AWARD NUMBER: W81XWH-22-1-0329

TITLE: Are Tau Proteins in Blood and PET Images Related to Gulf War Illness and Risk of Comorbid Neurological Disorders?

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CONTRACTING ORGANIZATION: Boston University Medical Campus, Boston, MA

REPORT DATE: July 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2023	Annual	01Jun2022-31May2023

4. TITLE AND SUBTITLE

Are Tau Proteins in Blood and PET Images Related to Gulf War Illness and Risk of Comorbid Neurological Disorders?

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-22-1-0329

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Boston University
Medical Campus
Boston, MA 02118

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The primary objective of this study is to compare total tau levels and types of tau species called p-tau 181 and p-tau 217 levels in stored blood samples from Gulf War veterans. Tau levels have been associated with neurological disorders. This study has two parts: first tau markers will be analyzed in the stored blood of 300 veterans from the BBRAIN repository. Second, 30 veterans will be recruited to participate in Tau PET brain scans to see where these markers are in the brain. We will compare tau levels in healthy GW veterans to those with Gulf War Illness as well as veterans with exposures to GW exposures including pesticides, PB anti-nerve gas pills, sarin and mild traumatic brain injury.

15. SUBJECT TERMS
Gulf War Illness, CNS, innate immunity, cytokines, pesticides, DFP, sarin, Tau

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	21	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

During the 1991 Gulf War (GW), U.S. veterans were exposed to chemicals, pesticides, and nerve gas agents leading to chronic illness. This chronic disorder, Gulf War Illness (GWI), is characterized by cognitive decline, fatigue, and chronic pain that affects approximately a third of all veterans who served in the Gulf War. In our prior research studies, we identified higher levels of autoantibodies as an indirect measure of proteins that are produced by the immune system in response to foreign substances, in the blood of veterans with GWI compared to healthy veterans without GWI. These autoantibodies are related to a build-up of damaging proteins in the brain. These damaging proteins are hallmark indicators of neurological diseases and illnesses related to aging such as Alzheimer's and Parkinson's disease. One specific protein that researchers have identified as related to neurological diseases is tau.

This study will compare total tau levels and types of tau species called p-tau 181 and p-tau 217 in larger numbers of stored blood samples from veterans with Gulf War Illness (GWI) compared with healthy Gulf War (GW) veteran controls. Additionally, we will do tau PET scans on a smaller group of veterans to see where the proteins are within the brain. In this study, we will determine whether veterans with GWI have higher levels of tau proteins than healthy veterans and whether the pattern or level of tau is associated with exposures during the war including pesticides, sarin nerve gas, anti-nerve gas pills and history of mild traumatic brain injury in GW veterans. Our study hypothesis is that Gulf War Illness has led to early aging in veterans, and we expect to see higher levels of tau in the blood samples of veterans with GWI compared to the healthy veterans.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Gulf War Illness, Biorepository Network, GW veterans, biomarkers, tau, PET imaging, MRI

3. ACCOMPLISHMENTS:

Major goals of the project.

The approved statement of work lists the major goals of the project and the timeline for achieving each goal:

Statement of Work: Tau Protocol

Major Tasks	Timeline
Major Task 1: Obtain Regulatory Reviews and Approvals	**Months**
1a. Obtain necessary IRB approvals for Boston University and Exempt status for Nova Southeastern University (NSU)	1-4
1b. Obtain IND for Tauvid PET imaging tracer if necessary	1-4
1c. Obtain DoD Human Research Protections Office (HRPO) approvals or exempt status	4-6
Milestones Achieved: IRB/ HRPO Approval	6
Major Task 2: Preparation and Training for tau blood laboratory analyses and clinical study procedures	**Months**
2a. Provide SOPs for NOVA lab staff on removing samples 300 stored from BBRAIN biorepository and scheduling assays to avoid freeze/thaw cycles	2-3
2b. Coordinate data entry plan, work with data management team for direct data entry and establishing data transfers between BU and NOVA sites	4-6
2c. Develop manuals for structural MRI and tau PET imaging and clinical blood draw examination protocols at BU	1-6
2d. Train researchers and staff on neuroimaging and clinical examination protocols and quality control measures.	4-6
Milestone(s) Achieved: Research staff trained and data management established.	6

