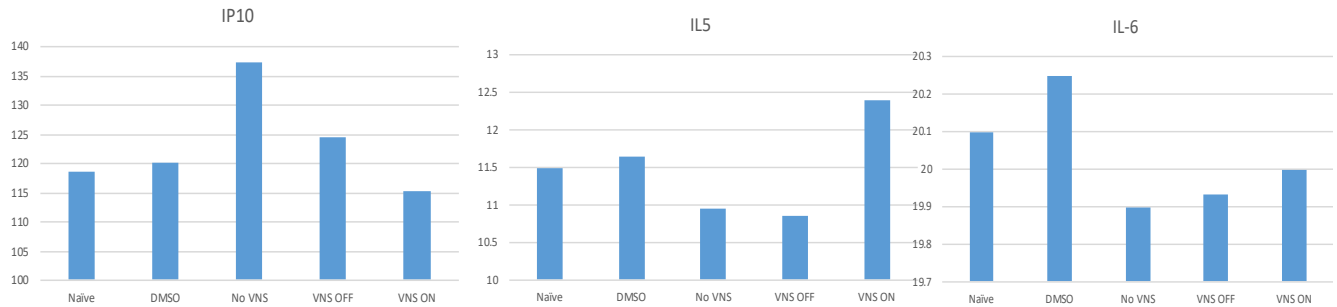
Figure 4. DCX-labeled newborn neurons in the infrapyraidal blade of the hippocampal dentate gyrus. DCX-labeled newborn neurons (arrows) are clearly depicted. Note, that these cells contain apical dendrites extending through the granule cell layer and into the molecular layer of the dentate gyrus. Also note that these cells do not appear to have basal dendrites extending into the hilus.

We are now in the process of finalizing the cell counts of GFAP-labeled astrocytes and DCX-labeled newborn neurons. The last remaining portion of this analysis is to determine if:

1) DCX-labeled immature neurons have hilar basal dendrites from any of the experimental groups.
2) If DCX-labeled hilar basal dendrites are growing along an ectopic glial scaffold.

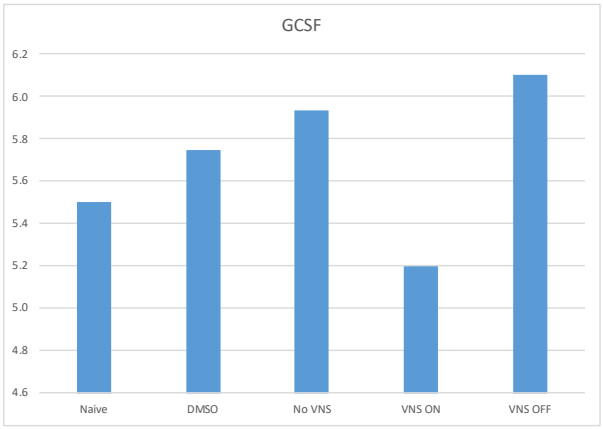
Once we complete these last two components of the analysis, we plan to submit another manuscript on these data.

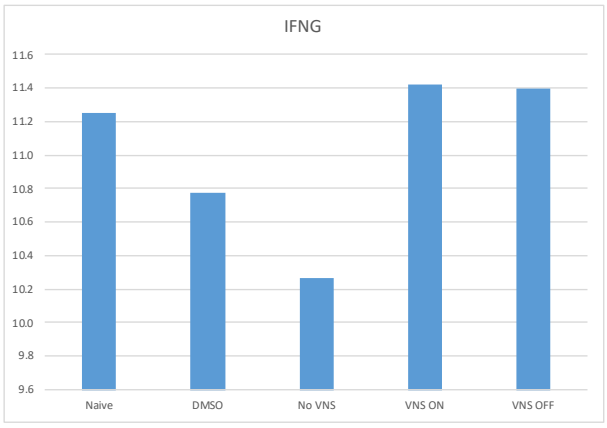
We have completed the data collection of the multiplex assays. Although we have not yet performed statistical analysis, we have created graphs of means. Graphs of some of the more notable cytokines and chemokines are included below:

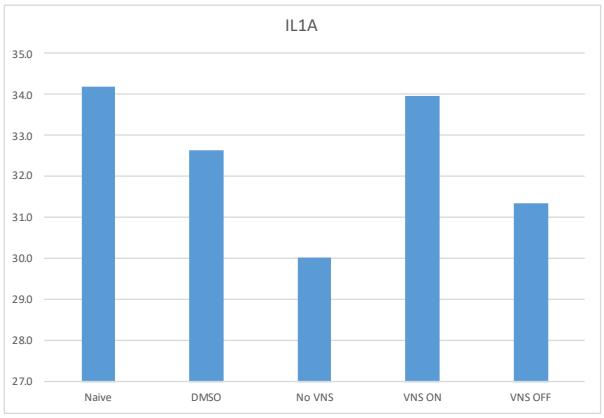


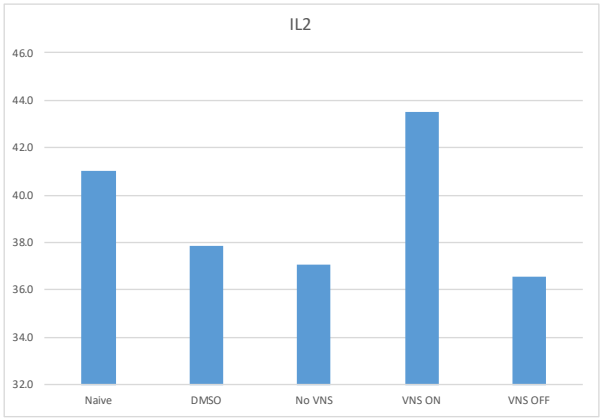
Hypothalamus

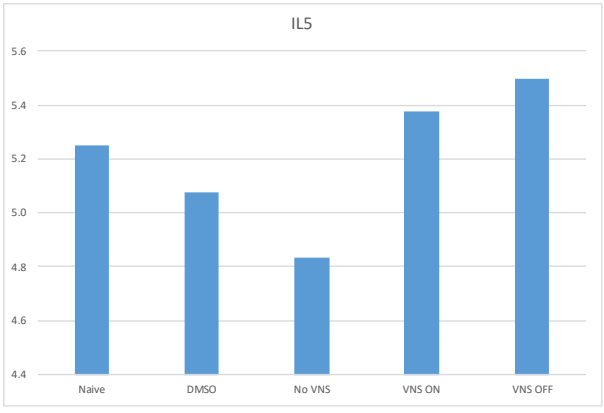
The following graphs were obtained from cytokine analysis in the hippocampus

