<u>Select Gulf War Illness Research Publications (2020-21)</u>

Aggregated and lay summaries by Veterans for Common Sense
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Select publications of Research funded by the Gulf War Illness Research Program (GWIRP-CDMRP):

Abou-Donia, Mohamed B., Elizabeth S. Lapadula, Maxine H. Krengel, Emily Quinn, Jessica LeClair Joseph Massaro, Lisa A. Conboy, Efi Kokkotou, Maria Abreu, Nancy G. Klimas, Daniel D. Nguyen, and Kimberly Sullivan, "**Using Plasma Autoantibodies of Central Nervous System Proteins to Distinguish Veterans with Gulf War Illness from Healthy and Symptomatic Controls**," *Brain Sciences*, 2020, 10(9), 610: https://doi.org/10.3390/brainsci10090610

ABSTRACT: For the past 30 years, there has been a lack of objective tools for diagnosing Gulf War Illness (GWI), which is largely characterized by central nervous system (CNS) symptoms emerging from 1991 Gulf War (GW) veterans. In a recent preliminary study, we reported the presence of autoantibodies against CNS proteins in the blood of veterans with GWI, suggesting a potential objective biomarker for the disorder. Now, we report the results of a larger, confirmatory study of these objective biomarkers in 171 veterans with GWI compared to 60 healthy GW veteran controls and 85 symptomatic civilian controls (n = 50 myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and n = 35 irritable bowel syndrome (IBS)). Specifically, we compared plasma markers of CNS autoantibodies for diagnostic characteristics of the four groups (GWI, GW controls, ME/CFS, IBS). For veterans with GWI, the results showed statistically increased levels of nine of the ten autoantibodies against neuronal "tubulin, neurofilament protein (NFP), Microtubule Associated Protein-2 (MAP-2), Microtubule Associated Protein-Tau (Tau), alpha synuclein (α-syn), calcium calmodulin kinase II (CaMKII)" and glial proteins "Glial Fibrillary Acidic Protein (GFAP), Myelin Associated Glycoprotein (MAG), Myelin Basic Protein (MBP), S100B" compared to healthy GW controls as well as civilians with ME/CFS and IBS. Next, we summed all of the means of the CNS autoantibodies for each group into a new index score called the Neurodegeneration Index (NDI). The NDI was calculated for each tested group and showed veterans with GWI had statistically significantly higher NDI values than all three control groups. The present study **confirmed the utility of the use of plasma autoantibodies for CNS proteins to distinguish among veterans with GWI and other healthy and symptomatic control groups**.

LAY SUMMARY: This study confirmed and validated prior preliminary results of increased autoantibodies in a much larger sample of veterans with GWI (as compared to controls), and provided evidence to support a blood test to measure these autoantibodies as an objective measurement of GWI.

Abou-Donia, Mohamed B., Maxine H. Krengel, Elizabeth S. Lapadula, Clara G. Zundel, Jessica LeClair, Joseph Massaro, Emily Quinn, Lisa A. Conboy, Efi Kokkotou, Daniel D. Nguyen, Maria Abreu, Nancy G. Klimas, and Kimberly Sullivan, "**Sex-Based Differences in Plasma Autoantibodies to Central Nervous System Proteins in Gulf War Veterans versus Healthy and Symptomatic Controls**," *Brain Sci.* 2021, *11*(2), 148: https://doi.org/10.3390/brainsci11020148

ABSTRACT: Veterans from the 1991 Gulf War (GW) have suffered from Gulf War illness (GWI) for nearly 30 years. This illness encompasses multiple body systems, including the central nervous system (CNS). Diagnosis and treatment of GWI is difficult because there has not been an objective diagnostic biomarker. Recently, we reported on a newly developed blood biomarker that discriminates GWI from GW healthy controls, and symptomatic controls with irritable bowel syndrome (IBS) and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). The present study was designed to compare levels of these biomarkers between men and women with GWI, as well as sex-specific effects in comparison to healthy GW veterans and symptomatic controls (IBS, ME/CFS). The results showed that men and women with GWI differ in 2 of 10 plasma autoantibodies, with men showing significantly elevated levels. Men and women with GWI showed significantly different levels of autoantibodies in 8 of 10 biomarkers to neuronal and glial proteins in plasma relative to controls. In summary, the present study addressed the utility of the use of plasma autoantibodies for CNS proteins to distinguish among both men and women veterans with GWI and other healthy and symptomatic control groups.

LAY SUMMARY: This research team was one of the first to propose and document that GWI is related to chemical exposures during the war and that those exposures adversely affected the brain and throughout the central nervous system (CNS). In addition, they have documented differences in autoantibodies between GWI and controls, and that male veterans with GWI are even more affected. After further validation, the researchers are hopeful that their newly developed blood test and scoring system can be used to develop objective diagnostic markers of GWI and to compare treatment trial effectiveness for both men and women GW veterans.

Alshelh, Zeynab, Daniel S. Albrecht, Courtney Bergan, Oluwaseun Akeju, Daniel J. Clauw, Lisa Conboy, Robert R. Edwards, Minhae Kim, Yvonne C. Lee, Ekaterina Protsenko, Vitaly Napadow, Kimberly Sullivan, and Marco L. Loggia, "**In-vivo imaging of neuroinflammation in veterans with Gulf War illness**," *Brain Behav Immun*, 2020 Jul;87:498-507: https://doi.org/10.1016/j.bbi.2020.01.020

ABSTRACT: Gulf War Illness (GWI) is a chronic disorder affecting approximately 30% of the veterans who served in the 1991 Gulf War. It is characterized by a constellation of symptoms including musculoskeletal pain, cognitive problems and fatigue. The cause of GWI is not definitively known but exposure to neurotoxicants, the prophylactic use of pyridostigmine bromide (PB) pills, and/or stressors during deployment have all been suspected to play some pathogenic role. Recent animal models of GWI have suggested that neuroinflammatory mechanisms may be implicated, including a dysregulated activation of microglia and astrocytes. However, neuroinflammation has not previously been directly observed in veterans with GWI. To measure GWI-related neuroinflammation in GW veterans, we conducted a Positron Emission Tomography (PET) study using [11C]PBR28, which binds to the 18 kDa translocator protein (TSPO), a protein upregulated in activated microglia/macrophages and astrocytes. Veterans with GWI (n = 15) and healthy controls (HC, n = 33, including a subgroup of healthy GW veterans, HCVET, n = 8), were examined using integrated [11C]PBR28 PET/MRI. Standardized uptake values normalized by occipital cortex signal (SUVR) were compared across groups and against clinical variables and circulating inflammatory cytokines (TNF-α, IL-6 and IL-1β). SUVR were validated against volume of distribution ratio (n = 13). Whether compared to the whole HC group, or only the HCVET subgroup, veterans with GWI demonstrated widespread cortical elevations in [11C]PBR28 PET signal, in areas including precuneus, prefrontal, primary motor and somatosensory cortices. There were no significant group differences in the plasma levels of the inflammatory cytokines evaluated. There were also no significant correlations between [11C]PBR28 PET signal and clinical variables or circulating inflammatory cytokines. Our **study provides the first direct evidence of brain upregulation of the neuroinflammatory marker** TSPO in veterans with GWI and **supports the exploration of neuroinflammation as a therapeutic target** for this disorder.