Major Task 3. Screening, recruitment and assessment of BBRAIN Gulf War veterans for call back study	**Months**
Subtask 3a. Identify candidates from BBRAIN study with high neurological Kansas Criteria and previous elevated tau samples for call-back study of 30 participants.	6-24
Subtask 3b. Perform blood draw for 30 (20 cases/10 controls) BBRAIN veterans as a call-back study for planned analyses at BU.	6-24
Subtask 3c. Perform tau PET imaging and structural and diffusion MRI imaging from 30 (20 cases/10 controls) Gulf War veterans for BBRAIN call back study at BU.	6-24
Subtask 3d. Identify and pull 300 BBRAIN repository plasma samples from subjects at the NOVA lab who have consented to share samples with future studies.	6-30
Milestones Achieved: subject recruitment completed for BBRAIN call-back study of 30 study participants and bloods pulled from BBRAIN repository from 300 GW veterans (200 cases/100 controls).	6-30
Major Task 4: Perform ELISAs for tau and phospho-tau	**Months**
4a. Optimize ELISA for total tau and phospho-tau ELISA at NOVA	6-10
4b. Run batched assays for total tau, phospho-tau on all 300 stored BBRAIN repository participant blood samples (200 GWI cases, 100 GW controls) at NOVA where samples are stored	11-28
4c. Run batched assays for newly collected blood samples from 30 additional GW veterans (20 GWI cases, 10 GW controls) with corresponding PET imaging at NOVA	11-30
4d. Perform data analysis for batched assays	11-32
Milestones Achieved: complete all assays for total tau and the different phospho-tau (pThr231, pThr181, pThr217) and their corresponding data analysis for 330 total blood samples.	32
Major Task 5. Perform post-processing of MRI and tau PET imaging and analyze newly drawn blood samples	**Months**
5a. Post-process MRI and PET tau imaging data for data analysis and calculate selected SUVR for areas of interest at BU	11-34
5b. Merge BBRAIN neuropsychological test data, demographics and t-tau and p-tau blood study outcomes with MRI imaging and PET tau datasets for data analysis.	11-34
5c. Ship newly collected blood samples (20 cases/10 controls) for t-tau and p-tau species analyses to NSU laboratory	11-24
5d. Obtain t-tau and p-tau results from NSU lab and merge with brain imaging for planned analyses at BU.	11-34

Task. 6. Merge Data and Perform Interim Data analyses	Months
6a. Merge BBRAIN data including questionnaires, cognitive evaluations and clinical evaluations with current brain imaging data and t-tau and p-tau results at BU	11-24
6b. Interim statistical analyses of data obtained from brain imaging outcomes, cognitive evaluations and blood markers will be performed periodically.	11-24
6c. Annual reports of progress will be written.	12-24
Milestones Achieved: Data merged and interim analyses completed	24-32
Major Task 7. Perform Final Data Analysis, Prepare Manuscripts for Publication and share brain imaging and t-tau and p-tau data results with BBRAIN repository	**Months**
7a. Perform analyses comparing t-tau and p-tau levels in BBRAIN GWI cases and controls	24-34
7b. Perform sub-analyses comparing t-tau and p-tau levels in GW veterans with neurotoxicant, mild traumatic brain (mTBI) injury or both exposures	24-26
7c. Perform analyses comparing PET tau ligand uptake in veterans with GWI compared with controls veterans.	32-36
7d. Perform analyses comparing PET tau imaging with t-tau and p-tau blood levels	32-36
7e. Write final study report	35-36
7f. Present findings at scientific meetings and prepare manuscripts for submission for t-tau and p-tau blood and PET imaging outcomes	24-36
Milestones Achieved: publish novel research findings in scientific journals and assess new objective diagnostic marker for GWI and risk of development of other neurodegenerative disorders	24-36

Statement of Work:

The statement of work for year 1 is inclusive of tasks 1-4 above.

Accomplished under these goals:

Major Task 1: Obtain Regulatory Reviews and Approvals

Subtask 1a. Obtain necessary IRB approvals for Boston University and Exempt status for Nova Southeastern University (NSU)

Major task 1 has been completed with all IRB and OHRO approvals obtained. Specifically, Boston University has obtained IRB approval and NOVA Southeastern University obtained exempt status for the study.

Subtask 1b. Obtain IND for Tauvid PET imaging tracer if necessary

An IND was determined not to be required for this study.

Subtask 1c. Obtain DoD Human Research Protections Office (HRPO) approvals or exempt status

OHRO has approved this study.

Major Task 2: Preparation and Training for tau blood laboratory analyses and clinical study procedures

Subtask 2a. Provide SOPs for NOVA lab staff on removing 300 samples stored from BBRAIN repository and scheduling assays to avoid freeze/thaw cycles

Major task 2 has been completed with Tau blood marker analysis planned to be completed by Quanterix Inc. Quanterix is now FDA approved to perform tau Simoa p-tau 181 and p-tau 217 assays and it was determined by the research team that having Quanterix perform these analyses would provide more sensitive and reliable analyses for comparison with other diagnostic groups. Contracts and protocol with Quanterix Inc., the company preforming Simoa ELISAs on samples, has been completed. Laboratory staff at NOVA have been informed of protocol for extracting samples from the BBRAIN repository and have begun removing 300 blood samples from BBRAIN repository for shipment to Quanterix Inc.

Subtask 2b. Coordinate data entry plan, work with data management team for direct data entry and establishing data transfers between BU and NOVA sites

Our data coordinating center at BU, has collaborated with our team to create REDcap electronic data capture forms for the in-person study visits. This includes a screener for eligibility into the study as well as data entry forms for the study visit. Additionally, the data coordinating center has shared data with NOVA to assist in the sample pull from the biorepository.

Subtask 2c. Develop manuals for structural MRI and tau PET imaging and clinical blood draw examination protocols at BU

Manuals for the structural MRI and tau PET imaging protocols have been drafted. The next step is final meeting with the radiology team at BU to finalize these protocols.