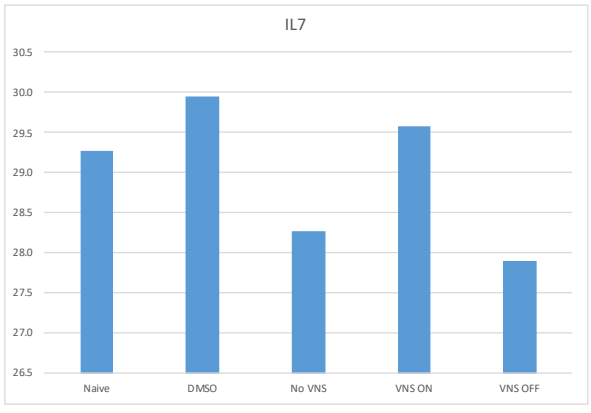


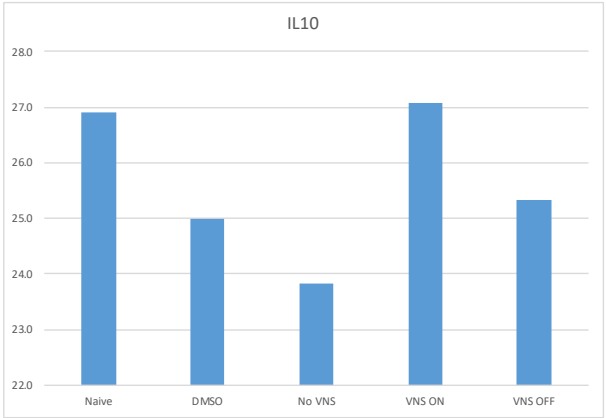


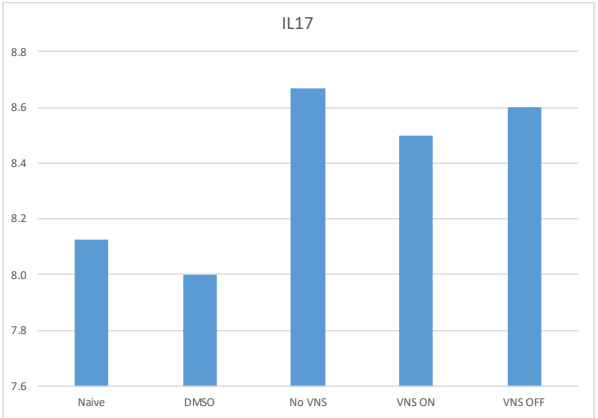


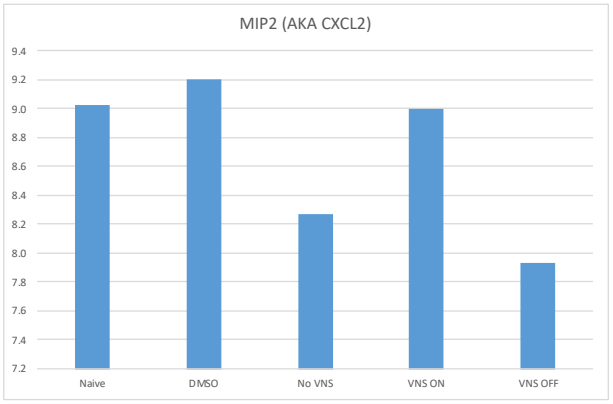


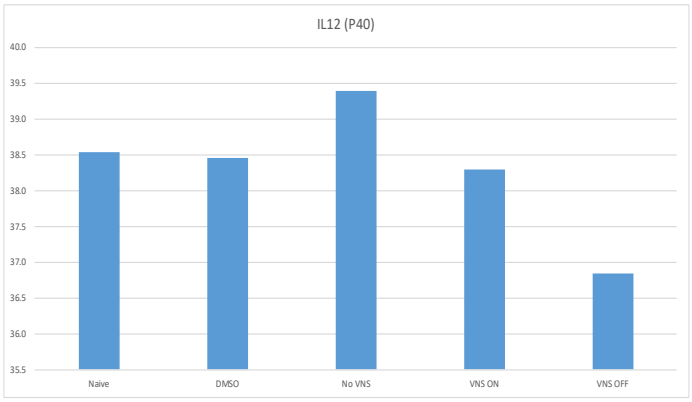




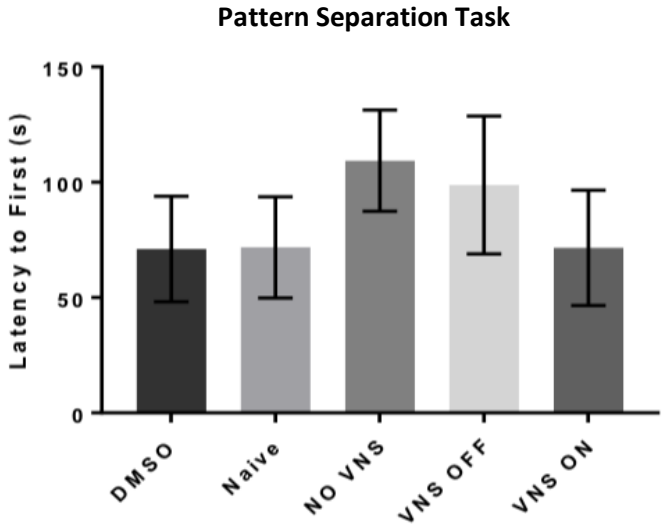








In the next series of graphs, data from the behavioral testing are shown. It is important to note that these graphs.



In this Figure, the results from the Pattern Separation Task are shown. Here, the latency to first visiting the novel object in the recall trial (3rd trial) are shown. As can be seen, both the DMSO and Naïve mice visit the novel object within about 70 seconds. Alternatively, the GWI mice, and the GWI + VNS Off mice take almost 100 seconds to visit the novel object. This deficit is reversed by VNS stimulation, in which the latency to visit the novel object is about 70 seconds.

Below, the data on velocity is shown to illustrate the significant reduction in velocity of mice that had the VNS implants.