LAY SUMMARY: Neuroinflammation has long been thought to underly Gulf War Illness. This study provides the first direct evidence of involvement of TSPO – a specific neuroinflammatory marker that can be targeted with treatments – in veterans with GWI.

Brandon S. Baksh, Kristen L. Zayan, Raquel Goldhardt, Elizabeth R. Felix, Nancy Klimas, and Anat Galor, "**Ocular manifestations and biomarkers of Gulf War Illness in US veterans**," *Sci Rep*, 11, 6548, 2021 Mar 21: https://doi.org/10.1038/s41598-021-86061-0

ABSTRACT: Gulf War Illness (GWI) is a multisystem disease with variable presentations, making diagnosis difficult. Non-invasive biomarkers would aid in disease diagnosis. We hypothesized that the eye could serve as a biomarker for GWI. We performed a retrospective case–control study using a sample of 1246 patients seen during a 5-month period in an optometry clinic. We identified veterans who were active duty during the Gulf War Era and either had a questionnaire-based diagnosis of GWI (cases) or did not (controls). Medical records were reviewed for eye and medical co-morbidities, medication use, and retinal macular and nerve fiber layer (NFL) thicknesses based on optical coherence tomography (OCT) images. Compared to controls (n = 85), individuals with GWI (n = 60) had a higher frequency of dry eye symptoms (50% vs 32.9%, p = 0.039). Multivariable analysis revealed average retinal NFL thickness (odds ratio; OR = 0.95), cup-to-disc ratio (OR = 0.005), age (OR = 0.82), and PTSD (OR = 20.5) were predictors of a GWI diagnosis. We conclude that GWI is associated with dry eye symptoms and RNFL thinning may serve as a biomarker for disease.

LAY SUMMARY: This study found that GWI is associated with dry eye disease (DE) symptoms, and that the chronic inflammation underpinning GWI may lead to inflammation in the surface of the eye and to nerve

abnormalities that lead to persistent dry-eye symptoms. The study also found changes in the retinal nerve fiber layer (RNFL) and that RNFL thinning may serve as a biomarker for GWI.

Baksh, Brandon S., Julia Costa Garcia, and Anat Galor, "**Exploring the Link Between Dry Eye and Migraine: From Eye to Brain**," *Eye Brain*, 2021 Mar 4;13:41-57: https://doi.org/10.2147/EB.S234073

ABSTRACT: Dry eye and migraine are common diseases with large societal and economic burdens that have recently been associated in the literature. This review outlines the link between dry eye and migraine, which may have implications for reducing their respective burdens. We highlight possible shared pathophysiology, including peripheral and central sensitization, as the potential link between dry eye and migraine. Finally, therapies targeting similar pathophysiological mechanisms between dry eye and migraine are discussed.

LAY SUMMARY: Involvement of eye conditions in Gulf War Illness is only just beginning to be recognized and investigated. This publication links between several funded studies, including one funded by GWIRP, to outline underlying links between dry eye disease (emerging among GWI patients) and migraines (commonly reported and well documented in a GWI subpopulation), and discusses potential treatments.

Bose, Dipro, Ayan Mondal, Punnag Saha, Diana Kimono, Sutapa Sarkar, Ratanesh K. Seth, Patricia Janulewicz, Kimberly Sullivan, Ronnie Horner, Nancy Klimas, Mitzi Nagarkatti, Prakash Nagarkatti, and Suarabh Chatterjee, "**TLR Antagonism by Sparstolonin B Alters Microbial Signature and Modulates Gastrointestinal and Neuronal Inflammation in Gulf War Illness Preclinical Model**," *Brain Sci*, 2020 Aug 8;10(8):532: https://doi.org/10.3390/brainsci10080532

ABSTRACT: The 1991 Persian Gulf War veterans presented a myriad of symptoms that ranged from chronic pain, fatigue, gastrointestinal disturbances, and cognitive deficits. Currently, no therapeutic regimen exists to treat the plethora of chronic symptoms though newer pharmacological targets such as microbiome have been identified recently. Toll-like receptor 4 (TLR4) antagonism in systemic inflammatory diseases have been tried before with limited success, but strategies with broad-spectrum TLR4 antagonists and their ability to modulate the host-microbiome have been elusive. Using a mouse model of Gulf War Illness, we show that a nutraceutical, derived from a Chinese herb Sparstolonin B (SsnB) presented a unique microbiome signature with an increased abundance of butyrogenic bacteria. SsnB administration restored a normal tight junction protein profile with an increase in Occludin and a parallel decrease in Claudin 2 and inflammatory mediators high mobility group box 1 (HMGB1), interleukin-1β (IL-1β), and interleukin-6 (IL-6) in the distal intestine. SsnB also decreased neuronal inflammation by decreasing IL-1β and HMGB1, while increasing brain-derived neurotrophic factor (BDNF), with a parallel decrease in astrocyte activation in vitro. Mechanistically, SsnB inhibited the binding of HMGB1 and myeloid differentiation primary response protein (MyD88) to TLR4 in the intestine, thus attenuating TLR4 downstream signaling. Studies also showed that SsnB was effective in suppressing TLR4-induced nod-like receptor protein 3 (NLRP3) inflammasome activation, a prominent inflammatory disease pathway. SsnB significantly decreased astrocyte activation by decreasing colocalization of glial fibrillary acid protein (GFAP) and S100 calcium-binding protein B (S100B), a crucial event in neuronal inflammation. Inactivation of SsnB by treating the parent molecule by acetate reversed the deactivation of NLRP3 inflammasome and astrocytes in vitro, suggesting that SsnB molecular motifs may be responsible for its anti-inflammatory activity.

