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14. ABSTRACT The primary objective of BBRAIN is to establish a retrospective and prospective biorepository network for GWI research by data mining from existing BBRAIN collaborator specimens and for recruiting 500 additional repository samples. The four prospective recruitment resource sites include Boston, Miami, Bronx and San Francisco. The BBRAIN structure provides centralized cataloguing and coordination of retrospective biorepository samples from 10 collaborating institutions who are sharing existing blood plasma, sera, PBMCs, cerebrospinal fluid, human-induced pluripotent stem cells (hiPSCs), DNA and saliva samples. Corresponding cognitive outcomes, brain imaging, demographics and health symptom surveys are included in the BBRAIN network datasets to allow for the comparison of biomarkers with behavioral outcomes. To date, sharing has begun with retrospective data and outcomes and several manuscripts have been published.					
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1. INTRODUCTION:

The primary objective of the Boston Biorepository and Integrative Network for Gulf War Illness (BBRAIN) is to establish a retrospective and prospective biorepository network for Gulf War Illness (GWI) research by data mining from existing BBRAIN collaborator specimens and by recruiting 500 additional participants' repository samples. The four prospective recruitment resource sites include Boston University, Miami VA, Bronx VA and San Francisco VA. The BBRAIN structure currently provides centralized cataloguing and coordination of retrospective biorepository samples from 10 collaborating institutions who will share existing blood plasma, sera, PBMCs, cerebrospinal fluid, human-induced pluripotent stem cells (hiPSCs), DNA and saliva samples from participants previously consented to share their samples for future research. Corresponding cognitive outcomes, brain imaging, demographics and health symptom surveys will be included in BBRAIN network datasets to allow for the comparison of biomarkers with behavioral outcomes.

2. KEYWORDS:

Gulf War Illness, Biorepository Network, GW veterans, biomarkers

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

Table 1. Brain-Immune Interactions as the Basis of Gulf War Illness: Gulf War Illness Consortium

Tasks	Timeline	Status
Major Task 1: Obtain IRB/HRPO approval	Months	
1a. Obtain necessary IRB approvals or Exempt status	1-3	X
1b. Obtain DOD Human Research Protections Office (HRPO) approvals or Exempt Status for use of de-identified samples	4-6	X
Milestone(s) Achieved: Regulatory reviews completed, final approvals obtained	6	X
Major Task 2: Obtain Biorepository Data for Analysis and Catalogue Available Biospecimens for sharing at Repository Resource Sites	Months	
2a. Obtain MRI, MRS, DTI, PET imaging data from retrospective resource (RR) sites and send to BUMC for cataloguing, post-processing and machine learning analyses	6-12	X
2b. Obtain demographic, health symptom and cognitive outcome data from RR Sites and send to BUMC for cataloguing, post-processing and analysis	6-12	X
2c. Catalogue and track available tissue, blood, saliva and CSF samples from RR sites	6-12	X
Milestone(s) Achieved: Biorepository data mining/cataloguing complete for 10 sites	12	X
Major Task 3: Perform Post-Processing of Imaging Data and Machine Learning Analysis with Combined Common Data Elements	Months	
3a: Perform Brain image post-processing and cataloguing of images for repository.	1-24	X
3b: Perform data analysis of brain imaging and machine-learning analyses including cognitive data and health symptom data in cases and controls.	1-24	X

Milestone(s) Achieved: Brain imaging data mining analysis studies complete and imaging, demographic, cognitive outcomes repository created.	24	X
Major Task 4: Preparation and Training for Clinical Study Procedures	Months	
4a. BUSPH Data Coordinating Center (DCC) will create website, data collection forms, specimen tracking system and databases for the entire repository network	1-6	X
4b. Develop manuals for the neuropsychological testing protocol, specimen collection protocols and recruitment screening procedures	1-9	X
4c. Train researchers and staff on protocols and quality control measures for the clinical study and repository collection	1-9	X
Milestone(s) Achieved: Staff trained and all databases and websites ready for subject recruitment and specimen tracking.	9	X
Major Task 5: Screening, Recruit and assessment of GW Veterans From Four Resource Sites	Months	
5a: Obtain informed consent from potentially eligible GW veterans	9-36	ongoing
5b: Assess subjects by obtaining demographics, medical history, self-report questionnaires, neuropsychological testing, Fitbit tracker, blood draw and saliva samples from 500 study participants.	9-36	ongoing
5c. Obtain participants urine and stool sample kits back by mail to BUSPH for biorepository storage.	9-36	ongoing
5d. Upload Fitbit sleep, heart rate variability and blood pressure data to BUSPH DCC to merge with other datasets.	9-36	ongoing
5e. Score neuropsychological tests and upload summary data to DCC for entry, cleaning and analyses.	9-36	ongoing
5f. Send blood and saliva samples to Nova University for analysis of cytokine and chemokine panels and cortisol measurements and biorepository banking.	9-36	ongoing
Milestone(s) Achieved: Obtained blood, saliva, urine, stool, autonomic, demographic and cognitive data from 500 participants for biorepository and analysis.	36	ongoing
Major Task 6: Process, Store and Manage samples for a total of 500 new participants.	Months	
6a. Process and store blood, saliva, urine and stool samples received from 4 recruitment sites at two biorepository sites	6-33	ongoing
6b. Perform local laboratory analyses (CBC) for 500 participants	6-33	Ongoing
6c. Extract DNA and RNA from 500 saliva samples and store in repository	6-33	Ongoing
6d. Run batched assays for cortisol and cytokine results (18 plex cytokine assay)	6-33	Ongoing
6e. Provide Sample request forms and track information regarding the use of samples	6-36+	Ongoing
6f. Ship samples to requesting investigators	6-36+	Ongoing
Milestone(s) Achieved: Processed all newly collected samples into biorepository storage, ran cytokine and cortisol measurements, extracted DNA/RNA for repository.	36	ongoing
Major Task 7: Merge Data and Perform interim Data Analyses	Months	
7a. Merge Clinical datasets data from prior studies for brain imaging, cognitive, demographic and health symptom report data	6-18	X
7b. Perform machine learning and/or meta-analytic techniques on common data elements from prior study epidemiological, cognitive and brain imaging data	6-18	X
7c. Discussion of results and preparation of abstracts for meeting presentations and initial manuscript for publication	18-24	X

7d. Annual reports of progress will be written	12-24	X
Milestone(s) Achieved: Preliminary analysis of results and presentation of initial results at scientific meetings and potential publication. Possible diagnostic markers from machine learning and meta-analyses.	6-24	X
Major Task 8. Perform Final Analysis, Prepare Manuscripts for Preparation and Finalize Biorepository for Sharing of Samples	Months	
8a. Merge clinical datasets for prospective and retrospective data sets for GWI cases and GW controls and other symptomatic controls (fibromyalgia, IBS, CFS)	24-30	ongoing
8b. Perform data analysis comparing cytokine, cortisol, cognitive outcomes, Fitbit and self-reported health symptom outcomes within GWI cases and controls from prospective data collection	25-26	ongoing
8c. Coordinate interim and final collaborative publications and/or meta-analyses and Final Study Report	24-36	Ongoing
8d. Finalize websites for BBRAIN biorepository with data dictionaries and a searchable indexed site	24-36	X
Milestone(s) Achieved: Final analyses performed, publications prepared and biorepository dataset and indexed repository website prepared	24-36	

The statement of work for year 5 is inclusive of Tasks 1-8 above. The statement of work for year 5 primarily describes the plan to complete recruitment of study participants for the study protocol including cognitive evaluations and specimen collection and with analysis of retrospective datasets and sharing of repository samples and data with GWI investigators. Progress toward completing each task is listed below.