Subtask 2d. Train researchers and staff on neuroimaging and clinical examination protocols and quality control measures

Upon finalization of the imaging protocol, study staff members will be trained on protocol details and quality control measures.

Major Task 3: Screening, recruitment and assessment of BBRAIN Gulf War veterans for a call back study

Subtask 3a. Identify candidates from BBRAIN study with high neurological Kansas Criteria and previous elevated tau samples for call-back study of 30 participants

We are preparing for the Simoa ELISA analysis of blood samples with Quanterix, Inc. Tau results from these samples will inform which previous BBRAIN participants are candidates for the call-back study.

Subtask 3b. Perform blood draw for 30 (20 cases/ 10 control) BBRAIN veterans as a call-back study for planed analyses at BU

This call-back blood draw will be completed after the Tau analysis with Quanterix, Inc.

Subtask 3c. Perform tau PET imaging and structural and diffusion MRI imaging from 30 (20 cases/ 10 controls) Gulf War veterans for BBRAIN call back study at BU

This call-back PET and MRI imaging protocol will be completed after the Tau analysis with Quanterix, Inc.

Subtask 3d. Identify and pull 300 BBRAIN repository plasma samples from subjects at the NOVA lab who have consented to share samples with futures studies

Our data coordinating center, provided information to NOVA on which participants consented to share samples for future research. NOVA is now preparing the shipment of 300 BBRAIN repository plasma samples to Quanterix, Inc. Quanterix will then preform the ELISAs for tau and phospho-tau.

Major Task 4: Perform ELISAs for tau and phospho-tau

Currently, the laboratory team at NOVA is pulling BBRAIN plasma samples to be batch shipped to Quanterix, Inc. Subsequently, the ELISAs will be performed and major task 4 will be underway.

Major Task 5: Perform post-processing of MRI and tau PET imaging and analyze newly drawn blood samples

This task will be initiated after completion of 30 BBRAIN participants call back study visits.

Major Task 6: Merge data and perform interim data analyses

This task will be completed upon completion of data collection from the call-back study as well as ELISA analysis.

Major Task 7: Perform final data analysis, prepare manuscripts for publication and share brain imaging and t-tau and p-tau data results with BBRAIN repository

This task will be initiated upon completion of data collection from the call-back study as well as ELISA analysis.

Opportunities for training and professional development:

Research Staff Training:
Personnel who will be using the web systems for either direct data capture, participant tracking, or viewing reports have been trained to ensure uniformity of procedures, to achieve the ultimate aim of ensuring high quality protocol implementation and data collection. As a large academic community, Boston University also provides opportunities for students to get real world experience. The BBRAIN studies and call-back studies can tap into this talent pool by taking practicum and intern students in the fields of health communication and promotion. Doctoral students in related fields such as cognitive neuroscience can also be brought on to aid in this project.

How results will be disseminated to communities of interest:
Research staff will use some of the outreach structure already in place from the BBRAIN cohort. The research group Facebook page has over 3,000 followers, many of whom regularly interact with and share page content. This allows researchers to freely spread information among the already existing GW network on social media. BBRAIN also has a study website that will be used to disseminate information about this tau study to the community. Boston University also has a very proactive media team that produces text and video news stories that get widely shared. As the research team has done for BBRAIN, they will write professional publications that state the findings of this Tau study and the implications for

the Gulf War veteran community. The first hypothesis paper on Tau in Gulf War Illness has already been published (see Baas et al., in appendix) and will be disseminated through these channels.

Plan for upcoming reporting period to accomplish goals:
In the next reporting period, we plan to:

- Complete Simoa ELISA analysis of 300 plasma samples from BBRAIN
- Initiate and continue recruitment of 30 BBRAIN participants for a call back tau PET scan, MRI and blood draw
- Begin analyses on findings from the ELISA and call back study

4. IMPACT:

This study proposal translates our prior Gulf War Illness Consortium and Boston, Biorepository and Integrative Network (BBRAIN) biomarker findings into a very focused study of hypothesized pathobiology of GWI based on tau abnormalities. Results of this study could have a dramatic impact on treatment strategies for the GWI field as a whole and for personalized treatments strategies for individual veterans with GWI based on their total tau and p-tau profiles. This study goal will be to provide a blood test for more severe forms of GWI and risk of chronic neurodegenerative disease before the onset of these additional age-related clinical disease states (tauopathies) emerge. These individuals will then be targeted for specific treatment trials through the already established Gulf War Illness Clinical Trials Consortium network.

By utilizing the large amount of stored blood samples from the well-characterized BBRAIN cohort, we can more definitively determine the impact of tau pathology on GWI, identify which tau species are most prevalent in GWI, and the risk of further development of neurodegenerative disorders including dementia, Parkinson's disease and chronic traumatic encephalopathy. From these results, we hope to develop a simple blood diagnostic test and objective biomarker for GWI that we will validate with tau brain imaging using positron emission tomography (PET). Understanding these relationships will provide the basis for transitioning these results into mechanistic-based treatment trials for GWI.

