

# Gulf War Illness Research Program

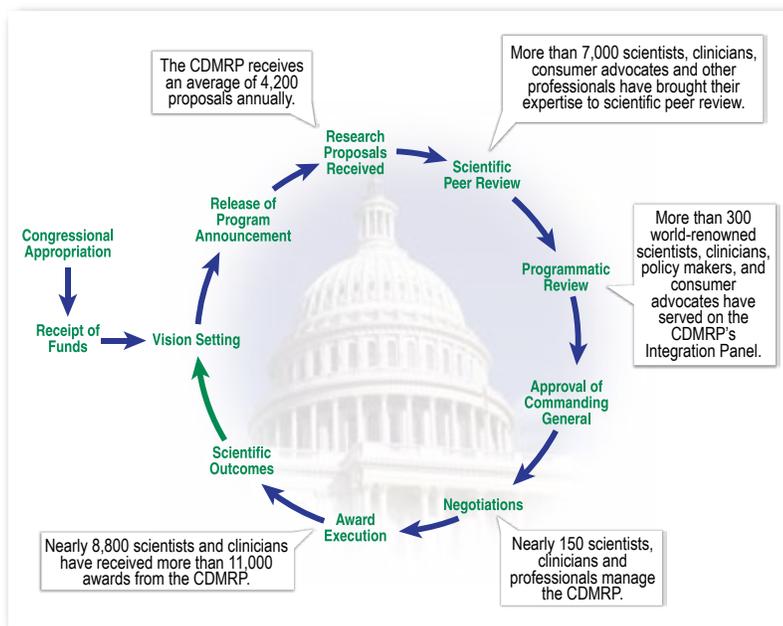


# Congressionally Directed Medical Research Programs

**HISTORY** The Congressionally Directed Medical Research Programs (CDMRP) office was born in 1992 from a powerful grassroots effort led by the breast cancer advocacy community that resulted in a congressional appropriation of funds for breast cancer research. This initiated a unique partnership among the public, Congress, and the military. Since then, the CDMRP has grown to encompass many targeted programs and has received over \$8 billion in appropriations from its inception in fiscal year 1993 (FY93) through FY13. Through FY13, nearly 12,000 awards have been made across 25 individual programs. Funds for the CDMRP are added by Congress to the Department of Defense (DOD) budget annually to provide support for targeted research programs in breast, prostate, and ovarian cancers; neurofibromatosis; autism; and other areas with military health interests including psychological health, traumatic brain injury, orthopedic injury, spinal cord injury, and Gulf War Illness (GWI). Under the auspices of the U.S. Army Medical Research and Materiel Command (USAMRMC), the CDMRP manages these programs from receipt of funds to competitive selection of applications, through individual project performance to closeout.



The CDMRP program management cycle includes a two-tier review process for application evaluation recommended by the National Academy of Sciences' Institute of Medicine. The first tier of evaluation is an external scientific peer review of applications against established criteria for determining scientific merit. The second tier is a programmatic review conducted by an Integration Panel (IP) composed of program-specific researchers, clinicians, and consumers who evaluate applications on innovation, potential impact, programmatic priorities, and mechanism-specific criteria. The Commanding General of the USAMRMC issues the final approval for funding prior to award negotiations and execution.



# Gulf War Illness Research Program

## VISION

Improve the health and lives of veterans who have Gulf War Illness.