Accomplishment under these goals.

TASK 1. OBTAIN IRB & HRPO APPROVAL (MONTHS 1-6)

Task 1a. Obtain necessary IRB approvals or Exempt status

Institutional Review Board (IRB) approvals for the prospective sites have been obtained at Boston University, Miami VA, NOVA Southeastern University, Bronx VA, and the San Francisco VA/UCSF.

The DUA and MTA documents have been finalized and approved between BU and SF VAMC, NOVA Southeastern University, Bronx VA, Mass General Hospital, Georgetown University, Beth Israel Deaconess Medical Center, Roskamp Institute and University of Alabama.

All retrospective sites/samples have received IRB approvals (this includes, BUSPH, SF VAMC, NOVA Southeastern University, Drexel University, Beth Israel/Deaconess/Harvard, Mass General Hospital, Georgetown University, and the University of Alabama).

Task 1b. Obtain final DOD Human subjects Research Protections Office (OHRO) approvals or Exempt Status for Use of de-identified samples.

All participating sites (BUSPH, SF VAMC, NOVA Southeastern University, Miami VA, and the Bronx VA) have received OHRO approvals.

TASK 2. OBTAIN BIOREPOSITORY DATA FOR ANALYSIS AND CATALOG AVAILABLE BIOSPECIMENS FOR SHARING AT REPOSITORY RESOURCE SITES (MONTHS 6-12)

The consortium coordinating center and Administrative Core at Boston University has led many monthly web and in-person meetings to maintain engagement from investigators in the consortium and update the group on the prospective and retrospective BBRAIN studies. During each of these monthly meetings, an investigator of the BBRAIN consortium was invited to speak in depth about their research topic. For example, Dr. Linda Chao spoke to the group about her Mild Cognitive Impairment in GWI research. These meetings were also used to update the larger consortium on any new publications and prospective study recruitment updates. Time was allotted for initiating collaboration and conversation of novel scientific questions. In addition, smaller working groups met throughout the year to discuss further planning efforts. Several planning meetings were held with the Data Management Service Group to track the data sharing from the biorepository with collaborating investigators. Variable names of specimens from retrospective resource (RR) sites were obtained and a common variable names and data dictionary for catalogued samples have been created. We now have obtained and cataloged samples to be shared with the consortium for analysis from the 10 RR sites. Table 2 details the status of RR data sharing and processing. To date, twenty-three requests have been reviewed and approved for sharing of samples through our online request forms. The details of these approved requests are outlined in table 3.

Table 2. Retrospective Resource Site Data Sharing Status

Retrospective Resource Site	Shared Data	Shared (Y/N)	Processed (Y/N)
<i>Boston University</i>	<u>GWIC samples</u> -Serum, plasma, cytokines, genetic SNP panels, saliva, cortisol -Health data, Symptoms, exposure data; medical conditions -Cognitive assessments, brain imaging, Cerebral Spinal Fluid	Y	Y
<i>Harvard University: Beth Israel Deconess Medical Center</i>	<u>GWI</u> -blood samples and demographic data	Y	Y
<i>VA Boston</i>	<u>VA Biorepository Brain Bank (VABBB)</u> -Brain tissue database	Y	Maintained by VA: https://www.research.va.gov/programs/tissue_banking/als/
<i>University of Alabama</i>	<u>GWI, Healthy controls, Chronic Fatigue syndrome, Fibromyalgia cohorts</u> -plasma, serum -brain imaging scans	Y	Y
<i>Georgetown University</i>	<u>GWI, CFS, healthy control</u> -blood and brain imaging	Y	Y
<i>Harvard University: Mass General Hospital</i>	<u>GWI</u> -PET scans, blood, demographic and cognitive data	Y	Y
<i>San Fransico VA</i>	<u>GWI and healthy controls</u> -Brain imaging -saliva samples, demographic, exposure assessment and survey	Y	Y
<i>Drexel University</i>	<u>Stem Cell Biorepository</u> -hiPSCs stem cells (GWI/Controls)	Y	Y
<i>Roskamp Institute</i>	-Omics (proteomics, lipidomics, and metabolomics)	Y	Y
<i>CDC/NIOSH</i>	-mouse brain samples from GWI studies	Y	Y

Task 2a. Obtain MRI, MRS, DTI, PET Imaging data from retrospective resource (RR) sites and send to BUMC for cataloguing, post processing and machine learning analyses

Imaging data obtained from retrospective sites including the San Francisco VA, Georgetown University, U-Alabama and Mass General Hospital were sent to Drs. Killiany and Koo at Boston University for cataloguing, post-processing and analysis. The MRI scans were transferred electronically in either extended DICOM or par/rec format to the Center for Biomedical Imaging at Boston University School of Medicine.

The San Francisco VA site has contributed the following de-identified neuroimaging data types to the BBRAIN Imaging repository from prior studies on GW veterans: 1.5T data (T1, T2, and proton density scans) from 257 GW veterans, 4T data (T1, T2, and FLAIR scans) from 207 GW veterans, and 3T data (T1, T2 and FLAIR scans) from 230 GW veterans. These images have been categorized and stored for query for researchers interested in using them for analyses.

Data from the Harvard University/Mass General Hospital PET and diffusion tensor imaging studies were also shared from 40 scans with Dr. Koo at Boston University as part of the ongoing BBRAIN imaging studies. This has resulted in a collaborative publication of these data (Cheng et al., 2021). These machine learning and multi-modal analyses are currently being conducted. Imaging data from Georgetown University and University of Alabama was also shared. Georgetown University shared T1, resting state fMRI, diffusion (DTI), and pCASL 3T data from 151 GW veterans. The University of Alabama shared T1 and ASL 3T data from 92 GW veterans. This data was added to the imaging post-processing pipeline and machine learning analyses.

Additional secured shared drive space has been purchased to store the vast amount of imaging data that will be shared. As the images were obtained, each scan underwent quality checking that consisted of a visual inspection for the presence of noise or artifact as well as a review of scan parameters to ensure that the appropriate ones were used in the acquisition. Scans that failed the quality check were rejected by the study and remediation discussed with the appropriate retrospective site investigator. Scans that passed the quality check were entered the post-processing pipeline for data mining and sharing.

Task 2b. Obtain demographic, health symptom and cognitive outcome data from RR sites and send to BUMC for cataloguing, post-processing and analysis

OHRO approvals and DUAs have been obtained from all retrospective study sites and the demographic, health symptom and cognitive outcome data have now been obtained from all RR sites. Data variables names, REDCap shells, and data dictionary were obtained from retrospective sites to create common variable names for specimens that were cataloged for sharing.