There are already FDA approved drugs for these purposes available for clinical trials. Treatment options based on our study findings could include FDA-approved compounds such as the HDAC6 inhibitor tubacin, and the kinesin-5 inhibitor monastrol as well as tau RNAi or antisense oligonucleotides, GSK3B inhibition by CHIR99021, PP2A activation via RNAi to its regulatory proteins and phosphatidylserine supplement which has been shown to increase tubulin acetylation, improve axonal transport and reduce neurodegeneration. Based on our results, we may also consider combining tau based medications with those known to reduce neuroinflammation that are already in clinical trials by our group as part of the planned and/or ongoing Gulf War Illness Clinical Trials Consortium (GWICTIC) trials including etanercept, n-acetyl cysteine, Co-Q10 or minocycline.

Identifying early markers of tau related changes that are associated with GWI is potentially important to subtyping of this chronic disorder and identifying personalized treatment approaches. In this way, there may be veterans who respond better to treatment that alters tau levels than those that reduce neuroinflammation. Alternatively, a dual-approach to treatment may be warranted to both reduce tau levels and neuroinflammation in subgroups with neurotoxicant exposures vs mTBI or combination exposures. Identifying not only potential biomarkers of subtypes of GWI but also which veterans may be at risk for early prodromal neurological illnesses based on their potential tauopathy before they develop clinical onset of these disorders could have a tremendous impact on determining who is at risk for these disorders and for identifying FDA approved targeted treatments for them when there is still time to prevent or slow the onset of some of these age-related disorders.

Impact on other disciplines

Nothing to report.

Impact on technology transfer

Nothing to report.

Impact on society beyond science and technology

Nothing to report.

5. CHANGES/PROBLEMS:

Nothing to report.

6. PRODUCTS:

Publications:
Baas PW, Sullivan KA, Terry AV, Case K, Yates PL, Sun X, Raghupathi R, Huber BR, Qiang L. Is Gulf War Illness a prolonged early phase tauopathy? Cytoskeleton (Hoboken). 2023 Sep 13. doi: 10.1002/cm.21786. Online ahead of print.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Individuals that have worked on the project:

Name: Kimberly Sullivan	Project Role:	*PI of Tau Study and Biorepository Network*
	Researcher Identifier (e.g. ORCID ID):	*https://orcid.org/0000-0001-7940-6123*
	Nearest person month worked:	*12*
	Contribution to Project:	*Oversee the biorepository at BUSPH; oversee the recruitment and participation of 30 former BBRAIN participants for PET and MRI. oversee data and sample coordination of BBRAIN plasma to Quanterix Inc.*
	Funding Support:	*Tau Award,* 81XWH-2-21-0329
Name: Nancy Klimas	Project Role	*Co-Investigator, PI of Miami Site Biorepository*
	Researcher Identifier (e.g. ORCID ID):	*https://orcid.org/0000-0003-1459-3268*
	Nearest person month worked:	*12*

	Contribution to Project:	*Oversee the BBRAIN biorepository at the NOVA; oversee shipment of 300 samples to Quanterix Inc.*
	Funding Support:	*Tau Award,* 81XWH-2-21-0329
Name: Kristina Aenelle	Project Role	*Director of NSU lab*
	Researcher Identifier (e.g. ORCID ID):	
	Nearest person month worked:	
	Contribution to Project:	*Overseeing batch shipment of BBRAIN plasma samples to Quanterix Inc.*
	Funding Support:	
Name: Emily Sisson	Project Role	*Data Manager/Analyst*
	Researcher Identifier: (e.g. ORCID ID):	
	Nearest person month worked	*12*
	Contribution to Project:	*Data Management Specialist*
Name: Bang-Bon Koo	Project Role	*Co-Investigator*
	Researcher Identifier (e.g., ORCID ID):	*https://orcid.org/0000-0001-7423-5572*
	Nearest person month worked	
	Contribution to Project	*Machine Learning and Imaging Expert; Conduct MRI imaging*
Name: Maxine Krengel	Project Role	*Co-Investigator*
	Researcher Identifier (e.g., ORCID ID):	*https://orcid.org/0000-0001-7632-590X*
	Nearest person month worked	*12*
	Contribution to project	*Neuropsychologist; Neurobehavioral Core Director*
Name: Timothy Heeren	Project Role	*Co-Investigator*
	Researcher Identifier (e.g., ORCID ID):	*https://orcid.org/0000-0001-5643-3559*
	Nearest person month worked	*12*

	Contribution to project	*Senior Biostatistician*
Name: Marco Loggia	Project Role	*Co-Investigator*
	Researcher Identifier (e.g., ORCID ID):	
	Nearest person month worked	
	Contribution to project	*PET imaging expert*
Name: Liang Qiang	Project Role	*Co-Investigator*
	Researcher Identifier (e.g., ORCID ID):	
	Nearest person month worked	
	Contribution to project	*Expert in Tauopathies*
Name: Peter Baas	Project Role	*Co-Investigator*
	Researcher Identifier (e.g., ORCID ID):	
	Nearest person month worked	
	Contribution to project	*Neurobiologist*
Name: James O'Callaghan	Project Role	*Consultant*
	Researcher Identifier (e.g., ORCID ID):	
	Nearest person month worked	
	Contribution to project	*Toxicologist*
Name: Sarah O'Shea	Project Role	*Consultant*
	Researcher Identifier (e.g., ORCID ID):	
	Nearest person month worked	
	Contribution to project	*Neurologist*

- **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.

