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TITLE: Tau Pathology as a contributor to Gulf War Illness and a basis for potential therapy

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14. ABSTRACT The study plan is to differentiate the hiPSCs, which were derived from Gulf War (GW) veterans with Gulf War Illness (GWI) and healthy control veterans without GWI, into neurons, and to use these neurons as models to probe for tau pathologies when they are exposed to the GWI toxicants (Cortisol+DFP). Also, we want to see if reduction of tau or inhibition of tau phosphorylation helps alleviate the pathological changes. For the two-year proposed study, the first year has been devoted to differentiating the hiPSCs, investigating tau related pathological phenotypes and examining the morphological changes and microtubule deficit among the hiPSC derived neurons from both control and GWI cases when exposed to cortisol+DFP.					
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1. INTRODUCTION: Gulf War Illness (GWI) is thought to have its origins in exposure of soldiers serving in the 1991 war to various neurotoxicants, including pesticides, anti-nerve gas pills, and low level nerve gasses including sarin/cyclosarin. Mechanistic clues from animal and cell studies of GW-relevant organophosphate neurotoxicants at low levels include abnormalities in neuronal microtubules (MTs), as well as deficits in the movement of vesicles and organelles along MTs by the process known as axonal transport. Rodents exposed to GW-relevant organophosphate pesticides and nerve agents display neuronal MTs with fewer associated proteins than the normal situation, leading to reduced MT width. This may be due to abnormal phosphorylation of proteins that normally decorate the tubulin backbone of the MTs, such as the MT-associated protein – tau. Our preliminary studies with peripheral blood serum from veterans with GWI compared with symptomatic non-veteran controls has demonstrated an increase of auto-antibodies against tau. Hyperphosphorylation of tau releases it from the MT, giving rise to defective MT behaviors and configurations, and also leads to intracellular tau inclusions that produce gain-of-function toxicity. Long-term low-level organophosphate exposure has also been associated with neuroinflammation, which can directly induce tau hyperphosphorylation. Thus tau pathology in GWI might explain why a limited exposure to neurotoxicants caused a persistent disease that was not reversible upon termination of the exposure. The fact that tau goes awry in so many disease and injury scenarios fortifies it as a suspect for GWI etiology and importantly provides potential avenues for treatment development. We hypothesize that exposure to GW-related toxicants together with the physical stress of the battlefield caused many but not all of the veterans to develop tau pathology. Our objective is to use the hiPSC lines to model the disease, test for evidence of tau pathology, evaluate the impact on MT stability, and then vet a small number of therapeutic interventions to reverse the effects of the tau pathology.

2. KEYWORDS: Gulf War Illness, hiPSC, neuron, tau, phosphorylation

3. ACCOMPLISHMENTS:

What were the major goals of the project?

- Aim 1. Differentiate various subtypes of human CNS neurons derived from the Gulf War hiPSC repository bank and examine tau pathology with and without GWI toxicant exposures.
- Aim 2. Screen for potential therapeutic inhibitory compounds and antibodies to alleviate tau phenotypes as well as MT stability defects.

What was accomplished under these goals?

- The hiPSCs from the veterans were differentiated into forebrain glutamatergic neurons. They were validated for specific markers like MAP2, Tbr1, β III tubulin, vGlut and they express high level of human tau (Figure 1). These differentiated human neurons also can form functional synapses in cultures supported by the multi-electrode array (MEA) system.
- We also confirmed that hiPSC-derived neurons from both control and GWI patients developed tau pathologies when they were exposed to cortisol (mimicking stress) and DFP (sarin analog). Briefly, cortisol significantly elevated the level of the tau protein in these neurons, whereas DFP increased the phosphorylation of tau. Therefore, our GWI toxicant regimen induced a unique tau pathology which could contribute to the

development of the disease (Figure 2). It is strikingly interesting that hiPSC-derived neurons from GWI cases seemed to express way more tau proteins (~4 folds higher) than controls, which potentially led to the vulnerability of hiPSC-derived neurons from GWI to the tau pathologies. We are currently analyzing the other potential changes of the tau phenotypes including: a) examining 3R and 4R ratios of tau; b) measuring the extracellular tau level via ELISA; c) probing other pathological forms of tau via multiple isotope specific tau antibodies, such as TOC1, TNT2, ALZ50 and PHF1. We are actively analyze these results and aim to submit a manuscript in several months.