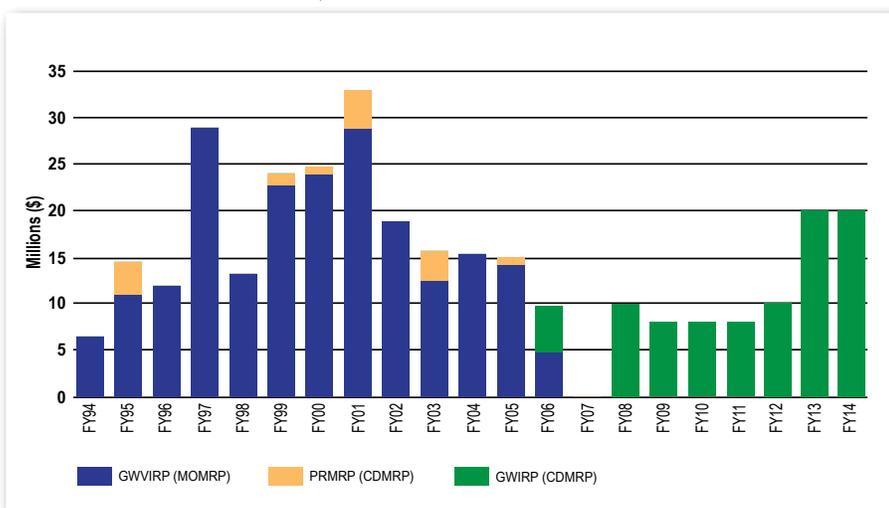
## MISSION

Fund innovative Gulf War Illness research to identify effective treatments, improve definition and diagnosis, and better understand pathobiology and symptoms.

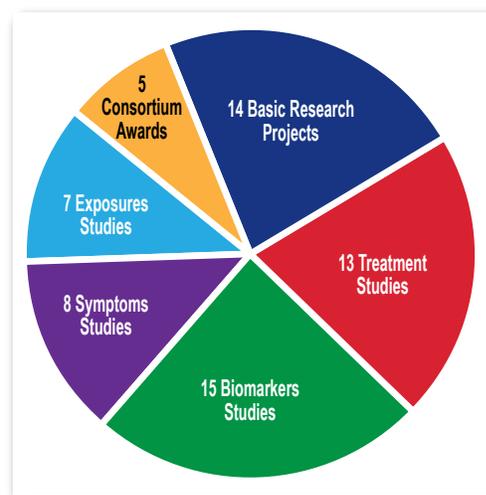
## HISTORY

DOD-funded GWI research began in 1994 with the establishment of a Gulf War Veterans' Illnesses Research Program (GWVIRP) to study the health effects of service members deployed in the 1990–1991 Persian Gulf War. From FY94 to FY05, the GWVIRP was managed by the USAMRMC Military Operational Medicine Research Program (MOMRP). Research pertaining to GWI also was funded intermittently through the CDMRP's Peer Reviewed Medical Research Program (PRMRP), which supports selected military health-related research topics each fiscal year. The MOMRP shared management responsibility for the GWVIRP with the CDMRP in FY06 with separate \$5 million (M) appropriations. Although the GWVIRP did not receive funding in FY07, a \$10M appropriation renewed the program in FY08, renamed the Gulf War Illness Research Program (GWIRP), to be managed fully by the CDMRP. Since that time, the GWIRP has been funded by an \$8M appropriation each year from FY09 through FY11, \$10M in FY12 and \$20M in FY13 and FY14. The program continues to support innovative, competitively peer-reviewed research to develop treatments addressing the complex symptoms that comprise GWI and their underlying causes, to identify objective markers (biomarkers) improving its diagnosis, and to better understand the pathobiology underlying GWI.

DOD GWI Research Investment



GWIRP-Funded Portfolio by Research Topic, FY06-FY12



## Scientists and Clinicians

The Integration Panel, or IP, is composed of prominent members of the GWI research community, including Gulf War veterans (i.e., consumers) suffering from the disease. During the annual vision setting meeting, the IP advises the GWIRP on programmatic focus and areas of research interest that can be addressed through certain award mechanisms. This is also where an investment strategy for the program year is recommended. Later in the program year, the IP meets to recommend to the GWIRP (through individual confidentially administered voting) which applications best fulfill the program's vision and mission while also demonstrating innovative science. The recommendations of IP members enable the GWIRP to find and fund cutting-edge research and set important program priorities designed to benefit ill Gulf War veterans.