To date, GWIC demographic, health symptom and cognitive data from 263 study participants has been added to the BBRAIN repository. In addition, the San Francisco VA has also shared demographic information, health symptom and cognitive outcomes from 224 GW veterans and the Roskamp Institute shared demographic information, health symptom and cognitive outcomes from 63 GW veterans.

Additional demographic, case status and health symptom data has been obtained from Georgetown University of 55 Gulf War veterans with GWI and 12 GW veterans with CFS.

Similar data has been obtained from the University of Alabama on 105 GW vets without GWI, 26 GW vets with CFS, 15 GW vets with TBI and 18 GW vets with RA.

Data from RR sites have been combined with prospective BBRAIN data and analyzed. These results have been presented at conferences, monthly meetings and submitted for publications (Keating et al., 2023; Kregel et al., submitted). Additional ongoing analysis for cognitive outcomes and health symptom outcomes are being prepared for manuscript submission.

Task 2c. Catalogue and track available tissue, blood, saliva and CSF samples from RR sites

We have obtained and catalogued specimen data information from all retrospective resource sites (RRS) to add to the list of available samples for sharing. This has included human induced pluripotent stem cell (hiPSC) lines, serum samples, plasma samples, DNA and RNA samples and cerebrospinal fluid.

Specifically for the BBRAIN repository of hiPSC cells, we have:

- Expanded capabilities to provide partially differentiated neurospheres
- Expanded technical know how into organoid technology
- Published manuscripts on organoid effects of GW-relevant neurotoxicants and link to tauopathy (Baas et al., 2023; Yates et al., 2022; Yates et al., 2021)
- Drs. Baas, Qiang and Sullivan have submitted DOD proposals using the hiPSC lines that would involve collaborations with Dr. James O’Callaghan of the CDC, Dr. Ashok Shetty of Texas A&M and Dr. Russ Huber of the VA GW Veterans Brain Bank.

TASK 3. PERFORM POST-PROCESSING OF IMAGING DATA AND MACHINE LEARNING ANALYSIS WITH COMBINED COMMON DATA ELEMENTS (MONTHS 1 - 24)

Task 3a. Perform brain image post-processing and cataloguing of images for repository

Images shared from all sites have been catalogued and inventoried. Post-processing and harmonization of images across sites is underway. Once data harmonization is complete, analysis with combined data elements will be conducted.

Task 3b. Perform data analysis of brain imaging and machine-learning analyses including cognitive data and health symptom data in cases and controls.

Machine learning multi-modal approach to brain connectomics analysis will to be conducted once brain scans are post-processed and added to the machine learning data analysis computational pipeline. This work will be used to validate Dr. Koo’s recent paper using GWIC data analyses (Cheng et al., 2020).

TASK 4. PREPARATION AND TRAINING FOR CLINICAL STUDY PROCEDURE (MONTHS 1 - 9)

This task has been completed as we are well underway in subject recruitment and enrollment. Additional training of the study procedure is conducted when

needed for new study personnel. As previously mentioned, study coordinators meet montly to discuss any outstanding questions and ensure consistency in study methods across sites.

Task 4a. BUSPH Data Coodinating Center (DCC) will create website, data collection forms, specimen tracking system and databases for the entire repository network

The Data Coordinating Center (DCC) now renamed ‘BEDAC’ at BUSPH has developed a website to assist in the management and conduct of the repository that contains the following components: a data entry application, participant and data tracking applications, reports for investigators to monitor status and research staff to manage day to day operations; public-facing web application for repository information, data requests, and data access. The website now contains publicly available informational pages, including descriptions of RS, projects, investigator profiles, and publications with links to PubMed; as well as informational areas to be used for dissemination of project results. This information is updated regularly to accuratetly inform veterans and other investegators. All project-specific applications (e.g., tracking, data entry and upload) are accessible via the website and restricted to project personnel through secure logins. The site also includes areas for NCC, RS and project level communications including news (announcements, meetings); manuals, forms and protocols; up-to-date personnel contact information; an area for secure transfer of data; reports for enrollment, follow up and data management. The sites are listed at the links below:

<http://sites.bu.edu/bbrain/> and https://wwwapp.bumc.bu.edu/BEDAC_BBRAINRetro

Progress to date includes BEDAC creating needed reports from the electronic data capture tool REDCap. These reports help to track study status and materials from a large volume of participants. Weekly, a generated report of outstanding stool, urine, survey and fitbit data is sent to each site. This report allows for easy access to follow up eith participants and remind them to complete data from the study. BEDAC has generated multiple analytic datasets from cleaned data to date. An updated analysis of the population’s demographics is periodically sent to investigators of the consortium. Other data is requested by investegators through the sample request electronic form. The sample request electronic form and approval process is shown below:



BOSTON UNIVERSITY

BBRAIN
Boston Biorepository, Recruitment
and Integrative Network for GMI

Welcome to the BBRAIN Data Repository Request Form.
What would you like to do?

Create a New Request

OR to view/edit an existing request, please enter the email address entered
for the primary investigator

Go

Task 4b. Develop manuals for the neuropsychological testing protocol, specimen collection protocols and recruitment screening procedures

Manuals for the neuropsychological testing protocol, blood, saliva, urine and microbiome specimen collection and recruitment protocols have been compiled and finalized. The table below describes the full study protocol for the four study sites in Boston, Miami, Bronx and San Francisco.

Table 3. BBRAIN study protocol

Study Protocol	Boston		Miami		Bronx		San Francisco		Total
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	
Questionnaires (demographics, general health and symptoms, pain, fatigue, sleep, medical conditions, deployment and exposure history)	150*	50*	50	50	50	50	50	50	n = 500 (300 cases/200 controls)
Clinical evaluation and autonomic measures (medical history, height, weight, supine/standing BP and pulse; FitBit BP, HRV, sleep quality)	150	50	50	50	50	50	50	50	n = 500 (300 cases/200 controls)
Clinical lab tests (CBC, metabolic profile, lipid panel, TSH, ANA, RF)	150	50	50	50	50	50	50	50	n = 500 (300 cases/200 controls)
Biorepository Samples and Research assays (plasma cytokines /chemokines, salivary cortisol; plasma, serum, whole blood, DNA, RNA, Urine, fecal sample)	150	50	50	50	50	50	50	50	n = 500 (300 cases/200 controls)
Neuropsychological assessment (executive function, attention, memory, psychomotor function, motivation, mood)	150	50	50	50	50	50	50	50	n = 500 (300 cases/200 controls)
Longitudinal assessment (clinical evaluation, clinical lab tests, plasma cytokines, neuropsychological assessment; biorepository samples)	100	50	25	25	-	-	-	-	n = 125 (cases/ 75 controls)

*100 participants will be recruited on site and 100 participants remotely.