LAY SUMMARY: There is increasing recognition of gut-brain involvement in GWI, and GWIRP has funded multiple relevant studies. Some of this work is related to the "good bacteria" found in the gut and the byproducts they produce that keep the gut wall healthy and intact – thereby preventing "leaky gut" and the resultant symptoms throughout the body. This study found that a particular treatment was helpful, in a mouse model of GWI, not only in restoring a healthy gut balance, but also in improving the brain inflammation found in GWI.

Baraniuk, James N., Grant Kern, Vaishnavi Narayan, and Amrita Cheema, "**Exercise modifies glutamate and other metabolic biomarkers in cerebrospinal fluid from Gulf War Illness and Myalgic encephalomyelitis / Chronic Fatigue Syndrome**," *PLoS One*, 2021 Jan 13;16(1):e0244116: https://doi.org/10.1371/journal.pone.0244116

ABSTRACT: Myalgic encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS) and Gulf War Illness (GWI) share many symptoms of fatigue, pain, and cognitive dysfunction that are not relieved by rest. Patterns of serum metabolites in ME/CFS and GWI are different from control groups and suggest potential dysfunction of energy and lipid metabolism. The metabolomics of cerebrospinal fluid was contrasted between ME/CFS, GWI and sedentary controls in 2 sets of subjects who had lumbar punctures after either (a) rest or (b) submaximal exercise stress tests. Postexercise GWI and control subjects were subdivided according to acquired transient postexertional postural tachycardia. Banked cerebrospinal fluid specimens were assayed using Biocrates AbsoluteIDQ® p180 kits for quantitative targeted metabolomics studies of amino acids, amines, acylcarnitines, sphingolipids, lysophospholipids, alkyl and ether phosphocholines. Glutamate was significantly higher in the subgroup of postexercise GWI subjects who did not develop postural tachycardia after exercise compared to nonexercise and other postexercise groups. The only difference between nonexercise groups was higher lysoPC a C28:0 in GWI than ME/CFS suggesting this biochemical or phospholipase activities may have potential as a biomarker to distinguish between the 2 diseases. Exercise effects were suggested by elevation of short chain acylcarnitine C5-OH (C3-DC-M) in postexercise controls compared to nonexercise ME/CFS. Limitations include small subgroup sample sizes and absence of postexercise ME/CFS specimens. Mechanisms of glutamate neuroexcitotoxicity may contribute to neuropathology and "neuroinflammation" in the GWI subset who did not develop postural tachycardia after exercise. Dysfunctional lipid metabolism may distinguish the predominantly female ME/CFS group from predominantly male GWI subjects.

LAY SUMMARY: While this study's findings are complex, one important takeaway is further evidence for the possible role of glutamate in the neuroinflammation found in GWI.

Gargas, Nathan M., Victoria T. Ethridge, Molly K. Miklasevich, and Joyce G. Rohan, "**Altered hippocampal function and cytokine levels in a rat model of Gulf War illness**," *Life Sci*, 2021 Mar 8;119333: https://doi.org/10.1016/j.lfs.2021.119333

ABSTRACT: Aims: Gulf War illness (GWI) is a disorder affecting military personnel deployed in the Gulf War (GW) from 1990 to 1991. Here, we will use a rat model of GWI to evaluate hippocampal function and cytokine levels. Materials and methods: Rats were exposed to diethyltoluamide and permethrin via dermal absorption and pyridostigmine bromide via gavage with or without a 5-min restraint for 28 days. Immediate and delayed effects of GW chemical exposure were evaluated using electrophysiology to quantitate hippocampal function, behavioral tests to assess cognitive effects and biochemical assays to measure neurotransmitter and cytokine levels. Key findings: Behavioral data revealed a statistically significant increase in motor activity at 3 months following completion of exposures, potentially indicating increased excitability, and/or restlessness. Electrophysiology data revealed statistically significant changes in paired pulse facilitation and input-output function of CA1 hippocampal neurons within 24 h and 3 months following completion of exposures. There was also a statistically significant reduction in the frequency of spontaneous firing activity of hippocampal neurons within 24 h following exposures. Naïve hippocampal slices directly incubated in GW chemicals also resulted in similar changes in electrophysiological parameters. Biochemical measurements revealed reduced hippocampal glutamate level at 3 months post-exposure. Furthermore, there was a statistically significant increase in plasma and hippocampal levels of IL-13, as well as decrease in plasma level of IL-1β. Significance: Our data support an effect on glutamate signaling within the hippocampus as indicated by changes in PPF and hippocampal level of glutamate, with some activation of T helper type 2 immune response.

LAY SUMMARY: This study provides further evidence, in an established rat model of GWI, of the role of glutamate and sustained immune system activation following exposure to Gulf War toxins.

Golomb, Beatrice A., Hayley J. Koslik, Jun Hee Han, Anna Helena Preger Guida, Gavin Hamilton, and Richard I. Kelley, "**A Pilot Study of Bioenergetic Marker Relationships in Gulf War Illness: Phosphocreatine Recovery vs. Citric Acid Cycle Intermediates**," *Int J Environ Res Public Health*, 2021 Feb; 18(4):1635: https://doi.org/10.3390/ijerph18041635

ABSTRACT: Impaired bioenergetics have been reported in veterans with Gulf War illness (VGWIs), including prolonged post-exercise recovery of phosphocreatine (PCr-R) assessed with ^{31}Phosphorus magnetic resonance spectroscopy. The citric acid cycle (CAC) is considered the most important metabolic pathway for supplying energy, with relationships among CAC markers reported to shift in some but not all impaired bioenergetic settings. We

sought to assess relations of CAC markers to one another and to PCr-R. Participants were 33 VGWIs and 33 healthy controls 1:1 matched on age–sex–ethnicity. We assessed seven CAC intermediates, and evaluated PCr-R in a subset of matched case–control pairs (N = 14). CAC markers did not significantly differ between cases and controls. Relationships of alpha-ketoglutarate to malate, isocitrate, and succinate were strongly significant in cases with materially weaker relationships in controls, suggesting possible shifts in these markers in concert in VGWIs. PCr-R correlated strongly with five of seven CAC markers in controls (succinate, malate, fumarate, citrate, isocitrate, range r = −0.74 to −0.88), but bore no relationship in VGWIs. In summary, PCr-R related significantly to CAC markers in healthy controls, but not VGWIs. In contrast, relations of CAC markers to one another appeared to shift (often strengthen) in VGWIs.