"GWIRP capitalizes on the CDMRP mechanisms so that investigators can pursue research on Gulf War Illness, and that such research can be strategically funded by the agency. The GWIRP scientific panel and its staff are focused on research that will facilitate treatment of the disorder and an understanding of the underlying pathways and physiological mechanisms of the illness, with the ultimate goal of improving the health of Gulf War veterans still suffering over 20 years after the conflict."

***Dr. Roberta White, FY10-FY13 IP Member and Chair Emeritus***



"Serving on the GWIRP IP is a highlight of my career as a medical provider and compensation and pension examiner for the VA. It is an unequalled opportunity to interact with some of the best research minds in the country and play a role in facilitating their efforts to tackle one of the most daunting medical challenges of our time. The knowledge gained could have broad implications across other medical areas as well. In addition, the program is increasing awareness and understanding of the scope, impact, and medical complexity of GWI among medical providers and examiners such as myself, thereby helping us to better serve our veterans."

***Dr. K. Jeffery Meyers, FY10-FY13 IP Member***



# Consumers: “Boots on the Ground”

A unique aspect of the CDMRP is the active participation of consumer advocates throughout the program. Consumers are a vital part of all CDMRP programs as they represent the collective views of survivors, patients, family members, and those affected by and at risk for a disease. Consumers for the GWIRP are Gulf War veterans who are experiencing symptoms and illnesses that may be related to their military service in theater. They sit side by side with research professionals on both peer and programmatic review panels, and their voices play a pivotal role in maintaining an appropriate focus within the program.



INTEGRATION  
PANEL MEMEBERS

“It’s unfortunate that 23 years after the Persian Gulf War there remain so many unanswered questions as to “how” and “why” more than one-third of the warriors who fought in that war became chronically ill. It is, however, extremely encouraging that the CDMRP Gulf War Illness Research Program has made enormous strides within the past 8 years uncovering the “what” of Gulf War Illness. In the end, defining the illness and developing effective treatments is of far more importance than knowing the cause. In this regard, there is great optimism among the veteran community that, thanks to the dedicated scientists and physicians involved in Gulf War Illness Research, we are on the cusp of developing the medical protocols that will bring much needed relief to the hundreds of thousands suffering from the debilitating symptoms associated with Gulf War Illness.”



***Dave Winnett, FY13 GWIRP Consumer***

“The GWIRP is focused solely on improving our health and lives, by finding and funding the best medical research to better understand and treat Gulf War Illness. As such, the GWIRP is a source of inspiration and hope that there is a real possibility of improved health. It is of critical national importance for current and future generations.”



***Anthony Hardie, FY10-FY13 GWIRP Consumer***



# Brain Immune Interactions as the Basis of Gulf War Illness



**Kimberly Sullivan, Ph.D.**  
**Boston University Medical Campus**

A growing body of evidence indicates that GWI is associated with diverse central nervous system (CNS) and immune alterations, but the specific pathobiological processes driving GWI symptoms have not been clearly elucidated. Animal studies indicate that a chronic CNS inflammatory state can develop in response to an insult—chemical injury, infection, or physical trauma—that mobilizes CNS defense systems via activation of microglia, the brain’s primary immune response cells, and release of chemical messengers that precipitate a complex of “sickness behavior symptoms” identified by measures of impaired memory and learning, increased pain sensitivity, and persistent fatigue, a symptom complex similar to that of GWI. Recent studies have also demonstrated CNS inflammatory effects of GW-related exposures and additional immune and cellular processes plausibly explain the mechanisms contributing to the full spectrum of GWI symptoms.

To leverage recent findings and bring a broad range of techniques to bear on GWI, the CMDRP GWIRP has funded the Brain-Immune Interaction as the Basis of Gulf War Illness Consortium (GWIC) whose objective is to provide a cohesive understanding of the pathobiological mechanisms responsible for the symptoms of GWI in order to provide a targeted and efficient basis for identifying beneficial treatments and diagnostic markers.