Task 4c. Train researchers and staff on protocols and quality control measures for the clinical study and repository collection

New study personnel who are working directly with study participants were extensively trained by the Boston University Coordinating Center to ensure adequate quality control of test administration and interview procedures among the study sites. All neuropsychological testing materials and survey instruments are routinely inventoried and restocked for the remaining clinical recruitment study sites. Quality control measures will continue to be instituted and monitored by experienced Administrative Core investigators including Dr. Toomey as the clinical studies proceed to ensure good inter-rater reliability and to reduce tester drift among the study sites.

TASK 5. SCREENING, RECRUIT AND ASSESSMENT OF GW VETERANS FROM FOUR RESOURCE SITES (MONTHS 9 - 36)

Due to the ramifications from the COVID-19 pandemic, and limited funding, recruitment has varied at the different study sites. Boston University has experienced no restriction

and have successfully been recruiting participants throughout the year. The San Francisco VA recruitment was completed in winter of 2023. The prospective data team at the San Francisco VA has been actively enrolling participants since March 2022. The Bronx VA ceased recruitment at the beginning of the report year and the Miami VA have been enrolling participants for the duration of the year. In the recent months, country-wide inflation and expensive travel have slightly hindered participant enrollment. However, we are providing reimbursements to those who travel a distance to participate and this has improved recruitment efforts.

In total, the recruitment plan of 500 study participants (300 GWI cases, 200 controls) was planned to be recruited from four RS sites; Boston, MA, Miami, FL, Bronx, NY and San Francisco, CA. At the Miami, Bronx and San Francisco sites, 100 study participants each will be recruited. At the Boston site, 100 study participants will be recruited to participate on site and an additional 100 participants not residing close to the any of the four recruitment sites will be given offered travel costs to participate at the Boston site.

To date, 556 participants have been screened. Of the 556 screened, 395 are eligible (337 GWI Cases and 58 healthy controls). Please see recruitment data tables below.

**BBRAIN: Screening and Enrollment Summary
October 13, 2023**

	Total	Boston	Bronx	Miami	San Francisco
Number of Subjects Contacted	583	296	55	61	171
Number of Subjects Screened	556 (95.4%)	293 (99.0%)	55 (100.0%)	52 (85.2%)	156 (91.2%)
Number of Subjects Eligible	395 (71.0%)	230 (78.5%)	32 (58.2%)	39 (75.0%)	94 (60.3%)
Number of Subjects with Appointments Made	245 (62.0%)	145 (63.0%)	18 (56.3%)	34 (87.2%)	48 (51.1%)
Number of Subjects Assessed	237 (96.7%)	137 (94.5%)	18 (100.0%)	34 (100.0%)	48 (100.0%)

	Total		Boston		Bronx		Miami		San Francisco	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Number of Subjects Screened	466	65	252	38	34	2	49	1	131	24
Number of Subjects Eligible	337	58	193	37	30	2	38	1	76	18
Number of Subjects Assessed	198	39	112	25	16	2	33	1	37	11

Task 5a. Obtain informed consent from potentially eligible GW veterans

To date, we have received informed consent from 237 eligible GW veterans.

Task 5b. Assess subjects by obtaining demographics, medical history, self-report questionnaires, neuropsychological testing, Fitbit tracker, blood draw and saliva samples from 500 study participants.

Two hundred and thirty seven study participants have been seen in-person to date, however all study protocols for the clinical studies have been finalized and are listed in the tables below and in Table 1 above. All study protocols that can be done remotely will also be done that way including study surveys. We will continue recruitment by re-contacting participants from GWIC and other GW studies at the Miami VAMC who have agreed to be re-contacted for future studies. The tables below lists the complete survey and cognitive

data that is collected at the prospective sites. To date, 237 participants have completed the study protocol.

Table 4. BBRAIN study surveys

Name	Description
Demographics	Subjects report information on age, education, gender, ethnicity, marital status, handedness, corrective lenses, hearing aid usage (SENSE), GW duty service (active vs. reserve/National Guard), military rank and current military status
SF36V	Veterans' version of the SF36 which assesses functional health-related quality of life in 8 domains and provides overall summary scores for physical and mental health status
Kansas Gulf War and Health Questionnaire	Queries veterans about demographics, military and deployment history, and chronic symptoms and diagnoses required to ascertain Kansas GWI and CMI case status.
Medical Conditions	A checklist with 21 medical conditions that the subject is asked to rate if they have ever had the condition, how it was diagnosed (self or doctor) and when it was diagnosed
Current Medications list	A list of current medications which participants are taking listing the medication and what symptoms it is taken for.
Profile of Mood States (POMS)	The POMS is a self-report assessment of current mood. The indicators of importance are current fatigue, confusion, tension and depression.
3 Question DVBIC TBI screening tool	A 3 question screening for deployment related TBI causes and symptoms from the Defense and Veterans Brain Injury Center.
American Academy of Neurology mTBI guidelines	Questions are asked regarding history of mild TBI (mTBI) during three time periods (before, during, after GW) and number of mTBIs.
Kansas Gulf War Experiences and exposures	A questionnaire that assesses veteran-reported experiences and exposures during their deployment to the 1991 Gulf War.
Structured Neurotoxicant Assessment Checklist (SNAC)	The SNAC assesses the degree of past and current exposure to neurotoxicants during civilian and military occupations and includes questions pertaining to recent occupational and environmental exposures.
Pittsburg Sleep Quality Index (PSQI)	PSQI assesses sleep quality during the past month. It covers domains of sleep quality, latency, duration, efficiency, disturbances, medications and daytime dysfunction. Total global scores range from 0-21.
Multidimensional Fatigue Inventory Questionnaire (MFI-20)	20 item self-report fatigue instrument that covers general fatigue, physical fatigue, mental fatigue, reduced motivation and reduced activity.
McGill Pain Questionnaire	A pain questionnaire that includes 3 sections including what the pain feels like, change over time and strength of pain. Scores range from 0-78.
Every day Cognition Scale	A self-report survey measuring aspects of everyday cognition related to memory, language, visuospatial and executive functioning.
Brief Pain Inventory (BPI) short form	The BPI is a brief 9 question self-report survey of severity, location and impact of pain on daily functioning.
Visual Analog Pain and Fatigue Scales	Participants measure their pain and fatigue by drawing a vertical line on a horizontal scale from 'not at all' to 'worst pain or fatigue ever'.
Fatigue Severity Scale	A brief survey where statements of fatigue affecting daily activities is endorsed on a scale from 1 to 7 for disagree to completely agree.
PTSD Checklist for DSM-5 (PCL-5)	A 20-item self-report measure that assesses the 20 DSM-5 symptoms of PTSD. It screens and provides a provisional PTSD diagnosis.
Center for Epidemiologic Studies Depression Scale (CES-D)	A 20-item scale which measures depressive feelings and behaviors during the past week. Provides a depression quotient.
COVID-19 Questionnaire	A 6 item questionnaire where the subject is asked to provide information on prior positive tests of COVID-19 and COVID-19 vaccination status.