LAY SUMMARY: This study found objective evidence of impaired bioenergetics, which has long been believed to be impaired in GWI following exercise or exertion. For context, the lead researcher made the initial findings of the ability for Coenzyme Q10 (CoQ10) to reduce multiple GWI symptoms. These new findings provide evidence for markers further explaining the mechanisms underlying impaired bioenergetics in GWI.

Guan, Yi, Chia-Hsin Cheng, Weifan Chen, Yingqi Zhang, Sophia Koo, Maxine Krengel, Patricia Janulewicz, Rosemary Toomey, Ehwa Yang, Rafeeque Bhadelia, Lea Steele, Jae-Hun Kim, Kimberly Sullivan, and Bang-Bon Koo, "**Neuroimaging Markers for Studying Gulf-War Illness: Single-Subject Level Analytical Method Based on Machine Learning**," *Brain Sci*, 2020 Nov; 10(11):884: https://doi.org/10.3390/brainsci10110884

ABSTRACT: Gulf War illness (GWI) refers to the multitude of chronic health symptoms, spanning from fatigue, musculoskeletal pain, and neurological complaints to respiratory, gastrointestinal, and dermatologic symptoms experienced by about 250,000 GW veterans who served in the 1991 Gulf War (GW). Longitudinal studies showed that the severity of these symptoms often remain unchanged even years after the GW, and these veterans with GWI continue to have poorer general health and increased chronic medical conditions than their non-deployed counterparts. For better management and treatment of this condition, there is an urgent need for developing objective biomarkers that can help with simple and accurate diagnosis of GWI. In this study, we applied multiple neuroimaging techniques, including T1-weighted magnetic resonance imaging (T1W-MRI), diffusion tensor imaging (DTI), and novel neurite density imaging (NDI) to perform both a group-level statistical comparison and a single-subject level machine learning (ML) analysis to identify diagnostic imaging features of GWI. Our results supported NDI as the most sensitive in defining GWI characteristics. In particular, our classifier trained with white matter NDI features achieved an accuracy of 90% and F-score of 0.941 for classifying GWI cases from controls after the cross-validation. These results are consistent with our previous study which suggests that NDI measures are sensitive to the microstructural and macrostructural changes in the brain of veterans with GWI, which can be valuable for designing better diagnosis method and treatment efficacy studies.

SUMMARY: This GWIRP-funded study involving imaging diagnostic methods found that a novel method, called neurite density imaging (NDI), is sensitive to the micro- and macro-structural changes in the brain of veterans with GWI, which is valuable for improving GWI diagnosis and, perhaps most importantly, measuring the efficacy of GWI treatments being tested.

Holton, Kathleen F., Anna E. Kirkland, Michael Baron, Shalini S. Ramachandra, Mackenzie T. Langan, Elizabeth T. Brandley, and James N. Baraniuk, "**The low glutamate diet effectively improves pain and other symptoms of Gulf War Illness**," *Nutrients*, 2020 Aug 26;12(9):2593: https://doi.org/10.3390/nu12092593

ABSTRACT: Gulf War Illness (GWI) is a multisymptom disorder including widespread chronic pain, fatigue and gastrointestinal problems. The objective of this study was to examine the low glutamate diet as a treatment for GWI. Forty veterans with GWI were recruited from across the US. Outcomes included symptom score, myalgic score, tender point count, dolorimetry and the Chalder Fatigue Scale. Subjects were randomized to the low glutamate diet or a wait-listed control group, with symptom score being compared after one month. Subjects then went onto a double-blind, placebo-controlled crossover challenge with monosodium glutamate (MSG)/placebo to test for return of symptoms. Symptom score was compared between diet intervention and wait-listed controls with an independent t-test and effect size was calculated with Cohen's *d*. Change scores were analyzed with Wilcoxon Signed Rank tests. Crossover challenge results were analyzed with General Linear Models and cluster analysis. The diet intervention group reported significantly less symptoms ($p = 0.0009$) than wait-listed controls, with a very large effect size, $d = 1.16$. Significant improvements in average dolorimetry ($p = 0.0006$), symptom score, tender point number, myalgic

score and the Chalder Fatigue Scale (all $p < 0.0001$) were observed after the 1-month diet. Challenge with MSG/placebo resulted in significant variability in individual response. These results suggest that the low glutamate diet can effectively reduce overall symptoms, pain and fatigue in GWI, but differential results upon challenge suggest that other aspects of the diet, or underlying differences within the population, may be driving these changes. Future research is needed to identify potential nutrient effects, biomarkers, and underlying metabolic differences between responders and non-responders.

LAY SUMMARY: This clinical pilot study found that a low-glutamate whole foods diet was effective in reducing multiple GWI symptoms.

Joshi, Utsav, James E. Evans, Andrew Pearson, Nicole Saltiel, Adam Cseresznye, Teresa Darcey, Joseph Ojo, Andrew P. Keegan, Sarah Oberlin, Benoit Mouzon, Daniel Paris, Nancy Klimas, Kimberly Sullivan, Michael Mullan, Fiona Crawford, and Laila Abdullah, "**Targeting sirtuin activity with nicotinamide riboside reduces neuroinflammation in a GWI mouse model**," *Neurotoxicology*, 2020 Jul;79:84-94: https://doi.org/10.1016/j.neuro.2020.04.006

ABSTRACT: Gulf War Illness (GWI) affects 30% of veterans from the 1991 Gulf War (GW), who suffer from symptoms that reflect ongoing mitochondria dysfunction. Brain mitochondria bioenergetics dysfunction in GWI animal models corresponds with astroglia activation and neuroinflammation. In a pilot study of GW veterans (n = 43), we observed that blood nicotinamide adenine dinucleotide (NAD) and sirtuin 1 (Sirt1) protein levels were decreased in the blood of veterans with GWI compared to healthy GW veterans. Since nicotinamide riboside (NR)-mediated targeting of Sirt1 is shown to improve mitochondria function, we tested whether NR can restore brain bioenergetics and reduce neuroinflammation in a GWI mouse model. We administered a mouse diet supplemented with NR at 100μg/kg daily for 2-months to GWI and control mice (n = 27). During treatment, mice were assessed for fatigue-type behavior using the Forced Swim Test (FST), followed by euthanasia for biochemistry and immunohistochemistry analyses. Fatigue-type behavior was elevated in GWI mice compared to control mice and lower in GWI mice treated with NR compared to untreated GWI mice. Levels of plasma NAD and brain Sirt1 were low in untreated GWI mice, while GWI mice treated with NR had higher levels, similar to those of control mice. Deacetylation of the nuclear-factor κB (NFκB) p65 subunit and peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) was an increase in the brains of NR-treated GWI mice. This corresponded with a decrease in pro-inflammatory cytokines and lipid peroxidation and an increase in markers of mitochondrial bioenergetics in the brains of GWI mice. These findings suggest that targeting NR mediated Sirt1 activation restores brain bioenergetics and reduces inflammation in GWI mice. Further evaluation of NR in GWI is warranted to determine its potential efficacy in treating GWI.