The GWIC will perform studies of microglial activation and neuroinflammation in GWI. The GWIC will build upon the prior work of very experienced GWI researchers from diverse specialties and other experts in the fields of the proposed mechanisms of GWI. This includes experts in the immune system, brain structure, signaling mechanisms and genetics coming together from government agencies, universities, and industry to help solve the perplexing pathobiology of GWI by working together and learning from each other. By working together as a collaborative team, the GWIC will maximize research study funds to translate results from human, animal, and cell studies into effective and targeted human treatment trials of GWI. This could be particularly important as new treatments are currently available that specifically target brain-immune cross-talk pathways and reduce chronic neuroinflammation. Initial studies with animals will be conducted to assess which treatments may be most promising for treating GWI and for identifying those that may not be as effective without the high costs associated with human clinical treatment trials.

# Understanding Gulf War Illness: An Integrative Modeling Approach

FY12 CONSORTIUM  
AWARD



*Dr. Mariana Morris*



*Dr. Nancy Klimas*

**Mariana Morris, Ph.D., and  
Nancy Klimas, Ph.D.  
Nova Southeastern University**

Current treatments used for GWI treat only the symptoms associated with the disease and do not target the underlying disease process. The GWI Research Program Consortium at Nova Southeastern University funded by the GWIRP will integrate our clinical understanding of the disease process with basic research efforts using a novel combination of animal and mathematical models. By increasing our understanding of the reasons for GWI, the consortium seeks to identify targets of dysfunction and find treatments that will address the causes of the disease – not just treat the symptoms associated with the disease. This approach will also enable identification of targets for improved diagnosis.

It has been established that GWI is caused by a disruption in normal cell signaling that results in disabling symptoms including fainting, low blood pressure, autonomic dysfunction, fatigue, and pain. This is primarily due to disruptions in normal immune, cardiovascular, and hormone signaling. However, the exact cause is not understood. It is the goal of the consortium to pinpoint the causes and tailor treatments

effectively. Specifically, a more detailed understanding of GWI dysfunction would greatly speed up the identification of promising targets to help improve diagnosis and treatment of GWI.

The exciting part of this consortium is the rapid identification of potential disease targets and effective treatments made possible by the computational systems biology approach that ties together the clinical and basic research understanding of the disease. The consortium approach combines cardiovascular and immunological data derived from Gulf War veterans and from animal models of GWI and uses it to simulate the signaling occurring in the immune and endocrine systems. This data can be used to suggest potential combination therapies and single possible targets for intervention and can also be used as a screen to evaluate proposed interventions. Screening will yield a short list of potential treatments. Compounds already known and approved by the FDA for other conditions would be preferred candidates. At the end of the 4-year study, the consortium will have completed early studies in human patients that will provide insight into disease targets and effective treatments, enabling the design of larger-scale clinical trials for further drug testing.

# Exercise-Induced Cerebrospinal Proteomic Biomarkers of Fatigue



**James N. Baraniuk, M.D.**  
**Georgetown University**

One of the hallmark features of Gulf War Illness is that patients have exaggerated or dysfunctional responses to physical stress. Thus, Dr. James Baraniuk in an FY98 GWIRP award proposed to study pre- and post-exercise outcomes for exercise-induced alterations in cognition function, fatigue, and systemic hyperalgesia in Gulf War Illness patients. Study participants engaged in physical exercise on consecutive days, and various subjective and objective measures were made before and at various times after exercise. Sophisticated magnetic resonance imaging (MRI) techniques were used to measure brain region integrity and activation.

Study participants completed a bicycle exercise stress test on consecutive days. GWI subjects reported greater pain with isometric exercise than healthy control subjects, as the bicycle exercise test increased the level of pain experienced by GWI subjects during a hand grip contraction test on Day 2, consistent with suspected central sensitization in GWI.