Table 5. BBRAIN Neuropsychological Test Battery

Test Name	Description	Outcome Measure
I. Executive System Functioning		
Controlled Oral Word Association Test (COWAT)	Spontaneous generation of words from letters F, A and S and animals category.	Total correct words generated
D-KEFS Category switching	Number of correct words generated switching between two categories	Total number of words Total number of correct category switches
D-KEFS Color-Word Interference Test (Stroop)	The Stroop is a test that measures the ability to inhibit competing responses in the presence of salient conflicting information. This ability can be called response inhibition, or behavioral inhibition. Secondly, the test also measures processing speed of verbal (words) and nonverbal (colors) stimuli.	Total Errors Self-corrected errors Uncorrected errors
D-KEFS Category switching	Number of correct words generated switching between two categories	Total number of words Total number of correct category switches
II. Tests of Attention, Vigilance and Tracking		
Trail-making Test (Reitan & Wolfson, 1985)	Timed connect-a-dot task to assess attention and motor control requiring sequencing (A).	Time to Completion Self-Corrected errors Uncorrected errors
Continuous Performance Test (Connors' CPT3)	Target letter embedded in series of distractors; to assess sustained attention and reaction time.	Reaction Time, Total Omission and Commission Errors
III. Tests of Motor Function		
Finger Tap Test	Continuous tapping of computer key with alternate hands; assesses simple motor speed.	Number of taps
IV. Tests of Visuospatial Function		
Rey-Osterrieth Complex Figure Test	Copy of a complex figure	Total correct out of 36
V. Tests of Memory		
California Verbal Learning Test (CVLT- II; Delis et al., 2000)	List of 16 nouns from 4 categories presented over multiple learning trials with recall after interference; assesses memory and learning strategies.	Total Trials 1-5 Long Delay
Rey-Osterrieth Complex Figure Test	Immediate and delayed recall of a complex figure	Total recall out of 36
VI. Tests of Motivation and General Intellectual Ability		
CVLT – Forced Choice Recognition	Forced choice of word pairs for each of the 16 target items.	Total correct
Wide-Range Achievement Test - Reading	Measures word reading skills and word decoding through letter identification and word recognition.	Total Correct Scaled Score

Task 5c. Obtain participants urine and stool sample kits back by mail to BUSPH for biorepository storage.

BUSPH serves as a biorepository storage (BS) site for urine and stool sample collection. Urine and stool sample collection kit are handed to participants on the day of their in person appointment at the four prospective sites. Subjects provide their first morning urine and ship it overnight to the BUSPH Exposure Assessment laboratory for analysis and storage. Subjects also collect stool samples using detailed instructions provided on the day of appointment and also ship it to BUSPH Exposure Assessment laboratory for analysis and storage. This procedure has been working well for the study participants. To date, we have received more than 370 samples total and are continuing to follow up communication with those participants who have yet to return their samples.

Task 5d. Upload Fitbit sleep, heart rate variability and blood pressure data to BUSPH DCC to merge with other datasets.

The BUSPH BEDAC will maintain the tracking database for the Fitbit sleep, heart rate variability and blood pressure data collection and transfer. The database resides on a secure, password-protected SQL server at the Boston University Medical Campus (BUMC). Because the server is part of the BUMC network, only connections from users authenticated from the domain controller will be accepted, thus providing a secure environment for all project data. The database will be automatically backed up on a nightly basis. Fitbits capable of collecting heart rate variability and sleep metrics have been purchased and shared with the recruitment sites to administer to participants when recruitment resumes. The BEDAC team has created a secure website for participants to upload their Fitbit data to promote higher compliance with obtaining Fitbit data. To date, we have received data from 140 participants and are continuing to follow up with those participants who have yet to send their Fitbit data.

Task 5e. Score neuropsychological tests and upload summary data to DCC for entry, cleaning and analyses.

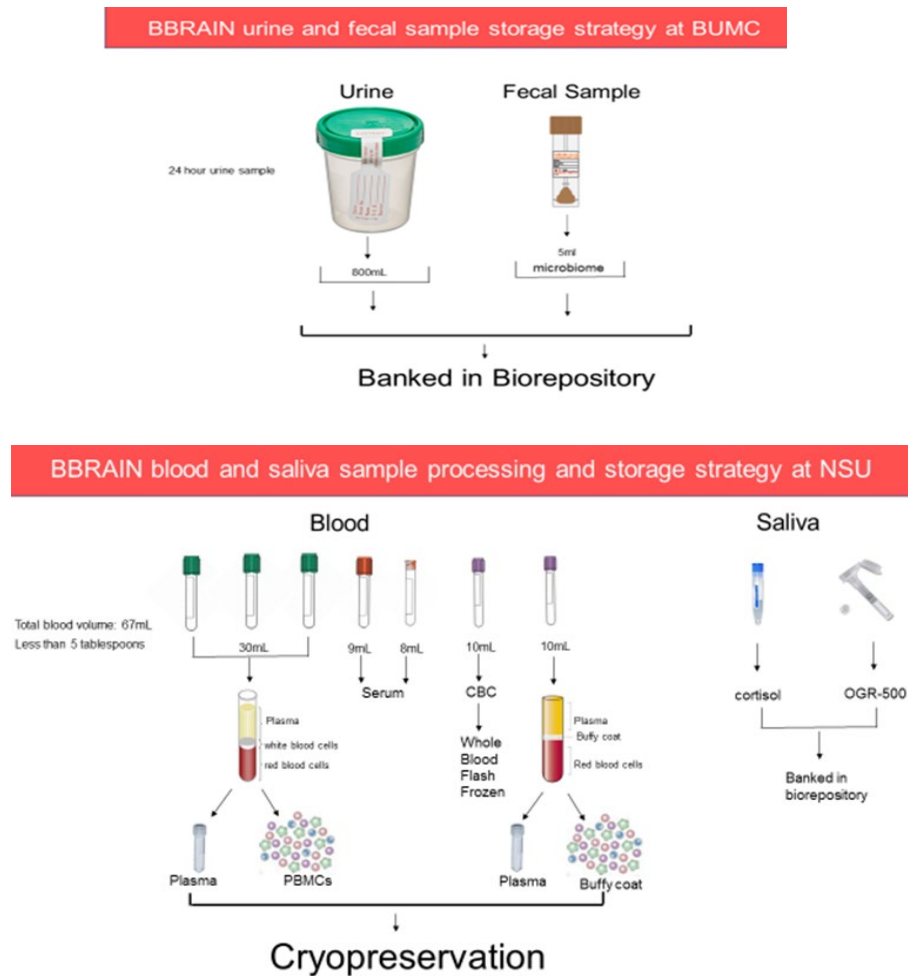
Neuropsychological protocols have been collected for 237 study participants to date and any new study staff at all sites have been trained for all test administration and standardized scoring procedures at the Boston Administrative Core. As data is collected, quality control procedures remain in place including double entry of data collection forms in the REDCap data collection website, built in range checks and quality control audits of all data collection by the Data Coordinating Center staff and the local BU Administrative Core neuropsychologists. Regularly monthly check-ins between the BU Administrative Core and study administrators are in place to answer any neuropsychological testing questions that may arise.

Task 5f. Send blood and saliva samples to Nova University for analysis of cytokine and chemokine panels and cortisol measurements and biorepository banking.