Source	Sum of Squa	Mean Square	F Ratio	Prob > F
Treatment	63.9368	15.9842	4.298	0.0044
Error	197.10537	3.719		
C. Total	261.04217			
Level	Mean	Std Error	Lower 95%	Upper 95%
DMSO	8.7914	0.5154	7.7576	9.825
Naïve	9.04804	0.5567	7.9314	10.165
NO VNS	9.38235	0.53486	8.3096	10.455
VNS OFF	6.18327	0.68181	4.8157	7.551
VNS ON	7.73178	0.58145	6.5655	8.898

In this figure, the ANOVA revealed a significant decrease in mean velocity of the mice implanted with the VNS stimulators as they navigate the open field. It is notable that the VNS treatment appears to increase the velocity compared to VNS off, although this effect is not signifcant. Nevertheless, both groups that had the VNS stimulators implanted have a reduced velocity. Thus, we are currently considering alternative plans for statistical analysis to take this decreased velocity into account, as discussed in “5 changes and problems”.

4) Other achievements and goals not met: As previously reported, in the group of mice that were assessed using other funds, we performed flow cytometry on the spleens and intestines of these mice. We found evidence of splenocyte activation, as well as activation and expansion of major histocompatibility complex (MHC) II-expressing B cells (**Fig. 1**).

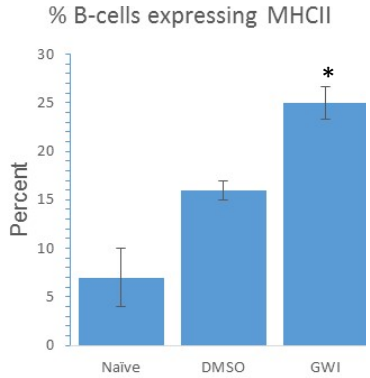


Figure 1. Percent of B cells in the spleen that also express MHCII. An increase in MHCII expression on B cells is indicative of T cell-dependent activation of the B cells. As can be seen in the graph, spleens harvested at the outset of behavioral testing have significantly more MHCII+ B cells in GWI mice, compared to DMSO or Naïve mice. It is pertinent to note that a trend toward an increase was observed in DMSO mice compared to Naïve, but this result was not significant.

Further evidence in support of the activation of B cells in the GWI mice is observed by staining the splenocytes for immunoglobulin D **(Fig. 2)**.

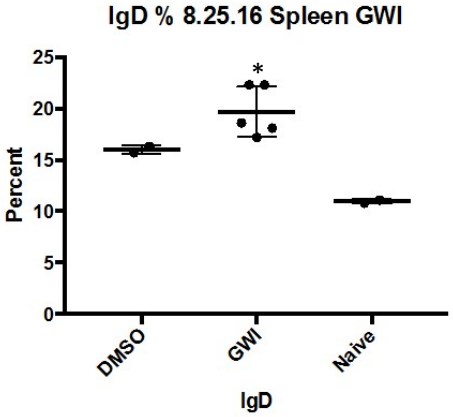


Figure 2. Flow cytometric analysis of immunoglobulin D (IgD) expression in splenocytes. IgD is an immunoglobulin that appears in species with an adaptive immune system. Among its numerous activities in the adaptive immune response, IgD is involved in B cell activation. As can be seen in the graph, IgD is significantly increased in GWI mice compared to DMSO mice and naïve mice. It is pertinent to note that we also examined IgM, but did not detect any significant differences).

We have now collected the spleens from the remaining mice that were freshly dissected for the multiplex analysis. We will add the analysis of these additional spleens in order to more fully power these immune cell experiments. This will allow us to further define some of the cellular mediators in the immune response to our model of GWI, and possibly identify therapeutic targets in the future. It should be further noted, that in consideration of gastrointestinal (GI) issues with GWI patients, we have also performed flow cytometric analysis of the intestines to our protocols. As with the spleens, this analysis has been and will continue to be done with money from the PI's lab that is separate from the grant.

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

Although this project was not intended to provide training and professional development opportunities, it continues to provide an excellent opportunity to train an up-and-coming scientist (Dr. Damir Nizamutdinov), on the rigors of carrying-out experiments as they are intended in a grant proposal, as well as the importance of being highly-organized, such that all of the data are optimally useful. Along these lines, Damir has also

gained expertise in a series of surgical, behavioral and neuroanatomical techniques, as well as significant one-on-one time with a mentor (Dr. Shapiro).