Physical exertion parameters measured for the bicycle stress test (V02, VC02, and watts generated) were comparable through two stages of the activity across 2 days of testing, indicating that the exercise protocol had no significant detrimental effect on cardiopulmonary or muscle performance in GWI. Importantly, however, perceptions of fatigue increased during the protocol for GWI subjects but not sedentary controls. Fatigue intensity scores remained constant during the lower heart rate threshold (70% pHR) of the activity, but increased significantly at 85% pHR and during recovery. These results also suggest an interoceptive or neurocognitive component to GWI fatigue.

Postural tachycardia and changes in heart rate and blood pressure induced by standing, was assessed before and after exercise. Approximately one-third of GWI subjects developed postural tachycardia 3 hours after their first exercise activity and associated diastolic hypertension 8 hours later. This postural tachycardia disappeared 24 to 36 hours after the second exercise activity, indicating a reversible condition. No control subjects exhibited this condition. This subgroup, labeled Stress Test Associated Reversible Tachycardia (START), demonstrated cognitive fatigue and showed cerebellar compensation on their pre-exercise cognitive task called the “2-back test” that was disrupted by exercise. In addition to these physical effects, the START subgroup displayed other ailments such as rhinitis, dyspnea, and gastrointestinal complaints compared to the other GWI subgroup, as well as tissue-specific white matter brain damage.

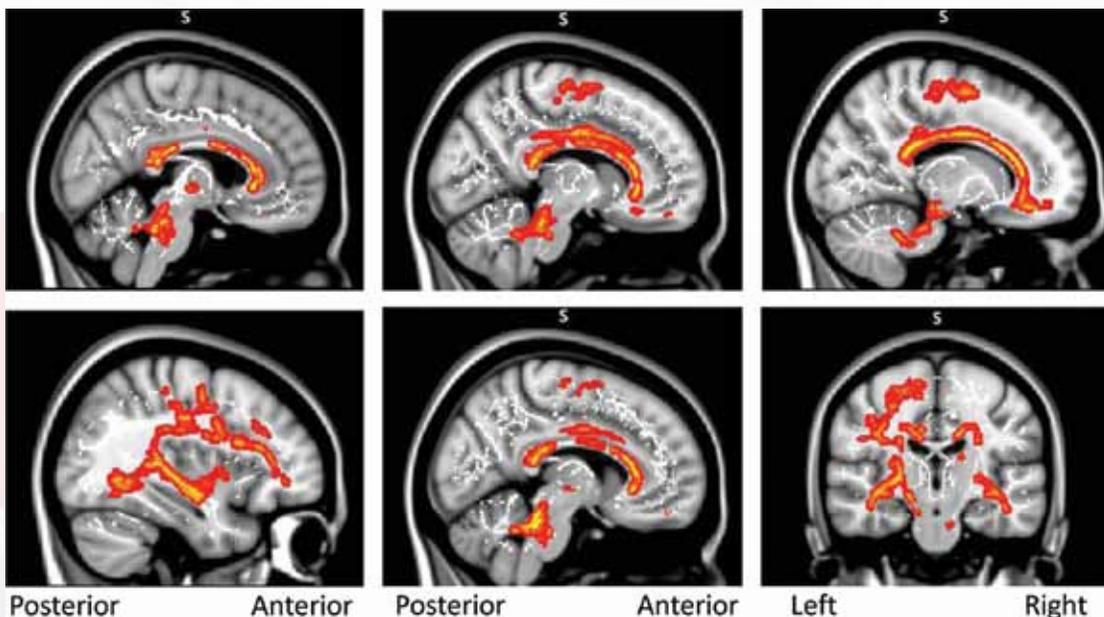
The remainder of GWI participants, labeled Stress Test Originated Phantom Perception (STOPP), had no increased heart rate after exercise, but had brain activation (in the insula), indicating increased perceptions of pain (phantom

perception), and basal ganglia compensation for cognitive testing that was disrupted by exercise. The two subgroups displayed disparate results in principal component analysis of fatigue scores, with the START subgroup showing cognitive fatigue in the first component and physical fatigue in the second, while the STOPP subgroup showed reversed scores.