Two hundred and thirty-seven participant blood and saliva samples have been sent to NOVA Southeastern University for this study. The analysis of cytokine, chemokine and cortisol measurements has included testing for neuroendocrine and immune alterations and for hypothalamic pituitary adrenal axis abnormalities. Specifically, blood samples have

been sent to NOVA Southeastern University for analysis of proinflammatory cytokine and chemokines and RNA analysis. Multiplex Quansys ELISA system have been used with an existing cytokine platform created by Dr. Klimas' research laboratory. Dr. Klimas' site is measuring 18 cytokines in plasma. Saliva samples will continue to be analyzed for DNA and cortisol measurements. See figure below for aliquotting and storage scheme for BBRAIN.

TASK 6. PROCESS, STORE AND MANAGE SAMPLES FOR A TOTAL OF 500 NEW PARTICIPANTS (MONTHS 6-33)



As previously mentioned, we have completed 237 subjects to date. Samples from these 237 subjects were sent to the NOVA (NSU) and BUMC laboratories for processing and storage and study protocols have been running smoothly. The INIM NSU lab is the biorepository site for all blood and saliva samples collected throughout the study. We have obtained all the required approvals from IRB and the exempt status for OHRO.

Dr. Abreu, and her successor Dr. Kristina Aenelle, have worked closely with Dr. Sullivan on the logistics of this project in regular meetings to ensure the project is on task and running efficiently. Reagents needed, such as blood collection tubes, saliva kits, and shipping kits are routinely purchased and distributed to each site. Regular inventory is tracked at each study site and new supplies are ordered as needed. Dr. Abreu established a protocol for the various study sites. The study coordinators were trained on blood and saliva sample collection and the sample shipment process. Study coordinators were also trained on urine and stool sample collection to be shipped to Boston University. Sample

accessioning, barcoding, and sample processing was reviewed with the laboratory technician at the INIM NSU lab. Blood and saliva samples are routinely accessioned, processed, and aliquoted for storage. Saliva, plasma, and serum samples are stored at -80°C freezers. Flash-frozen whole blood, buffy coat, and isolated peripheral blood mononuclear cells (PBMCs) are stored in liquid nitrogen. Sample tracking is maintained in WebLDMS, a laboratory information management system (LIMS) for managing collections of biological specimens.

Task 6a. Process and store blood, saliva, urine and stool samples received from 4 recruitment sites at two biorepository sites

We have processed and stored the blood, saliva, urine and stool samples from 237 participants and will continue with our study protocols.

Task 6b. Perform local laboratory analyses (CBC) for 500 participants

We have performed local laboratory analyses for our first 237 participants and will continue this protocol for the rest of the study participants.

Task 6c. Extract DNA and RNA from 500 saliva samples and store in repository

We have performed multiple batch analysis of DNA and RNA from saliva samples. These analysis will continue to be run in batches at Nova and are ready to be shared with other studies.

Task 6d. Run batched assays for cortisol and cytokine results (18 plex cytokine assay)

We have performed multiple batch assays for cortisol and cytokine results from blood and saliva samples. These analysis will continue to be run in batches at Nova.

Task 6e. Provide Sample request forms and track information regarding the use of samples

To date we have received 26 requests for samples/data to that have been sent to our steering committee for approval. 23 of the 26 requests have been approved and we have shared brain imaging data, CSF samples, urine, stool and blood samples with 15 participating investigators. Some of the requests were for grant submissions and the investigators are not yet ready to receive the samples/data at this time.

Task 6f. Ship samples to requesting investigators

We have shipped CSF and blood samples to the requesting investigators who have been approved to date through the online BBRAIN sample request form. We have also shipped urine and stool samples that have been requested and approved through the repository.

Table 6. Approved Data Requests from the BBRAIN Repository

Institution	Lead Investigator	Project Title	Project Description	Data Shared
Baylor College	Marjin Lijffijt,	Glutamate receptor	Test neuroinflammatory pathways in a well-	Y

of Medicine	PhD	and Kynurenin pathway functioning in the pathobiology of GWI	characterized group of Gulf War veterans with and without GWI. The aim is to determine quinolinic acid, kynurenic acid, select inflammatory cytokines, and reactive oxygen species in CSF and in blood, and associate those biomarkers with GWI symptoms.	
Boston University of Medicine	Bang-Bon Koo, PhD	Comparison of complex diffusion markers with glial PET imaging in GWI	For a better understanding of the biological basis underlying GWI pathology, Dr. Koo has previously applied a novel MRI diffusion processing method to estimate microstructural alterations of the GW veterans. In this project, we will compare diffusion imaging with glial PET imaging data to explore the relationship between the microstructural damage and glial activations in GWI.	Y
The Roskamp Institute	Laila Abdullah, PhD	Gulf War pesticide metabolite mediated autoimmune dysfunction in Gulf War Illness	The purpose of this research is to identify GW pesticide autoantibodies in the blood and CSF of veterans with GWI in order to determine if these pesticide-hapten autoantibodies are associated with various symptom/exposure profiles and if they are correlated with disease severity. Dr. Abdullah used western blot/ELISA to determine the presence of autoantibody against different GW pesticide-albumin haptens.	Y
Boston Children's Hospital	Yangming Ou, PhD	Brain Aging in Gulf War Veterans	Dr. Ou's big-data (N=16,705) AI-algorithm paper was just accepted that can predict a person's brain age from T1-weighted brain MRI. The differences between AI-predicted and actual chronological ages can quantify accelerated or delayed brain aging associated with disease, lifestyle, environment or other factors. Dr. Ou will apply his algorithm to quantify abnormal brain aging in Gulf War veterans' existing brain MRIs in BBRAIN, possibly stratified by sex, comorbidities, years in war and other factors.	Y
Texas A&M Health Science Center	Ashok Shetty, PhD		These studies are for generating preliminary data for DOD grant proposal to be submitted jointly by Dr. Ashok K Shetty and Dr. Kimberly Sullivan. The main aim of this study is to generate microglia from veterans with and without GWI.	Y
UCSF & San Francisco VA Health Care System	Linda Chao, PhD	Investigating the Incidence of Mild Cognitive Impairment in GW veterans	This study will examine the prevalence of mild cognitive impairment (MCI) in deployed GW veterans via retrospective data analysis of de-identified neuropsychological and neuroimaging data from the PI's VA Merit grant and from study co-investigator Dr. Sullivan's Boston Gulf War Illness Consortium (GWIC) cohort.	Y
Columbia University	Ian Lipkin, MD, PhD		This project is to study the metabolomics in Gulf War Illness. 100 GW cases and 100 GW controls collected by Dr. Sullivan will be analyzed (n = 200). We will also be looking at 50 GW cases (baseline and 24 hours post exercise tolerance test) and 50 GW controls (baseline and 24 hours post exercise tolerance test) from Dr. Klimas (n=200). Metabolomic analysis on the GWI cases and controls will be performed.	Y
Miami VA	Kristina Aenlle, PhD and Saurabh Chatterjee, PhD	Studying the unique virome signature in Gulf War Illness to treat dysbiosis and symptom persistence	To analyze the virome signature of GWI in mouse models and GWI veterans. Experiments will be designed to study the gut virome signature in existing GW veteran cohorts. For this aim, stool collected from 150 (50 GWI cases with reported GI disturbances, 50 GWI cases without GI disturbances, 50 controls) veteran samples will be processed for nucleic acid isolation and library preparation	Y