In addition, we are pleased that this funding has provided a training opportunity for Jaclyn Jenkins, an Army Veteran. Jaclyn has been undergoing a number of training activities in my lab. The initial idea for the training was to expose Jaclyn to the lab setting, and enhance her skill set, giving her a number of opportunities that include: 1) Working as a technician in the future; 2) Pursuing higher education (BS, MA); 3) Pursuing a Ph.D. While all of the options remain in play, currently Jaclyn has demonstrated a high capacity for laboratory work, and a growing interest in doing so. Jaclyn is nearing completion of her Associate degree program and is interviewing for her subsequent 4 year college. It needs to be emphasized that the ability to work on a project that involves exposure to GWI chemicals (some of which she was also exposed to in her military service) was the initial impetus for her interest. Jaclyn also collected data on the radial glial astrocytes in the hippocampus this summer. She presented a poster highlighting her findings (some of which are also included in this report). This poster was presented as part of the Temple College and Temple Biosciences Institute Summer Research Programs. We are honored to be able to facilitate Jaclyn's growing interests!

Most recently, this funding provided training opportunities for two additional undergraduate students, both of whom are members of underrepresented minority groups. Manuel Ramirez performed analysis on the DCX-labeled immature neurons and Vanessa Evans performed analysis on GFAP-labeled astrocytes. Both students presented posters on their findings, and they will be included as Co-Authors on the forthcoming manuscript.

How were the results disseminated to communities of interest?

We are presenting findings on the astrocytes from this project at the 2018 the annual Society for Neuroscience conference, an International conference attended by over 40,000 scientists each year. Through our discussions at this meeting, we will then finalize the analysis and interpretations of the astrocyte and immature neuron data, and plan a manuscript for publication. This is the same formula we used in 2017, when we first presented the nociception data, then based on our discussions at the meetings, constructed the manuscript that was recently accepted for publication.

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

We have generated extremely large behavioral data sets and multiplex data sets on cytokines and chemokines from these mice. One of the main goals of the next reporting period is to not only analyze these data, but to reconcile the data from each of the behavioral tasks, with each component of the neuroanatomical data. As such, we expect to continue our analysis as originally planned, and fully intend to accomplish the goals set forth, as well as pursuing other highly intriguing avenues of GWI research. As specified in the grant, we will complete all analysis of the remaining mice, interpret the data, and disseminate the data at conferences, meetings, and through manuscripts.

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?

Pain and neuroinflammation, with specific relevance to the astrocytes, have all ben interconnected. A major area of impact of our studies is to address questions relating to these symptom domains, and to determine if

vagus nerve stimulation would improve these symptoms. The findings indicate that the vagus nerve stimulation does reduce the astrocyte activation in the GWI model. If our analysis further confirms this finding, it will be a highly impactful finding in the GWI field. Of note, is that our preliminary analysis indicates that neurogenesis is decreased in the GWI mice. Our preliminary findings also indicate that these mice are deficient in the pattern recognition task. As the pattern recognition task is known to be dependent on intact neurogenesis, these data would not only show long term behavioral and neurogenic deficits in response to GW agent administration (PB and PER), but would also show a potential neuroanatomical correlate of the behavioral deficit.

What was the impact on other disciplines?

Based on our ability, and now expertise, at implanting the vagus nerve stimulators, as well as the collaboration with Dr. Stauss, we have submitted an NIH R21, a U01, and a pending R01 proposal. We propose to assess the potential of vagus nerve stimulation on treating diabetes. This collaborative effort would never had happened, had this current GWI proposal not been funded. Interestingly, in human epileptic patients receiving VNS for intractable seizures, our preliminary data indicates that these patients have elevated gluose levels. Thus, there is a potentially profound effect of VNS on glucose metabolism. Moreover, vagus nerve stimulation is FDA approved to treat a number of disorders. Therefore, our multiplex data will provide input on the neuroinflammatory mechanisms that are influenced by vagus nerve stimulation has important implications in numerous disciplines.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report. However, it is pertinent to note that during my grant review responsibilities, I had a number of very positive interactions with GWI Veterans, and all were highly enthusiastic about the possibilities represented by our studies.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