Notably, in studies of brain integrity and function, Dr. Baraniuk found very specific detrimental changes in brain white matter in GWI patients that correlated with postural tachycardia observed in those GWI patients. The particular brain regions involved suggest these changes may result in a decreased ability to concentrate.

Dr. Baraniuk also studied cognitive ability and how it changed in response to exercise. Study participants performed a cognitive task called the “2-back test” at baseline and after each day of a 2-day exercise challenge. The 2-back test results after the first day of exercise revealed little; after the second day of exercise, the 2-back test showed the group was clearly divided into two subgroups: one subgroup whose test scores improved on the second day (Increasers) and another whose scores deteriorated (Decreasers). MRI tests showed the members of the two subgroups actually recruited different portions of the brain during testing, with Decreasers activating a region that is not usually activated in tasks similar to the “2-back” test. Furthermore, Decreasers’ brain metabolism of lactate was found to be abnormal. Discovery of these distinct subgroups of GWI patients highlights the need for tailored treatments.

Microarray studies are planned to assess serial changes in peripheral leukocyte messenger Ribonucleic acid (mRNA) and are predicted to provide insights into differences in transcriptional control in GWI.



***Damage to areas of white matter observed in GWI***

# Theory-Driven Models for Correcting “Fight or Flight” Imbalance in Gulf War Illness



**Gordon Broderick, Ph.D.**  
**Nova Southeastern University**

The body’s response to stress and exertion is controlled by a group of hormones known collectively as the hypothalamic-pituitary-adrenal (HPA) axis. Abnormalities in this control system have been observed in veterans with GWI. These hormones control the “fight or flight” response and are profoundly intertwined with the immune system and the control of sex hormones by the hypothalamic pituitary-gonadal (HPG) axis.

In control systems in general, some combinations of circumstance and control settings are “unstable” and will not persist, while others are “stable” equilibrium points that can be maintained chronically: The combination of a locomotive traveling at 40 miles per hour (MPH) with a control speed setting of 25 MPH is unstable because the speed will not remain the same but will decrease until a stable operating point is reached where the actual speed matches the control setting.

The normal interplay between the HPA axis, the immune system, and the sex hormone axis constitutes a stable “rule set” for this control network, one that can be maintained over a lifetime. Because of the chronic nature of GWI, Dr. Broderick’s team suspected that GWI might represent a different stable rule set for these control systems—stable, yet well away from the normal range of settings and behavior. In the example above, if the locomotive speed is set to 9000 MPH, an unattainable speed, the system will eventually arrive at a stable operating point where the train is traveling as fast as it possibly can under maximum power with the speed controller futilely calling for more speed.

To test the possibility that GWI represents an abnormal stable rule set for hormone and inflammation control systems, Dr. Gordon Broderick and colleague Dr. Travis Craddock used high-performance computers to construct a “mock-up” of the HPA control system and its connections both to the immune system and to the HPA and HPG hormone axes. Then they used established engineering control theory practice to identify stable operating points.

Such a simulation is an exceedingly complex undertaking, and high fidelity simulations require accurate values from experiments to describe how the level of one control molecule influences the activities of the other molecules. Unfortunately, such values are generally unavailable or have not been accurately measured in the human body, so the researchers used special methods for portraying biological systems as discrete logical networks that were originally developed from robust engineering control theory principles. This approach allows the development of reasonable simulations when many interaction parameters are not accurately known.



# Identification of Biological Pathways Implicated in Hippocampal Dysfunction and Cognitive Impairment in Gulf War Illness



**Fiona Crawford, Ph.D.**  
**The Roskamp Institute**

There had been a lack of research in well-characterized animal models until Dr. Fiona Crawford and her team used an FY08 GWIRP Investigator-Initiated Research Award to develop two mouse models of chemical exposures from the Gulf War. Studies included characterization of the cognitive and neurobehavioral impairment in the animals and proteomic analyses on blood and brain samples.