LAY SUMMARY: This study honed in on bioenergetics issues and chronic inflammation underlying GWI. It found that the particular compound being studied, nicotinamide riboside (NR) was effective in an established animal (mouse) model of GWI in reducing inflammation and restoring bionenergetics in the brain. This study provided the needed evidence to advance this potentially promising compound (NR) to clinical evaluation in veterans with GWI.

Kaimal, Girijah, and Rebekah Dieterich-Hartwell, "**Grappling with Gulf War Illness: Perspectives of Gulf War Providers**," *Int J Environ Res Public Health*, 2020 Nov; 17(22):8574. https://doi.org/10.3390/ijerph17228574

ABSTRACT: *Background*: Although the Gulf War occurred almost 30 years ago, the chronic symptoms of Gulf War illness (GWI), which include respiratory, gastrointestinal, and skin problems, as well as fatigue, pain, and mood alterations, currently affect over 200,000 veterans. Meanwhile, healthcare providers lack clear guidelines about how to best treat this illness. The objective in this study was to learn about the perceptions and experiences of healthcare providers of GWI veterans in terms of medical symptoms, resources for treatment, and quality of care. *Methods*: We interviewed 10 healthcare providers across the United States and subsequently conducted a qualitative grounded theory study which entailed both systematic data analysis and generating a grounded theory framework. *Results*: Our findings indicated multiple challenges for providers of veterans with GWI, including gaps in knowledge about GWI, lack of treatment options, absence of consistent communication within the Department of Veterans Affairs (VA) system, and personalized care that was limited to validation. *Conclusion*: While this study had several limitations, it supported the notion that healthcare providers have inadequate knowledge and awareness about GWI, which leads to continued uncertainty about how to best care for GWI veterans. This could be remedied by the creation of a

comprehensive curriculum for a Massive Open Online Course (MOOC) to serve as an educational tool for those attending to this largely overlooked veteran population.

LAY SUMMARY: This GWIRP-funded study of healthcare providers of veterans with GWI, an "overlooked and underserved veteran population," supported the widely held concern among veterans with GWI that there are still large gaps in knowledge about GWI among healthcare providers, which leads to continued uncertainty about how to best treat GWI veterans. The findings indicate that it is imperative that providers have access to educational materials on GWI and recommend providing readily accessible educational resources to healthcare providers, including presentations, articles, and an evidence-based, current curriculum for a MOOC for GWI patients' healthcare providers, both in and outside of the VA system. [NOTE: A MOOC development project of this type has now been funded by the GWIRP].

Phillips, Kristin F., and Laxmikant S. Deshpande, "**Calcium Hypothesis of Gulf War Illness: Role of Calcium Ions in Neurological Morbidities in a DFP-Based Rat Model for Gulf War Illness**," *Neurosci Insights*, 2020; 15: 2633105520979841: https://doi.org/10.1177/2633105520979841

ABSTRACT: Gulf War Illness (GWI) refers to a multi-system disorder that afflicts approximately 30% of First Gulf War (GW) veterans. Amongst the symptoms exhibited, mood and memory impairment are commonly reported by GW veterans. Exposure to organophosphate (OP) compounds which target the cholinergic system is considered a leading cause for GWI symptoms. It is hypothesized that chronic OP-based war-time stimulation of cholinergic signaling led to recruitment of excitatory glutamatergic signaling and other downstream signaling cascades leading to neuronal injury, neuroinflammation, generation of reactive oxygen species, oxidative stress, and mitochondrial damage within the central nervous system. These findings have been observed in both experimental models and GWI veterans. In this context the role of calcium (Ca^{2+}) signaling in GWI has come to the forefront. Here we present our Ca^{2+} hypothesis of GWI that suggests sustained neuronal Ca^{2+} elevations serve as a molecular trigger for pathological synaptic plasticity that has allowed for the persistence of GWI symptoms. Subsequently we discuss that therapeutic targeting of Ca^{2+} homeostatic mechanisms provides novel targets for effective treatment of GWI-related neurological signs in our rodent model.

LAY SUMMARY: This study further investigated elevated intracellular calcium (Ca2+) levels in the brain cells (neurons) of rats in a Gulf War exposure model of GWI. The study's findings suggest this elevation may explain in part the persistence of GWI symptoms. A treatment targeting analysis shows promising new targets for treatment of GWI-related neurological problems.

Dickey, Brandon, Leelavathi N. Madhu, and Ashok K. Shetty, "**Gulf War Illness: Mechanisms Underlying Brain Dysfunction and Promising Therapeutic Strategies**," *Pharmacol Ther*, 2021 Apr;220:107716: https://doi.org/10.1016/j.pharmthera.2020.107716

ABSTRACT: Gulf War Illness (GWI), a chronic multisymptom health problem, afflicts ~30% of veterans served in the first GW. Impaired brain function is among the most significant symptoms of GWI, which is typified by persistent cognitive and mood impairments, concentration problems, headaches, chronic fatigue, and musculoskeletal pain. This review aims to discuss findings from animal prototypes and veterans with GWI on mechanisms underlying its pathophysiology and emerging therapeutic strategies for alleviating brain dysfunction in GWI. Animal model studies have linked brain impairments to incessantly elevated oxidative stress, chronic inflammation, inhibitory interneuron loss, altered lipid metabolism and peroxisomes, mitochondrial dysfunction, modified expression of genes relevant to cognitive function, and waned hippocampal neurogenesis. Furthermore, the involvement of systemic alterations such as the increased intensity of reactive oxygen species and proinflammatory cytokines in the blood, transformed gut microbiome, and activation of the adaptive immune response have received consideration. Investigations in veterans have suggested that brain dysfunction in GWI is linked to chronic activation of the executive control network, impaired functional connectivity, altered blood flow, persistent inflammation, and changes in miRNA levels. Lack of protective alleles from Class II HLA genes, the altered concentration of phospholipid species and proinflammatory factors in the circulating blood have also been suggested as other aiding factors. While some drugs or combination therapies have shown promise for alleviating symptoms in clinical trials, larger double-blind, placebo-controlled trials are needed to validate such findings. Based on improvements seen in animal models of GWI, several antioxidants and anti-inflammatory compounds are currently

being tested in clinical trials. However, reliable blood biomarkers that facilitate an appropriate screening of veterans for brain pathology need to be discovered. A liquid biopsy approach involving analysis of brain-derived extracellular vesicles in the blood appears efficient for discerning the extent of neuropathology both before and during clinical trials.