- **Other organizations serving as partners:**

  Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:**
  Nothing to report.

9. APPENDICES:

Appendix 1. Baas PW, Sullivan KA, Terry AV, Case K, Yates PL, Sun X, Raghupathi R, Huber BR, Qiang L. Is Gulf War Illness a prolonged early phase tauopathy? Cytoskeleton (Hoboken). 2023 Sep 13. doi: 10.1002/cm.21786. Online ahead of print.

Appendix 2. Inclusion Enrollment Form

Received: 24 July 2023 | Revised: 21 August 2023 | Accepted: 28 August 2023

DOI: 10.1002/cm.21786

PERSPECTIVE

CYTOSKELETON WILEY

Is Gulf War Illness a prolonged early phase tauopathy?

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Funding information
College of Medicine, Drexel University, Grant/Award Numbers: 4100083087, 4100072545; National Institutes of Health, Grant/Award Numbers: R21AG068597, R01 ES012241; U.S. Department of Defense, Grant/Award Numbers: GW160151, GW170023, GW110073, GW170055, GW120037

Abstract

The work of the Gulf War Illness (GWI) Consortium and that of basic and clinical researchers across the USA have resulted in a better understanding in recent years of the pathological basis of GWI, as well as of the mechanisms underlying the disorder. Among the most concerning symptoms suffered by veterans with GWI are cognitive decrements including those related to memory functioning. These decrements are not severe enough to meet dementia criteria, but there is significant concern that the mild cognitive impairment of these veterans will progress to dementia as they become older. Recent studies on GWI using human brain organoids as well as a rat model suggest that one potential cause of the cognitive problems may be elevated levels of tau in the brain, and this is supported by high levels of tau autoantibodies in the blood of veterans with GWI. There is urgency in finding treatments and preventive strategies for these veterans before they progress to dementia, with added value in doing so because their current status may represent an early phase of tauopathy common to many neurodegenerative diseases.

KEYWORDS
antisense oligonucleotides, Gulf War Illness, microtubule, tau, tauopathy

Gulf War Illness (GWI) encompasses a constellation of debilitating symptoms suffered by a third of the nearly 700,000 U.S. soldiers who served in the 1990–1991 war (RAC, 2008). These symptoms reflect decrements in musculoskeletal, gastrointestinal and Central Nervous System (CNS) functioning. CNS symptoms include diminished short-term memory, poor attention/concentration, chronic headaches, and impaired sleep, and are consistent with CNS symptoms found in people with chronic exposure to neurotoxicants, including organophosphate (OP) pesticides and nerve agents (Brown & Brix, 1998; Landrigan, 1997; Proctor et al., 2006; Winkenwerder, 2003). OPs and carbamates, two subsets of pesticides used during the GW, are known to produce chronic health and cognitive symptoms at sufficient exposure levels (Rauh et al., 2012; Rowe et al., 2016). Examples of chronic decrements associated with exposures to OP and carbamate pesticides include decreased processing speed, learning/memory deficits and increased mood complaints in occupationally exposed workers

(Mackenzie Ross et al., 2010). Recent mechanistic studies have identified novel OP effects that are characterized by neuronal cell death or dysfunction through oxidative stress, neuroinflammation, metabolic and dopaminergic effects, and altered axonal transport of cellular organelles (O'Callaghan et al., 2015; Parihar et al., 2013; Pearson & Patel, 2016; Terry et al., 2003, 2007). Some benefit in health status has been reported in veterans with GWI taking supplements of coenzyme Q10 (CoQ10), which is an antioxidant essential for energy production (Golomb et al., 2014). However, there are no treatments currently available that improve cognitive functioning or reverse other chronic CNS maladies of veterans with GWI.

Animal and cell models exposed to GW-relevant OP neurotoxicants have shown deficits in axonal transport, as well as aberrations in microtubules, which are the structural railways for axonal transport (Gearhart et al., 2007; Grigoryan & Lockridge, 2009; Jiang et al., 2010; Prendergast et al., 2007; Terry et al., 2003). These aberrations include fewer proteins associated with the microtubules than normal, leading to reduced microtubule width (Gearhart et al., 2007; Grigoryan & Lockridge, 2009; Jiang et al., 2010; Prendergast et al., 2007; Terry et al., 2003). In addition, molecular motor proteins move less efficiently on the microtubules, and there is an expectation of altered stability properties of microtubules lacking their normal complement of associated proteins (Baas & Ahmad, 2013). Several studies have shown that GW-relevant OP exposures directly affect facets of axonal transport and mitochondrial dynamics that could lead to the cognitive complaints and fatigue suffered by veterans with GWI (Middlemore-Risher et al., 2011; Terry et al., 2003, 2007, 2012). Altered axonal transport mechanisms can result in deficits ranging from slowed information processing speeds and cognitive complaints to the development of various neurodegenerative disorders (Falnikar & Baas, 2009; Hernandez et al., 2015; Morfini et al., 2009). One hypothesis is that the neurotoxicants acted synergistically to create a self-perpetuating neuroinflammatory state, which in turn has an ongoing negative impact on microtubules. Another possibility is that the toxicants acted directly to damage the microtubules in the neurons in a manner that the neuron is not able to self-repair.