In our preliminary behavioral analysis, one of the parameters that we found significant differences on was locomotion. The difference was that the mice that had the VNS stimulators implanted were less ambulatory. This effect did not extend to the Morris Water Maze testing. Thus, we conclude that the implanted stimulators likely influenced ambulatory behavior in land, but not in water. With the exception of the Morris Water Maze and the Von Frey Nociception test, this reduction of ambulation could impact the results of the cognitive testing. Thus, we are working with a biostatistician to determine the best way to account for this confound. Thus far, the simplest and most straightforward way will be to first look at GWI compared to DMSO and Naïve mice, using ANOVA, to see if GWI impacts cognitive behavioral performance. Then, in a second t test, we will compare GWI + VNS on to GWI + VNS off using a t test. We have performed power analysis and we still maintain adequate power to perform the analysis in this way.

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

No anticipated problems or delays.

Changes that had a significant impact on expenditures

No changes.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

The internal approval dates for our protocol are: 7/28/2015 expires 7/28/2018

The DoD/US Army approval dates for our protocol are: 10/21/2015

We've adhered strictly to the approved protocol, and do not have any changes to report at this time.

6. **PRODUCTS:** Nothing to Report

Publications, conference papers, and presentations

Journal publications. Neurotoxicolgy (In Press).

Title: Gulf War agents pyridostigmine bromide and permethrin cause hypersensitive nociception that is restored after vagus nerve stimulation

Authors: Damir Nizamutidinov*, Sanjib Mukherjee*, Chenghao Deng, Harald M. Stauss, Lee A. Shapiro

*Authors contributed equally

Books or other non-periodical, one-time publications. Nothing to report

Other publications, conference papers, and presentations. Our abstract for the 2017 Society for Neuroscience was accepted and presented at the November, 2017 meeting. The title of this poster is: **Altered Von Frey Pain thresholds in a model of Gulf War Illness are restored by vagus nerve stimulation.**
A poster was also presented in August, 2017, at the Temple Biosciences Institute Summer Research Program Poster Session. The title of this poster was: **Analysis of GFAP Positive Radial Glial Processes in the Hippocampus of Gulf War Illness Mice.**

A poster was presented in April, 2018, at the Texas A&M Undergraduate Research Symposium. The title of this poster was: **Utilization of DCX-labeling of newborn neurons to observe effects of adult neurogenesis within the PB and PER model of Gulf War Illness.**

A poster was presented in April, 2018, at the Texas A&M Undergraduate Research Symposium. The title of this poster was: **Alterations to hippocampal astrocytes in a model of Gulf War Illness.**

Our abstract for the 2018 Society for Neuroscience was accepted for presentation. The poster will be presented in the November 2018 meeting. The tile of the poster is: **Vagus nerve stimulation reverses the pyridostigmine bromide and permethrin-induced increase in astrocytes in the hippocampus in a model of Gulf War Illness.**

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. Together with our collaborators, we've identified a role of vagus nerve stimulation in regulating glucose metabolism. We are actively seeking funding to further investigate the therapeutic potential of this finding.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Lee A. Shapiro*
Project Role:	*PI (No change)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	*No change*
Funding Support:	*Internal lab funds (to validate model and perform flow cytometry above and beyond funded project).*

Name:	*Ashok Shetty*
Project Role:	*No change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	*No change*
Funding Support:	

Name:	*Harald Stauss*
Project Role:	*No change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	

Contribution to Project:	*No change*
Funding Support:	

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?

- **Organization Name:** University of Iowa
- **Location of Organization:** *Iowa, USA*
- **Partner's contribution to the projec**t. Dr. Harald Stuass; manufactures the custom vagus nerve stimulators. Harald also came to TX to demonstrate how to implant the stimulator coils around the carotid sheath, and also how to place the stimulator in the subcutaneous space.
  - **Financial support;** *Co-I on grant, U of Iowa funds*
  - **In-kind support** *Partner makes the vagus nerve stimulators*
  - **Facilities** *NA*
  - **Collaboration** *Dr. Stauss visited our lab in March, 2016, in order to instruct us on the proper implantation of the vagus nerve stimulators, as well as the correct way to ensure proper activation/de-activation of the stimulators.*
  - **Personnel exchanges** *N/A*
  - **Other.** *All work with Dr. Stauss occurred as specified in the proposal.*

8. **SPECIAL REPORTING REQUIREMENTS:** None
9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report.* ***DO NOT RENUMBER PAGES IN THE APPENDICES.***