LAY SUMMARY: This publication is a review, with a particular focus on the issues impacting the brain in GWI, of other peer-reviewed published findings about the mechanisms underlying GWI and strategies for effectively treating them. Animal model studies have linked brain impairments to chronic oxidative stress, chronic inflammation, negative impact on certain types of key brain cells (called interneurons), and numerous other negative outcomes found in GWI. Other animal studies have discussed chronic inflammation, chronic immune system activation, and a negative impact on the "good" bacteria in the gut. In human studies of Veterans with GWI, multiple negative outcomes have been linked to brain dysfunction, immune dysfunction, chronic inflammation. While pilot studies have found potential treatments, those that had positive findings need to be validated in gold standard clinical trials to ensure they are both effective and safe. Among the treatments showing promise are several antioxidants and anti-inflammatory compounds. There remains a need for good blood biomarkers to appropriately screen veterans and to help objectively determine the degree of effectiveness of the treatments being tested.

ABSTRACT: *Background:* A new national cohort of Gulf War (GW) veterans of 1,318 participants was created from the Veterans Affairs Cooperative Studies Program 585 Gulf War Era Cohort and Biorepository (GWECB) pilot study. However, female veteran health outcomes have not been reported separately for those deployed versus nondeployed to the 1990-1991 GW. *Methods:* Using data from the cooperative studies program (CSP) #585 GWECB, this study examined whether excess prevalence and patterns of Gulf War Illness (GWI) symptoms were present among female veterans who served during the GW compared with female veterans who did not deploy to the GW (GW-Era). *Results:* A total of 301 women veterans participated in the survey (203 GW, 98 GW-era). Mean ages in 2016 were 53 years among GW women veterans and 54 years among GW-era women. Participant groups did not differ by age, race, ethnicity, or education, but GW women were more likely to have served in the army or navy and less likely to have served in the air force. Compared with GW-era women, GW-deployed women were significantly more likely to report 7 out of 34 symptoms related to cognitive, neurological, and mood problems and respiratory complaints when controlling for age, race, GW deployment, branch of service, and smoking status in logistic regression analyses. Ordered logistic regression was also used to estimate the association between the total number of self-reported symptoms and deployment status, age, race, branch of service, and smoking status. Results showed deployed GW veterans to have a nearly twofold risk of reporting more symptoms than GW-era women, with younger, nonwhite, army-enlisted GW women significantly more likely to report more total symptoms. *Discussion:* Twenty-five years after the war, GWECB women GW veterans continued to report a wide variety of symptoms at a significantly higher excess frequency prevalence than GW-era women. Our results showed at least a 14% excess frequency prevalence in all seven significantly different symptoms encompassing two out of the six Kansas GWI criteria, including neurological/mood/cognition, and respiratory domains. These results suggest that further study of these symptom domains is warranted in GW women veterans.

LAY SUMMARY: This study of women Gulf War veterans, who made up seven percent of the troops deployed in the 1990-91 Gulf War, continued at 25 years after the war to report rates of multiple symptoms at double the rate of their female non-deployed counterparts, demonstrating that GWI remains a serious chronic health issue for this cohort.

ABSTRACT: Gulf War illness is associated with a combination of exposure to war-related chemical agents and traumatic stress. Currently, there are no effective treatments, and the pathophysiology remains elusive. Neurological problems are among the most commonly reported symptoms. In this study, we investigated the glutamatergic system in the hippocampi of mice exposed to war-related chemical agents and stress. Mice developed Gulf War illness-like symptoms, including mood deficits, cognitive impairments, and fatigue. They exhibited the following pathological changes in hippocampi: elevated extracellular glutamate levels, impaired glutamatergic synapses, astrocyte atrophy, loss of interneurons, and decreased neurogenesis. LDN/OSU-215111 is a small-molecule that can strengthen the structure and function of both the astrocytic processes and the glutamatergic synapses that together form the tripartite synapses. We found that LDN/OSU-215111 effectively prevented the development of mood and cognitive deficits in mice when treatment was implemented immediately following the exposure. Moreover, when symptoms were already present, LDN/OSU-215111 still significantly ameliorated these deficits; impressively, benefits were sustained one month after treatment cessation, indicating disease modification. LDN/OSU-215111 effectively normalized hippocampal pathological changes. Overall, this study provides strong evidence that restoration of tripartite glutamatergic synapses by LDN/OSU-215111 is a potential therapy for Gulf War illness.

LAY SUMMARY: This study focused on the glutamatergic system in the brains of mice in an established animal model of GWI. It found that a particular compound (LDN/OSU-215111), which is also being investigated for other neurologic conditions such as Alzheimer's Disease, was effective as both a prevention for the development of GWI symptoms soon after exposure and for a treatment of GWI long after the original exposure. This study provided the evidence needed to advance this compound to a clinical evaluation in veterans with GWI.

Washington, Stuart D., Rakib U. Rayhan, Richard Garner, Destie Provenzano, Kristina Zajur, Florencia Martinez Addiego, John W. VanMeter, and James N. Baraniuk, "**Exercise alters cerebellar and cortical activity related to working memory in phenotypes of Gulf War Illness**," *Brain Commun,* 2020;2(1):fcz039: https://doi.org/10.1093/braincomms/fcz039

ABSTRACT: Gulf War Illness affects 25-32% of veterans from the 1990-91 Persian Gulf War. Post-exertional malaise with cognitive dysfunction, pain and fatigue following physical and/or mental effort is a defining feature of Gulf War Illness. We modelled post-exertional malaise by assessing changes in functional magnetic resonance imaging at 3T during an N-Back working memory task performed prior to a submaximal bicycle stress test and after an identical stress test 24 h later. Serial trends in postural changes in heart rate between supine and standing defined three subgroups of veterans with Gulf War Illness: Postural Orthostatic Tachycardia Syndrome (GWI-POTS, 15%, n = 11), Stress Test Associated Reversible Tachycardia (GWI-START, 31%, n = 23) and Stress Test Originated Phantom Perception (GWI-STOPP, no postural tachycardia, 54%, n = 46). Before exercise, there were no differences in blood oxygenation level-dependent activity during the N-Back task between control (n = 31), GWI-START, GWI-STOPP and GWI-POTS subgroups. Exercise had no effects on blood oxygenation level-dependent activation in controls. GWI-START had post-exertional deactivation of cerebellar dentate nucleus and vermis regions associated with working memory. GWI-STOPP had significant activation of the anterior supplementary motor area that may be a component of the anterior salience network. There was a trend for deactivation of the vermis in GWI-POTS after exercise. These patterns of cognitive dysfunction were apparent in Gulf War Illness only after the exercise stressor. Mechanisms linking the autonomic dysfunction of Stress Test Associated Reversible Tachycardia and Postural Orthostatic Tachycardia Syndrome to cerebellar activation, and Stress Test Originated Phantom Perception to cortical sensorimotor alterations, remain unclear but may open new opportunities for understanding, diagnosing and treating Gulf War Illness.