Several years of work on the mechanistic basis of GWI, conducted by members of the Gulf War Illness Consortium (GWIC) and others, have led us to posit that GWI might be an early phase tauopathy. Tau, a prominent microtubule-associated protein in the axon, is prone to pathological changes that contribute to many neurological disorders (Wang & Mandelkow, 2016). Aberrantly phosphorylated tau detaches from the microtubule, which results in altered microtubule properties (Baas et al., 2016), while the abnormal tau, both in soluble form and aggregated into intracellular inclusions, produces a variety of toxic effects (Kneynsberg et al., 2017; Spillantini & Goedert, 2013). Interestingly, auto-antibodies against tau are present at higher levels in peripheral blood serum from veterans with GWI compared to non-veteran controls (Abou-Donia et al., 2017, 2020). Thus, potential tau pathologies in GWI might explain why a limited exposure to neurotoxicants led to a persistent disease condition that was not reversed upon termination of the exposure and may reconcile mechanistic models based on either neuroinflammation or microtubule abnormalities. A predisposition to tauopathy might also explain a key mystery of GWI, which is why some veterans acquired the disease while their similarly exposed colleagues did not.

Despite the appeal of this hypothesis, caution is due because elevation of tau antibodies in the blood of GWI patients may be a consequence of cellular degeneration caused by an effect of organophosphates and stress not directly related to tau, with elevated antibodies to many proteins appearing in the blood without any of these proteins displaying elevated intracellular levels in the CNS. Moreover, even if there are intracellular changes in tau levels in the CNS, this could occur while not being the main cause of the cognitive symptoms. However, if our tau hypothesis is correct, there is heightened concern of impending dementia for the veterans, which fortifies the need to investigate this hypothesis.

A significant challenge in studying GWI is the degree to which preclinical models reflect the disease pathways that led to GWI in the veterans. In neuronal culture models, a wide range of concentrations of OPs have been evaluated that can produce structural and/or functional deficits including concentrations that are well below the threshold for producing acetylcholinesterase (AChE) inhibition. In the animal models, doses both below and above the threshold for producing acute signs of cholinergic toxicity have been evaluated, but in virtually all of the cases, the doses are well above the threshold for producing AChE inhibition. However, in both types of scenarios (in vitro and in vivo models) the addition of corticosterone or physical stress (e.g., restrain stress) appears to exacerbate the pathophysiology and/or behavioral deficits supporting the hypothesis that GWI cannot be explained simply as poisoning of the soldiers but rather as a disease pathway activated by the synergy of the toxicants with stress.

Tau represents its own challenges in identifying appropriate experimental models for GWI. Rodent models are powerful because they can be used for behavioral testing, such as tests for memory deficits relevant to GWI, but disease pathways in humans are often not well represented in rodents. In particular, tau is quite different in humans and rodents, with human tau forming neurofibrillary tangles that are not formed by rodent tau. In our studies to date, we have used rats, but we have also used human-derived experimental models. For the latter, we have been using human induced pluripotent cells (hiPSCs) differentiated into two-dimensional neuronal cultures (Yates et al., 2021) or three-dimensional multi-cellular brain organoids (Yates et al., 2022). For the toxicant regimen, we have been using diisopropyl-fluorophosphate (DFP; a sarin analogue) together with corticosterone (CORT) or cortisol (for rat and human cells, respectively, to mimic battlefield stress). We showed that exposure to this toxicant regimen resulted in robust and consistent tau pathology in hiPSC-derived two-dimensional cultures and organoids, with elevated levels of tau both inside and outside of the neuron. In the rat, these same tau effects were documented in response to the toxicant regimen, but only in the CA3 region of the hippocampus, not in the CA1 region or the prefrontal cortex. The toxicant regimen also resulted in loss of cells from the CA3 region of the hippocampus, but not from the CA1 region or the prefrontal cortex, further highlighting a selective vulnerability of the CA3 region to GW-relevant toxicants.

Consistent with our findings, smaller hippocampal CA3 volumes have been identified via magnetic resonance imaging analyses in veterans with GWI (Chao et al., 2014; Chao & Zhang, 2018; Yates et al., 2021). Taken together with a mild memory deficit in the rats in response to the toxicant regimen, these results implicate tau abnormalities as a potential cause of the cognitive defects in GWI.

There is precedent for stress or stress hormones exacerbating tauopathy (Lopes et al., 2016; Okawa et al., 2003; Rissman et al., 2007; Sierra-Fonseca & Gosselink, 2018; Yan et al., 2010), which may be the key to understanding GWI. Without the stress, there is no GWI pathology in the experimental models, and it may be that without the stress, the toxicants would not produce enough of an effect on tau to produce symptoms. In our cell biological work, we have analyzed the effects of DFP alone, the stress hormone alone, or the two in combination (Rao et al., 2017), but more work needs to be done along these lines specifically with tau.