			followed by 16S rRNA sequencing of the V4 regions and detailed bioinformatics.	
University of Texas Health Science Center at Houston	Deborah Little, PhD	Expanding Characterization and Application of Clinical MRI Markers in Gulf War Illness	Our overall objective is to utilize systematic neuroradiologic assessments of MRI scans in GWI cases and controls to develop and validate an evidence-based assessment tool, the GWI Brain Injury Scale (GW-BIS) to characterize the types and degree of brain pathology affecting GW Veterans.	Y
Nova Southeastern university	Travis Craddock, PhD	Machine Learning and Network Modeling in Gulf War Illness Paradigm	Use a combination of machine learning and network modeling to train computational algorithms to identify patterns in bio-symptom dataset in GW veterans, and rat GWI+mTBI serum and brain. Evaluate the congruency between the GW veteran data and animal model data to aid in the validation of the PCI-instigated mTBI in rats as a reliable model of mTBI in GW veterans, specifically the effects of timing and number of “hits” in the animal model.	Y
The Roskamp Institute	Laila Abdullah, PhD	Validating blood biomarkers of brain immune and metabolic dysfunction in Gulf War Illness	Identify blood lipid/metabolites that correlate with cognitive decline in the presence of the APOE allele among veterans with GWI. Identify blood lipid/metabolite profiles that correlate with bioenergetics deficits and glial activation in the brains of GWI. Validate blood biomarker signatures of GWI using APOE genotyping and blood lipids/metabolites that correlate with the CNS dysfunction in GWI using BBRAIN stored blood samples.	Y
Boston University School of Public Health	Junenette Peters, ScD	Metal Exposure Assessment in Gulf War Veterans Update	This study proposes to characterize levels of heavy metals in new, existing biospecimens (i.e., urine) from Gulf War military veterans. Results of this study will allow us to put in a strong grant proposal to DOD to contribute to knowledge of the extent of military exposures to metals and ultimately provide insights into mechanisms of metals toxicity in veterans as they age with an eye to improving long-term health outcomes.	Y
UCSF/San Francisco VA Health Care System	Linda Chao, PhD	An investigation of the relationship between toxicant exposures during GWdeployment and prodromal Parkinson’s disease	The goal of this project is to examine the long-term consequences of GWI in the context of aging and Parkinson’s disease	Y
National Institutes of Health	Jay Chung, MD, PhD	VDAC Oligomer formation in Gulf War Illness	It is believed that chronic inflammation is a component of the Gulf War Illness (GWI). We hypothesize that the chronic inflammation in GWI is caused by mitochondrial release of mtDNA. Therefore, we propose to measure the levels of VDAC oligomerization and cytoplasmic mtDNA in PBMCs of GWI and healthy controls. If our hypothesis is correct, this work will have identified a new biomarker and a potential therapeutic strategy for treatment of GWI.	N
UCSF/San Francisco VA Health Care System	Linda Chao, PhD	Examination of plasma PON1 paraoxonase activity and genotype in Gulf War Veterans	The goal of this project is test the hypothesis that PON1-192 polymorphisms and deployment-related exposures account for variance in the risk for developing Gulf War Illness.	Y
Boston University School of Medicine	Bang-Bon Koo, PhD	Multi-site Gulf War wartime-related exposure analysis	We plan to process all available GWIC/BBRAIN MRI data and build a multi-site, longitudinal study framework. We currently have imaging data but will need demographics and additional exposure	Y

			information for further analysis.	
University of Adelaide	Janet Coller, PhD	Immune-genetic Biomarkers of Risk and Resiliency for GWI	Currently, there is no validated biomarker for risk susceptibility to GWI but our preliminary findings indicate this difference is associated with genetic variability in specific immune mediators that may confer susceptibility for developing chronic inflammation (risk) or conversely for anti-inflammatory compensation (resilience). The proposed study will validate our preliminary findings relating immune genetic and epigenetic variants in conjunction with deployment exposures to risk of GWI from BBRAIN DNA samples.	N
Nova Southeastern University	Konstantinos Sidiropoulos, MD, PhD	Large Scale Computational Analysis of Gulf War Illness / Deep Phenotypic Analysis	Our group will be conducting a large scale computational analysis of Gulf War Illness including a deep phenotypic analysis using public datasets, our institutes data, and other available datasets in order to help GWI patients for improved diagnosis and treatment.	N
Boston University School of Medicine	Bang-Bon Koo, PhD	Grant resubmission with the addition of MGH GW participants with PET scans	We plan to inspect PET and DTI patterns in a subset of GW participants from MGH cohort by focusing on changes in the substantia nigra for an upcoming grant resubmission.	N
University of South Florida	Marcus Cooke, PhD	Nucleic Acid Adductomics for the Identification and Monitoring of Environmental Toxic Exposures	Notably, 80-90% of chronic disease risks are related to environmental agents. The emerging technique of nucleic acid (NA) adductomics aims to report on the totality of DNA, and RNA modifications i.e., the NA adductome, which is strongly implicated in the pathogenesis of many chronic diseases. Our aim is to demonstrate that urinary NA adductomics is a useful tool to dissect the complex, multi-exposure combinations in military personnel, and hence develop biomarkers of exposure and disease risk.	N
Sandia National Laboratories	Joshua Podlevsky	Proposal for HT9425-23-TERP-IIRA	Toxic Exposures Research Program Investigator-Initiated Research Award inquiry	N

TASK 7: MERGE DATA AND PERFORM INTERIM ANALYSES (MONTHS 6-24)

Data merging has been performed from cleaned data sets and interim analyses have been completed.

7a. Merge Clinical datasets data from prior studies for brain imaging, cognitive, demographic and health symptom report data

Retrospective data sets were merged for neuroimaging, cognitive, demographic and health symptom outcomes by the BEDAC data management team and interim data analysis was performed. Multiple reports of these data have been presented at conferences and are in draft for publication. Preliminary data shows that veterans with GWI had significantly poorer performance on neuropsychological tests than healthy controls, indicating deficits in sustained attention and verbal memory (Table 7, Figure 1; Keating et al., 2023). Further, these cognitive decrements were associated with environmental exposures during the Gulf War (Table 8). Subjective cognitive complaints in veterans with GWI have been positively correlated with objective neuropsychological scores (Table 9) which builds on the initial report of memory problem self-report and objective validation from Dr. Chao (Chao, 2017). Additionally, exposure to chemical weapons in the GW were associated with increased reported sleep