LAY SUMMARY: This study of post-exertional malaise, a core symptom of GWI, found objective patterns of cognitive dysfunction following exercise in veterans with GWI. While the underlying mechanisms remain unclear, these identified patterns may impact improved understanding of GWI's underlying pathobiology, its definition and diagnosis, and GWI treatment development.

Yee, Megan K., Clara G Zundel, Alexis L. Maule, Timothy Heeren, Susan P. Proctor, Kimberly A. Sullivan, and Maxine H. Krengel, "**Longitudinal Assessment of Health Symptoms in Relation to Neurotoxicant Exposures in 1991 Gulf War Veterans: The Ft. Devens Cohort**," *J Occup Environ Med*, 2020 Sep;62(9):663-668: https://doi.org/10.1097/JOM.0000000000001910

ABSTRACT: Objective: This analysis examined the relationship between Gulf War (GW) exposures and health symptoms reported in three time periods over 20 years in Ft. Devens Cohort veterans. Methods: Repeated logistic regression models examined the association of exposures and health symptoms over time. Models included baseline age, active duty status, post-traumatic stress disorder status, sex, and time since deployment as covariates. Results: Exposure to tent heaters was associated with increased odds of crying easily and muscle twitching. Exposure to pyridostigmine bromide (PB) pills was associated with increased odds of depression and fatigue. Exposure to the Khamisiyah sarin plume was associated with increased odds of trouble concentrating and crying easily. Conclusion: This longitudinal analysis demonstrated an association between neurotoxicant exposures and increased odds of cognitive/mood, fatigue, and neurological symptoms. In addition, most symptoms increased over time since deployment regardless of exposure.

LAY SUMMARY: This study of Gulf War veterans from a large, established, cohort of Gulf War veterans, the Ft. Devens Cohort, found evidence implicating several Gulf War exposures in GWI symptoms, including the Khamisiyah sarin nerve agent plume, pyridostigmine bromide (PB) nerve agent protective pills (NAPP's), and tent heaters. The study also found that these GWI most of these symptoms increased over time since deployment. Together, these added findings further link Gulf War toxic exposures to GWI symptoms, and provide further evidence that GWI has not only not improved, but worsened over time.

Donovan, Emily K., Sophia Kekes-Szabo, Joanne C. Lin, Rebecca L. Massey, James D. Cobb, Kathleen S. Hodgin, Timothy J. Ness, Carl Hangee-Bauer, and Jarred W. Younger, "**A Placebo-Controlled, Pseudo-Randomized, Crossover Trial of Botanical Agents for Gulf War Illness: Curcumin (Curcuma longa), Boswellia (Boswellia serrata), and French Maritime Pine Bark (Pinus pinaster)**," *Int. J. of Environ. Rsch & Public Health*, 2021 Mar; 18(5): 2468: https://doi.org/10.3390/ijerph18052468

ABSTRACT: This report is part of a larger study designed to rapidly and efficiently screen potential treatments for Gulf War Illness (GWI) by testing nine different botanicals. In this placebo-controlled, pseudo-randomized, crossover clinical trial of 20 men with GWI, we tested three botanical agents with putative peripheral and central anti-inflammatory actions: curcumin (*Curcuma longa*), boswellia (*Boswellia serrata*), and French maritime pine bark extract (*Pinus pinaster*). Participants completed 30 +/− 3 days of baseline symptom reports, followed by 30 +/− 3 days of placebo, 30 +/− 3 days of lower-dose botanical, and 30 +/− 3 days of higher-dose botanical. Participants then repeated the process with a new botanical until completing up to three botanical cycles. Data were analyzed using linear mixed models. Curcumin reduced GWI symptom severity significantly more than placebo at both the lower ($p < 0.0001$) and higher ($p = 0.0003$) dosages. Boswellia was not more effective than placebo at reducing GWI symptoms at either the lower ($p = 0.726$) or higher ($p = 0.869$) dosages. Maritime pine was not more effective than placebo at the lower dosage ($p = 0.954$) but was more effective than placebo at the higher dosage ($p = 0.006$). This study provides preliminary evidence that curcumin and maritime pine may help alleviate symptoms of GWI. As a screening study, a final determination of the efficacy of these compounds for all individuals with GWI cannot be made, and further studies will need to be conducted to determine strength and durability of effects, as well as optimal dosage. These results suggest that GWI may, at least in part, involve systemic inflammatory processes. This trial was registered on ClinicalTrials.gov (NCT02909686) on 13 September 2016.

LAY SUMMARY: This study investigated nine compounds as potential treatments for GWI. This publication details the results for three of them, including Curcumin (contained in turmeric), Boswellia, and French maritime pine bark extract. Both Curcumin and the pine bark extract were found to reduce some GWI symptoms, with Curcumin effective at both low and higher doses. [**Of note, Curcumin is currently being tested as a treatment by the GWIRP-funded Gulf War Illness Clinical Trials and Interventions Consortium (GWICTIC).**]

Hodgin, Kathleen S., Emily K. Donovan, Sophia Kekes-Szabo, Joanne C. Lin, Joseph Feick, Rebecca L. Massey, Timothy J. Ness, and Jarred W. Younger, "**A Placebo-Controlled, Pseudo-Randomized, Crossover Trial of Botanical Agents for Gulf War Illness: Resveratrol (Polygonum cuspidatum), Luteolin, and Fisetin (Rhus succedanea)**," *Int J Environ Res Public Health*, 2021 Mar; 18(5): 2483: https://doi.org/10.3390/ijerph18052483