Our tau-based hypothesis for GWI is tauopathy is shown in Figure 1. As a general principle, we posit that most or all tauopathies begin as a pre-dementia early phase in which tau levels are elevated, after which the more broadly studied and recognized late phase ensues, corresponding to dementia, in which tau becomes aberrantly phosphorylated, detaches from microtubules, and forms pathological aggregates and filaments. As shown in Figure 1, microtubules in the axon consist of a stable domain and a labile domain, with elevated tau levels associated with the microtubule in the early phase of tauopathy resulting in an elongation of the labile domain and a shortening of the stable domain, and diminished tau levels associated with the microtubule in the late phase resulting in the inverse. In the early phase, the symptoms result from too much tau on the microtubules, while in the late phase the symptoms arise in part because of too little tau on the microtubules and in part because of the pathological properties of the misfolded, aggregated, and filamentous forms the tau acquires upon losing association with the microtubules.

Veterans with GWI suffer cognitive problems including memory loss, but they do not typically display dementia—at least not yet. On this basis, we posit that veterans suffering from GWI are currently in a persistent early phase tauopathy since returning home from the battlefield. This idea raises concerns that with aging, late phase tauopathy could eventually hit these veterans very hard. Interestingly, though, these veterans may provide an ideal model in which to understand if treatment of early phase tauopathy is reversible and if successful treatment of the early phase prevents the late phase of tauopathy from happening.

The early phase symptoms, consisting of prodromal pre-dementia cognitive problems, do not likely result from loss of tau's normal functions nor from gain-of-function toxicity of aberrant tau species, but rather from an increase in the normal activities of tau. Curiously, over the 50 years of tau research conducted in laboratories around the world, relatively less has been done to understand the normal functions of tau compared to the properties of pathological tau. The popular view of tau as a microtubule stabilizer has become dogma, and yet was never well justified in studies on neurons. In fact, we recently established that experimental depletion of tau from neurons does not, as the dogma would have predicted, result in destabilization of microtubules, but rather in a partial loss of the microtubule fraction from the axon that is the most labile (Qiang et al., 2018). Moreover, the remaining microtubules became more stable, not less stable, as tau was depleted. Additional studies provided an explanation for these findings, namely

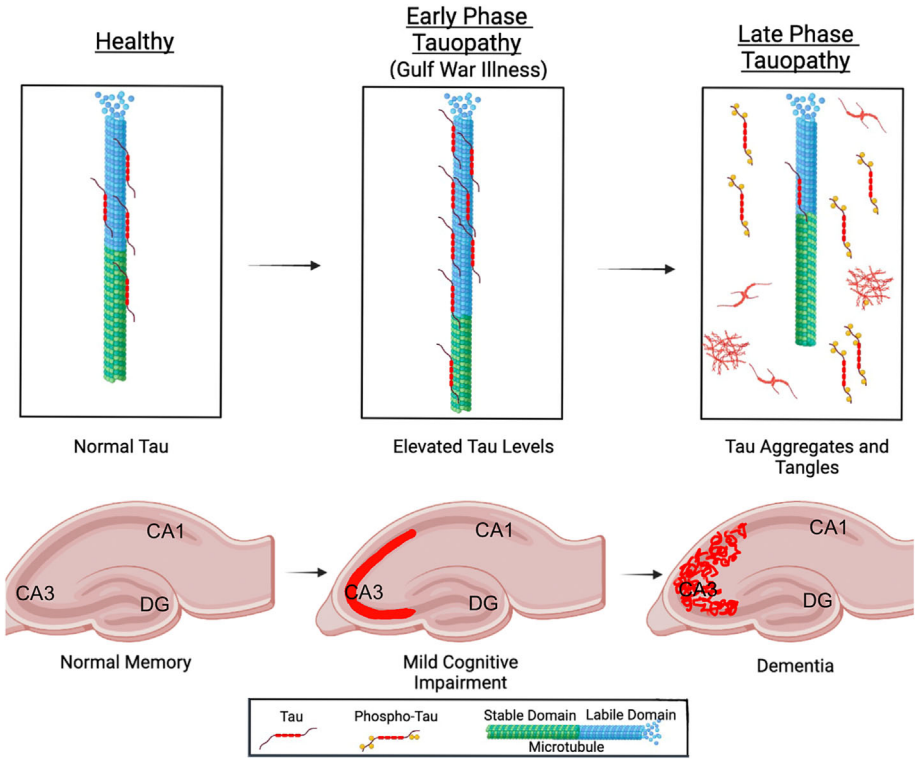

FIGURE 1 Schematic illustrating the progression from healthy tau levels to heightened tau levels in early phase tauopathy, which results in elevated microtubule levels and reduced microtubule stability, to late phase tauopathy, which results in the elevated tau detaching from the microtubules (while becoming aberrantly phosphorylated), thus causing a reduction in microtubule levels, with a greater proportion being stable. In the late phase, the detached tau molecules form abnormal aggregates, filaments and tangles with toxic properties. We posit that veterans with GWI are in an early phase tauopathy (manifesting as mild cognitive problems due to tau elevations in layer CA3 of the hippocampus) and are at risk of advancing to late phase tauopathy (manifesting as dementia due to the elevated tau forming abnormal aggregates filaments and tangles).

that tau normally outcompetes microtubule-stabilizing proteins, such as MAP6, for binding to the labile microtubules in the axon, thus preventing those microtubules from being stabilized (Qiang et al., 2018). These findings would suggest that elevated tau levels (as opposed to diminished tau levels) cause microtubule destabilization, which is exactly what we found in our rat and hiPSC-based models of GWI.