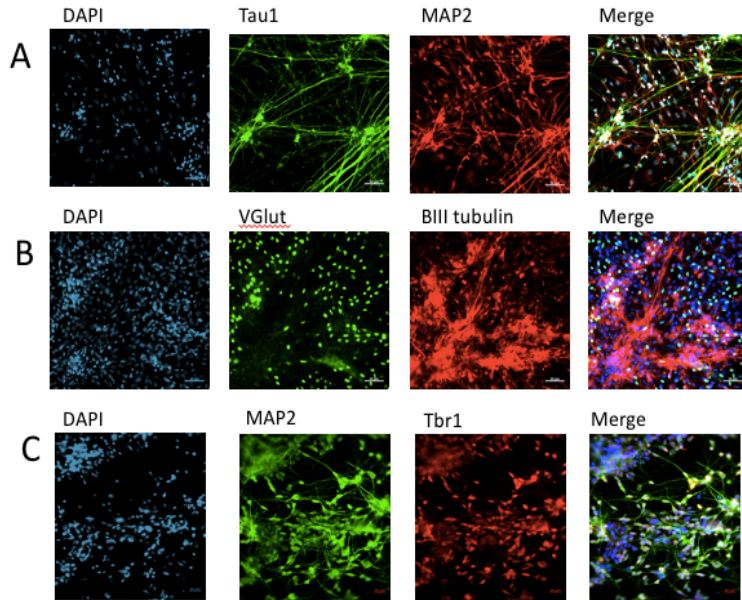


Figure 1. Neuronal validation of hiPSC-derived neurons. (A) Tau1 stains for total tau and is used to mark mature neurons. MAP2 is a microtubule-associated protein. (B) VGlut marks glutamatergic neurons and excitatory synapses. BIII tubulin is a neuron-specific tubulin maker. (C) Tbr1 is a transcription factor for mature forebrain glutamatergic neurons.

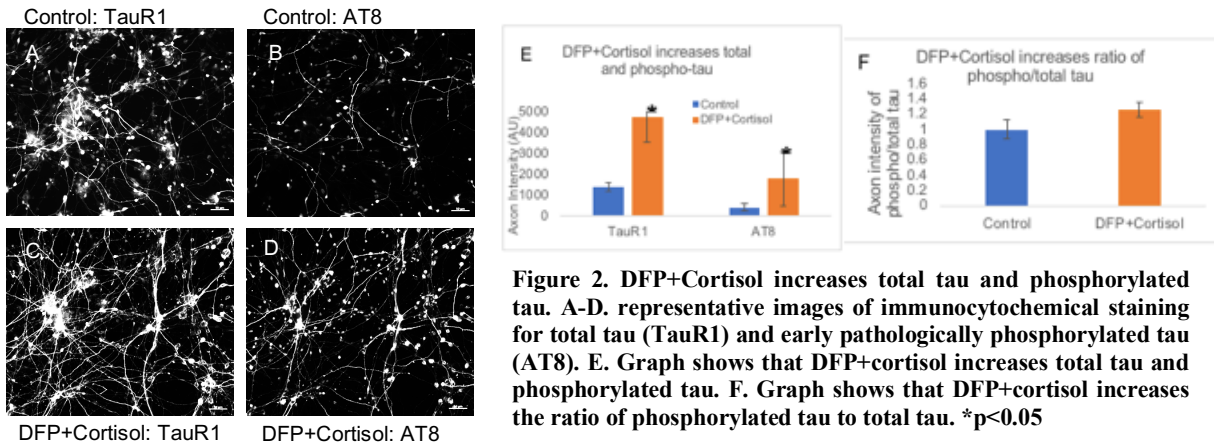


Figure 2. DFP+Cortisol increases total tau and phosphorylated tau. A-D. representative images of immunocytochemical staining for total tau (TauR1) and early pathologically phosphorylated tau (AT8). E. Graph shows that DFP+cortisol increases total tau and phosphorylated tau. F. Graph shows that DFP+cortisol increases the ratio of phosphorylated tau to total tau. *p<0.05

- We also confirmed that our GWI regimen (cortisol+DFP) causes deficits in the axonal transport of mitochondria in hiPSC-derived neurons (Figure 3). The cell-permeable dye tetramethylrhodamine, ethyl ester (TMRE) was used for the study. TMRE is actively taken up by mitochondria with an intact membrane potential, and so the dye can be used to track mitochondrial transport. The GWI treatment regimen decreased the percentage of mitochondria that move, and among moving mitochondria, the neurotoxicants reduced the distance and speed of traveling mitochondria (Figure 3).

These impairments in axonal transport, and of mitochondria in particular, can have a variety of deleterious effects on neurons, including disrupting energy production in the cell by mitochondria, as well as disrupting the transport and release of secretory vesicles carrying neurotransmitters to the synapse. Alterations in these processes might contribute to some of the long-lasting cognitive symptoms of GWI, such as chronic fatigue, reduced information processing speeds and memory deficits, synaptic dysfunction, and other cognitive complaints.

- No obvious morphological differences were identified in between all the hiPSC derived neurons. There is a decrease in the acetylation in the hiPSCs from both control an GWI cases when they were exposed to cortisol+DFP.