In the first model, Dr. Crawford aimed to replicate the Gulf War exposure profile demonstrated in a rat model by Abou-Donia, et al., in C57Bl/6 mice. This model combined exposure to the anti-sarin agent pyridostigmine bromide (PB), the pesticide permethrin (PER), and insect repellent N,N-Diethyl-meta-toluamide (DEET) and incorporated stress in the form of bodily restraint. After 28 days of exposure, mice exhibited sensorimotor deficits and astrogliosis (an abnormal increase in the number of astrocyte cells in the brain). They also exhibited novel anxiety-related behavior, with female mice generally more anxious than males, but they did not exhibit cognitive impairment, a primary complaint of those with GWI. This prompted Dr. Crawford to modify the exposure paradigm and time points of analyses.

In the second mouse model, Dr. Crawford used the CD1 mouse strain, removed the stress component, and gave a higher dose of PB and PER for 10 days by intraperitoneal injection. Exposed mice initially outperformed controls in memory tests but then exhibited delayed cognitive impairment, a phenomenon also observed in Gulf War veterans. A significant increase in astrogliosis was also detected in exposed mice compared to controls after cognitive impairment was evident, suggesting an association between the events. These neurobehavioral findings were subsequently confirmed in the more commonly used C57Bl/6 mouse strain.

Proteomic studies performed on brain and blood identified 31 proteins that were differentially expressed in brain tissue in response to the chemical exposure and were mostly associated with lipid metabolism, molecular transport, and endocrine and nervous system function (based on Ingenuity Pathway Analysis). They have now replicated the effects of PB+PER exposure in many different cohorts of mice, with analyses extending to more than a year after exposure in an effort to mimic the years post-exposure of our GWI patients today. The evidence for disruption of lipid metabolism and mitochondrial function are now key targets for Dr. Crawford's team.

In follow-on studies, Dr. Crawford is collaborating with clinicians to collect and investigate GWI patient blood samples for disruption of molecules and pathways related to those revealed in her mouse model, and it is hoped that these studies will uncover biomarkers for GWI diagnosis, prognosis, and monitoring treatment response.

# Proteomic Immune Profiling for the Therapeutic Modulation of Cognitive Impairment in a Novel GWI Mouse Model



## **Ghania Ait-Ghezala, Ph.D.** **The Roskamp Institute**

Dr. Ghania Ait-Ghezala used an FY10 Investigator-Initiated Research Award to develop mouse models of exposure to agents used in the Gulf War, including the acetylcholinesterase inhibitor pyridostigmine bromide (PB) and the pesticides permethrin (PER). Using these models she sought to better understand the functional and combinatorial protein changes evident in response to Gulf War agent exposures. Dr. Ait-Ghezala's

PB+PER exposure model turned out to be particularly relevant to GWI as it demonstrates delayed cognitive impairment accompanied by neuropathological changes. For this reason, Dr. Ait-Ghezala conducted a detailed molecular characterization of this model.

Dr. Ait-Ghezala evaluated neuropathology and cognitive impairment, and assayed the expression of thousands of proteins in the PB+PER exposure model using an unbiased proteomic approach. She applied bioinformatic analysis to identify specific protein changes and biomolecular pathways impacted by exposure. This work has identified several targets for potential therapeutic intervention in GWI, namely neuroinflammation and mitochondrial dysfunction. She demonstrated that there is a chronic inflammatory CNS and peripheral disturbance in the brains of exposed versus non-exposed mice. Dr. Ait-Ghezala also found evidence of an inflammatory response that is accompanied by a profound increase in astrogliosis, which is a consistent feature at chronic time-points post-exposure in every cohort of mice she has examined. This finding indicates that neuroinflammatory mechanisms and/or reactive oxygen species (ROS) should be investigated as specific targets for therapeutic intervention in GWI. She also observed reduced neurogenesis, reduced synaptophysin (a marker of pre-synaptic vesicles) as well as evidence for mitochondria and calcium signaling dysfunction. In addition, she has shown that compared to controls, exposed mice showed a significant decrease in a series of plasma-based pro-inflammatory cytokines: TNF- $\alpha$ , IL-6, IL-1 $\beta$ , IL-1 $\alpha$ , IL17 and INF- $\gamma$ , and in particular, the anti-inflammatory cytokine IL10.