There is urgency in providing treatment for the veterans because, if our hypothesis is correct, late phase tauopathy could hit them fairly soon. Because tau is not a microtubule-stabilizing protein in the axon, we have argued that microtubule stabilizing drugs are the wrong approach for late phases of tauopathies such as Alzheimer's disease (Baas & Qiang, 2019); however, there is logic to this approach for early phase tauopathy, when heightened tau levels presumably cause decreased microtubule stability. We have consistently observed that GWI toxicant regimens result in a decrease in microtubule acetylation, which correlates with a decrease in microtubule stability (Janke & Montagnac, 2017; Yates et al., 2021, 2022). Consistent with this, we have shown that pharmacologically increasing microtubule acetylation or directly stabilizing microtubules ameliorates some of the symptoms of GWI in cultured neurons (Naughton et al., 2020; Rao et al., 2017). Even so, treatment with such drugs nevertheless carries risks (Baas & Ahmad, 2013), and hence we suspect that the better and more direct therapy for translation to human patients will be lowering the elevated tau levels to normal with contemporary antisense oligonucleotides (ASOs). Such treatment, currently in clinical trials (Clinicaltrials.gov registration number: NCT03186989) for other diseases (Lozupone et al., 2023; Mummery et al., 2023) may reverse the cognitive problems suffered by these veterans and also protect them from advancing into dementia.

A great deal of work will be necessary to investigate our tau-based hypothesis with necessary rigor. A high priority will be studies on postmortem tissue from deceased veterans who had GWI, as well as more experimental work beyond correlation. Ascertaining the effects of the GWI toxicant regimens on tau knockout mice would likely be informative, as would conducting the studies on mice expressing human tau rather than mouse tau, due to the fact that mouse tau does not form aggregates or filaments similarly to human tau and because there are differences in the isoform expression of murine vs human tau in adulthood (Hernandez et al., 2019, 2020; Saito et al., 2019). Crucial will be studies in both rodent and human organoid models in which tau levels are experimentally reduced to ascertain whether cellular and cognitive symptoms are ameliorated. For now, our hypothesis remains speculative, but the appropriate experiments are within our grasp as is a potentially effective therapy.

ACKNOWLEDGMENTS

Research relevant to this Perspective was supported by grants from the Department of Defense to Liang Qiang (GW160151), Peter W. Baas (GW170023) and Alvin V. Terry (GW110073), and grants from the CURE program via Drexel University College of Medicine to Liang Qiang (CURE Grant SAP Number: 4100083087) and Peter W. Baas (CURE Grant SAP Number: 4100072545). Additional funding was provided by the National Institutes of Health to Peter W. Baas (R21AG068597) and Alvin V. Terry (R01 ES012241). The work was further supported by a Department of Defense grant to the Boston Biorepository, Recruitment, and Integrative Network (GW170055) and to the Gulf War Illness Consortium (GW120037) directed by Kimberly A. Sullivan.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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How to cite this article: Baas, P. W., Sullivan, K. A., Terry, A. V., Case, K., Yates, P. L., Sun, X., Raghupathi, R., Huber, B. R., & Qiang, L. (2023). Is Gulf War Illness a prolonged early phase tauopathy? *Cytoskeleton*, 1–6. https://doi.org/10.1002/cm.21786

PHS Inclusion Enrollment Report

This report format should NOT be used for collecting data from study participants.

OMB Number: 0925-0001
Expiration Date: 3/31/2020

***Study Title (must be unique):** Are Tau Proteins in Blood and PET Imaging Related to Gulf War Illness and Risk of Comorbid Neurological Disorders?

* Delayed Onset Study? ☐ Yes ☒ No

If study is not delayed onset, the following selections are required:

Enrollment Type ☐ Planned ☒ Cumulative (Actual)

Using an Existing Dataset or Resource ☒ Yes ☐ No

Enrollment Location ☒ Domestic ☐ Foreign

Clinical Trial ☐ Yes ☒ No

NIH-Defined Phase III Clinical Trial ☐ Yes ☒ No

Comments:

Participants from this study will be recruited from the BBRAIN Gulf War veteran cohort of 1991 Gulf War veterans who were primarily male and caucasian but were oversampled as much as possible for women veterans and other ethnic groups. No participants have been recruited to date for this study.

Racial Categories	Ethnic Categories									
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			Total
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	0	0	0	0	0	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0
More than One Race	0	0	0	0	0	0	0	0	0	0
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0	0	0

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