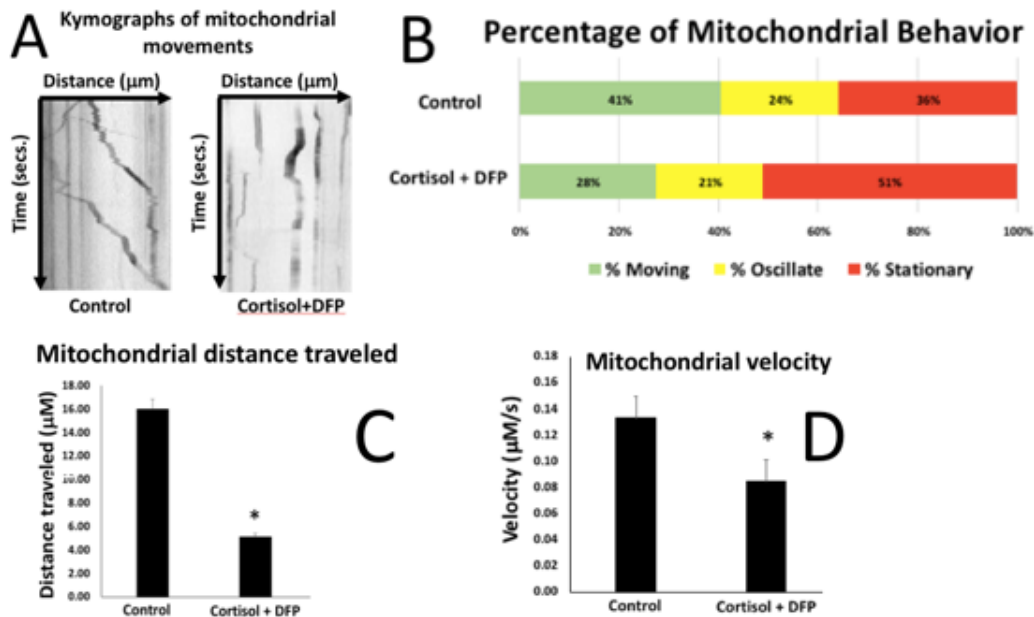


Figure 3. Neurotoxicant/stress-mediated impairment of mitochondrial transport. A. Representative kymographs showing mitochondrial movements along axons over time. B. Percentage of mitochondrial behavior. The graph shows a significant decrease in the number of moving mitochondria and a significant increase in the number of stationary mitochondria after Cortisol+DFP treatment. C. Distance traveled by mitochondria along the axon. The graph shows a significant decrease in distance traveled after Cortisol+DFP treatment. D. Velocity of mitochondria along the axon. The graph shows a significant decrease in velocity after Cortisol+DFP treatment. * $p < 0.05$

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

- This project provides an excellent opportunity for me to train Mr. Phil Yates, who is an MD/PhD student. Via this training process, Mr. Yates obtained the clinical and basic research knowledge of GWI and mastered the skills of conducting experiments using human stem cells. He also gained a lot of experience of interacting and communicating with other GWI research scientists by attending to GWI related conferences and meetings.

How were the results disseminated to communities of interest?

- Mr. Phil Yates (the MD/PhD student working under my supervision) and I are going to attend SFN 2018 annual conference presenting a poster titled “tau pathology as a mechanism underlying microtubule-based deficits in Gulf War Illness”.
- The news forum at Drexel University reported our hiPSC related GWI studies. This report was disseminated by some other medical news websites, such as www.medicalexpress.com and www.laboratoryequipment.com.
- The plans are underway to submit the one or two manuscripts indicated above for publication in high visibility journals

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

- During the next funding period, Aim 2 will be pursued, precisely as proposed.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

- This study is the first of its kind using neuronal cells and tissues generated from the veterans with and without GWI to probe for human specific pathological changes.
- Human iPSC derived neuronal cells were generated and available to be used for the collaborative investigations for the GWI researchers.

What was the impact on other disciplines?

- Nothing to report.

What was the impact on technology transfer?

- The resource of GWI hiPSC derived neurons will not be used for commercial profit. We will work through BBRAIN to make our developed protocols and technology available for all of the GWI investigators for collaborative projects.

What was the impact on society beyond science and technology?

- Our study indicated that hiPSC derived neurons can be effectively used as models to study human neurological disorders.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

- Nothing to report.

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

- Nothing to report.

Changes that had a significant impact on expenditures

- Nothing to report.

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

- Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

- One manuscript will be prepared for submission in several months.

Website(s) or other Internet site(s)

- <http://sites.bu.edu/gwic/>
- <https://www.facebook.com/gwicboston>

Technologies or techniques

- hiPSCs from veterans with and without GWI were differentiated to neuronal cultures. We are providing the knowledge and protocols for the GWI investigators who are interested in developing the neuronal cells from the veterans upon collaborative requests via BBRAIN.

Inventions, patent applications, and/or licenses

- Nothing to report.

Other Products

- Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

- Liang Qiang, Peter W. Baas, Kimberly Sullivan, Phil Yates: No change.

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:** Boston University
- **Location of Organization:** 715 Albany Street, Boston MA 02118
- **Partner's contribution to the project:** Supplying the hiPSCs stocked in the stem cell facility in Boston University; Dr. Kimberly Sullivan and Dr. Ronald Killiany are the collaborators in this project.

**8. SPECIAL REPORTING REQUIREMENTS
COLLABORATIVE AWARDS**

- n/a.

QUAD CHARTS

- n/a.

9. APPENDICES

- n/a.