These data suggest an anergic immune status in Gulf War agent-exposed mice compared to placebo-treated controls. Dr. Ait-Ghezala is continuing to scrutinize the altered neurobehavioral and neuropathological mechanisms in this animal model in the hope that it will help identify potential targets for therapeutic interventions to treat GWI.

# Effectiveness of Acupuncture in the Treatment of Gulf War Illness



**Lisa Conboy, M.A., M.S., Sc.D.**  
**Harvard School of Medicine**  
**New England School of Acupuncture**

Despite much research into the possible causes of GWI, few new treatments have emerged. In a Clinical Trial Award funded by the GWIRP in FY08, Dr. Lisa Conboy tested the hypothesis that acupuncture therapy could improve the physical functioning and overall quality of life for ill Gulf War veterans.

In this trial, 104 Gulf War veterans from the New England area meeting the inclusion criteria for GWI were randomized to either a regimen of biweekly individualized acupuncture treatments for 6 months, or wait-listed (control period) for 2 months, and then a regimen of weekly individualized acupuncture treatments for 4 months. Outcomes of the study measured changes in self-rated overall health in the Physical Functioning Subscale of the SF-36 (PFS), a short-form health survey of functional health and well-being, as well as other self-rated health inventories.

The study found that PFS scores improved significantly in the biweekly treatment group compared to the weekly treatment group. This improvement was led by the largest jump in scores in the final period (Months 4-6) in the biweekly treatment group, suggesting that a 6-month dose of treatment may be required to see clinically important effects in many veterans. Beyond the primary outcome, the study showed statistically significant improvement for both treatment groups in self-rated pain. In addition, participants reported a positive experience with acupuncture and acupuncturists. They expressed high confidence in the treatment and in recommending it to others.

The type of acupuncture treatment used in this study allowed for a highly individualized approach. As such, Dr. Conboy is planning further studies aimed at arriving at a better understanding of which veterans and which symptoms are most responsive to acupuncture, and the characteristics of the most successful individualized acupuncture treatments.

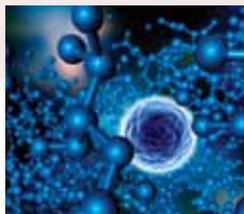
Dr. Conboy also plans to probe the results further to examine the effects of acupuncture on psychosocial factors and possibly discover blood-based biomarkers for improved status.

# Gulf War Illness Research on the Horizon

## **FY12 GWIRP Investigator-Initiated Research Awards:**



**Michelle Block, Ph.D.**  
Virginia Commonwealth University  
“The Role of Protein Radicals in Chronic Neuroimmune Dysfunction and Neuropathology in Response to a Multiple-Hit Model of Gulf War Exposures”



**Brian Cooper, Ph.D.**  
University of Florida  
“Persistent Neural Membrane Protein Misregulation Following Neurotoxicant Exposure”



**Beatrice Golomb, M.D., Ph.D.**  
University of California at San Diego  
“Gulf War Illness: Assessment of Bioenergetics in Brain and Muscle”



**Lisa Pierce, D.Sc.**  
Tripler Army Medical Center  
“Role of microRNAs in the Pathobiology of Gulf War Illness: Identification of Potential Novel Therapeutic Targets”





For more information, visit

<http://cdmrp.army.mil>

or contact us at:

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