ABSTRACT: A chronic multi-symptom illness of unknown etiology, Gulf War Illness (GWI) affects 175,000 to 250,000 veterans of the Gulf War. Because inflammation has suspected involvement in the pathophysiology of GWI, botanical treatments that target inflammation may be beneficial in reducing symptoms. No FDA-approved treatments currently exist for GWI, and rapid prioritization of agents for future efficacy testing is important. This

study is part of a larger project that screened nine different botanical compounds with purported anti-inflammatory properties for potential treatment of GWI. We tested three botanicals (resveratrol [*Polygonum cuspidatum*], luteolin, and fisetin [*Rhus succedanea*]) on symptom severity of GWI in this placebo-controlled, pseudo-randomized clinical trial. Twenty-one male veterans with GWI completed the study protocol, which consisted of 1 month (30 days ± 3) of baseline symptom reports, 1 month of placebo, 1 month of lower-dose botanical, and 1 month of higher-dose botanical. Participants completed up to 3 different botanicals, repeating the placebo, lower-dose, and higher-dose cycle for each botanical assigned. Linear mixed models were used for analyses. Resveratrol reduced GWI symptom severity significantly more than placebo at both the lower ($p = 0.035$) and higher ($p = 0.004$) dosages. Luteolin did not decrease symptom severity more than placebo at either the lower ($p = 0.718$) or higher dosages ($p = 0.492$). Similarly, fisetin did not reduce symptom severity at either the lower ($p = 0.504$) or higher ($p = 0.616$) dosages. Preliminary findings from this screening study suggest that resveratrol may be beneficial in reducing symptoms of GWI and should be prioritized for future testing. Larger trials are required to determine efficacy, response rates, durability of effects, safety, and optimal dosage. This trial was registered on ClinicalTrials.gov (NCT02909686) on 13 September 2016.

LAY SUMMARY: This study investigated nine compounds as potential treatments for GWI. This publication details the results for three of them, including resveratrol, luteolin, and fisetin. This study provided further evidence that Resveratrol, a readily available antioxidant, was found to reduce some GWI symptoms and was effective in doing so at both low and higher doses.

Select publications of research funded by VA:

Chao, Linda, Jennifer C. Kanady, Nicole Crocker, Laura D. Straus, Jennifer Hlavin, Thomas J. Metzler, Shira Maguenab, and Thomas C. Neylan, "**Cognitive behavioral therapy for insomnia in veterans with gulf war illness: Results from a randomized controlled trial**," *Life Sci*, 2021 Feb 4;119147: https://doi.org/10.1016/j.lfs.2021.119147

ABSTRACT: Aims: To examine whether cognitive behavioral therapy for insomnia (CBT-I), delivered by telephone, improves sleep and non-sleep symptoms of Gulf War Illness (GWI). Main methods: Eighty-five Gulf War veterans (21 women, mean age: 54 years, range 46–72 years) who met the Kansas GWI case definition, the Centers for Disease Control and Prevention (CDC) case definition for Chronic Multisymptom Illness (CMI), and research diagnostic criteria for insomnia disorder were randomly assigned to CBT-I or monitor-only wait list control. Eight weekly sessions of individual CBT-I were administered via telephone by Ph.D. level psychologists to study participants. Outcome measures included pre-, mid-, and post-treatment assessments of GWI and insomnia symptoms, subjective sleep quality, and continuous sleep monitoring with diary. Outcomes were re-assessed 6-months post-treatment in participants randomized to CBT-I. Key findings: Compared to wait list, CBT-I produced significant improvements in overall GWI symptom severity, individual measures of fatigue, cognitive dysfunction, depression and anxiety, insomnia severity, subjective sleep quality, and sleep diary outcome measures. The beneficial effects of CBT-I on overall GWI symptom severity and most individual GWI symptom measures were maintained 6-months after treatment. Significance: GWI symptoms have historically been difficult to treat. Because CBT-I, which is associated with low stigma and is increasingly readily available to veterans, improved both sleep and non-sleep symptoms of GWI, these results suggest that a comprehensive approach to the treatment of GWI should include behavioral sleep interventions.

[**NOTE: A follow-on clinical evaluation by this PI for comorbid insomnia and sleep apnea has been funded by the GWIRP (FY20 award).**]

Keledijan, Kaspar, Orest Tsymbalyuk, Stephen Semick, Mitchell Moyer, Serban Negoita, Kevin Kim, Svetlana Ivanova, Volodymyr Gerzanich, and J. Marc Sijan, "**Analysis of neuroinflammation and neurofunction,**

including cognition, found that an off-the-shelf insulin sensitizing drug, Rosiglitazone (trade name Avandia), was beneficial in a rat model of Gulf War Illness and Gulf War exposures," *PLoS One*, 2020; 15(11): e0242427: https://doi.org/10.1371/journal.pone.0242427

ABSTRACT: Background: Gulf War (GW) Illness (GWI) is a debilitating condition with a complex constellation of immune, endocrine and neurological symptoms, including cognitive impairment, anxiety and depression. We studied a novel model of GWI based on 3 known common GW exposures (GWE): (i) intranasal lipopolysaccharide, to which personnel were exposed during desert sand storms; (ii) pyridostigmine bromide, used as prophylaxis against chemical warfare; and (iii) chronic unpredictable stress, an inescapable element of war. We used this model to evaluate prophylactic treatment with the PPARγ agonist, rosiglitazone (ROSI). Methods: Rats were subjected to the three GWE for 33 days. In series 1 and 2, male and female GWE-rats were compared to naïve rats. In series 3, male rats with GWE were randomly assigned to prophylactic treatment with ROSI (GWE-ROSI) or vehicle. After the 33-day exposures, three neurofunctional domains were evaluated: cognition (novel object recognition), anxiety-like behaviors (elevated plus maze, open field) and depression-like behaviors (coat state, sucrose preference, splash test, tail suspension and forced swim). Brains were analyzed for astrocytic and microglial activation and neuroinflammation (GFAP, Iba1, tumor necrosis factor and translocator protein). Neurofunctional data from rats with similar exposures were pooled into 3 groups: naïve, GWE and GWE-ROSI. Results: Compared to naïve rats, GWE-rats showed significant abnormalities in the three neurofunctional domains, along with significant neuroinflammation in amygdala and hippocampus. There were no differences between males and females with GWE. GWE-ROSI rats showed significant attenuation of neuroinflammation and of some of the neurofunctional abnormalities. Conclusion: This novel GWI model recapitulates critical neurofunctional abnormalities reported by Veterans with GWI. Concurrent prophylactic treatment with ROSI was beneficial in this model.