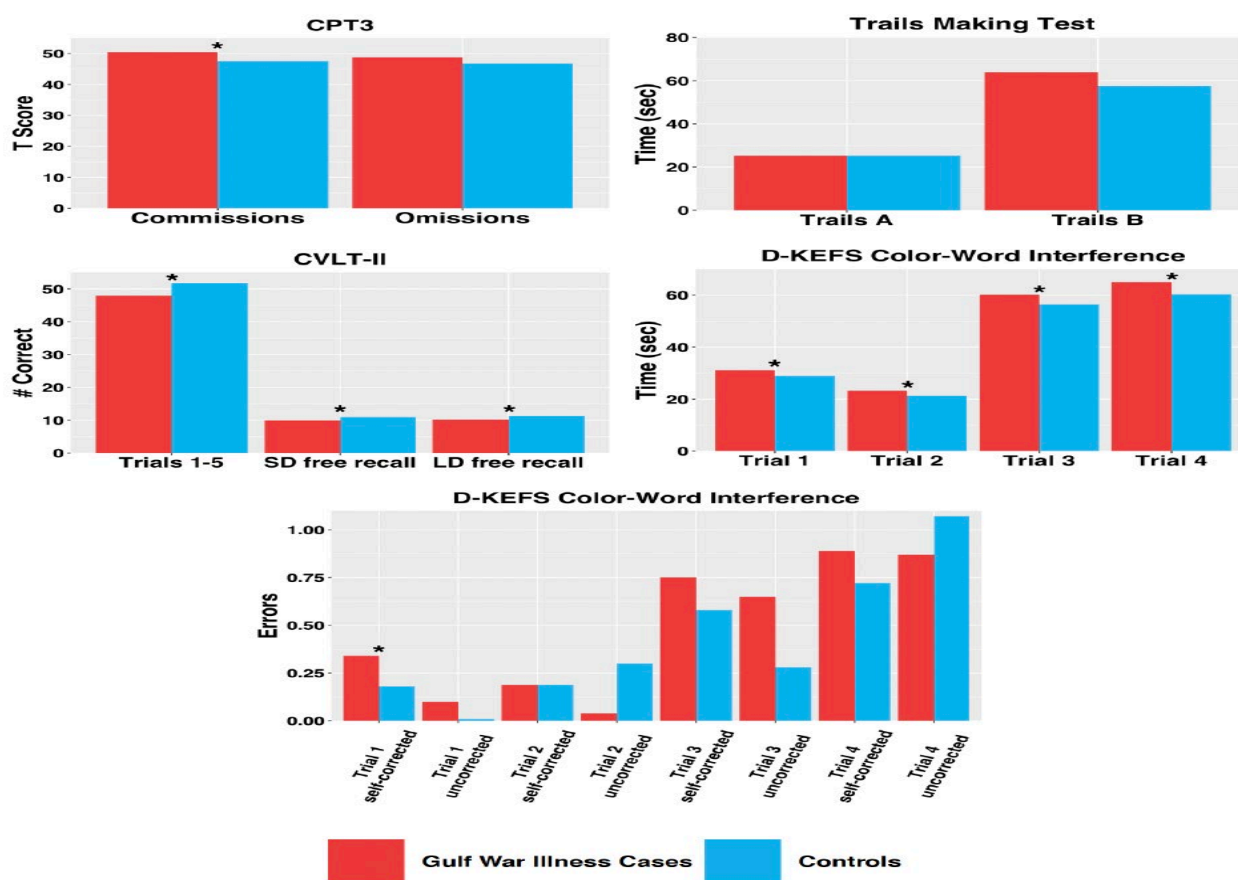
problems while exposure to PB pills was associated with increased pain reporting (Tables 10-11).

Table 7. Retrospective Neuropsychological Measures from BBRAIN Repository by GWI Status

		GW Cases (n=312)	Controls (n=99)
		Means	
<i>CPT 3</i>	Omissions*	49.72	47.05
	Commissions**	53.13	48.17
<i>CVLT-II</i>	Correct in trials 1-5**	46.34	50.82
	Correct in SD Recall**	9.36	10.61
	Correct in LD Recall*	9.69	10.94
<i>D-KEFs Stroop</i>	Trial 1 time (sec)**	32.71	29.51
	Trial 2 time (sec)**	24.21	21.53
	Trial 3 time (sec)**	62.43	56.85
	Trial 4 time (sec)**	67.43	60.63
<i>Trails</i>	Trail A time (sec)**	32.00	26.79
	Trail B time (sec)**	73.73	63.22

*p<0.05
**p<0.001

Figure 1: Retrospective Neuropsychological Measures from BBRAIN Repository by GWI Status



*p<0.05

Table 8. Retrospective Neuropsychological data by exposure status

Exposures			Controls	<7 days Exposed Cases	7+ days Exposed Cases
			Mean		
Heard chemical alarms sounded	CPT 3	Omissions*	49.09	49.71	48.29
		Commissions*	45.4	52.67	55.67
	CVLT-II	# correct in long-delay free recall*	11.79	9.77	8.9
	Trails	Trail A(sec)*	22.94	32.33	34.38
		Trail B(sec)*	59.65	71.04	80.17
Saw Smoke from oil well fires	CVLT-II	# correct in short-delay free recall*	11.2	9.71	8.79
	Trails	Trail A(sec)*	23.2	32.15	33.78

Table 9. Subjective cognitive symptoms correlated to objective cognitive tests among GWI Prospective Cases

Cognitive Domain	Neuropsychological Test	Spearman Correlation Coefficient	p-value
Memory	CVLT-II (n = 136)		
	Trial 1 words correct*	-0.160	0.062
	Trial 2 words correct*	-0.248	0.004
	Trial 3 words correct*	-0.202	0.018
	Trial 4 words correct*	-0.223	0.009
	Trial 5 words correct	-0.149	0.082
	Trials 1-5 words correct*	-0.214	0.012
	Short Delay Recall words correct*	-0.301	<0.001
	Short Delay Recall words correct – Cued*	-0.249	0.003
	Long Delay Recall words correct*	-0.261	0.002
	Long Delay Recall words correct– Cued*	-0.267	0.002
	REY-Osterrieth complex figure		
	Immediate recall	-0.163	0.063
	Delayed recall	-0.158	0.071
Visuospatial Abilities	REY (n=131)		
	Total Copy	-0.196	0.025
	Trail Making Test (n = 138)		
	Trials A time*	0.243	0.004
	Subject Corrections	0.018	0.838
	Examiner Corrections	0.107	0.211
Planning	COWAT (n=127)		
	A total correct words*	-0.191	0.032
	F total correct words	-0.105	0.240

	S total correct words	-0.125	0.160
	D-KEFS Category Switching (n=125)		
	Correct Responses (1st quarter)	-0.0849	0.347
	REY-Osterrieth complex figure		
	BQSS Organization score	-0.038	0.665
	Organizational T score	-0.014	0.872
	Organizational percentile	-0.004	0.967
	D-KEFS Color Word Inference		
	Trial 3 time*	0.275	0.001
Organization	COWAT		
	A total correct words*	-0.171	0.055
	F total correct words	-0.088	0.327
	S total correct words	-0.096	0.285
	D-KEFS Category Switching		
	Correct Responses (1st quarter)*	-0.219	0.014
	REY-Osterrieth complex figure		
	BQSS Organization score	0.004	0.960
	Organizational T score	0.031	0.726
	Organizational percentile	0.007	0.944
	D-KEFS Color Word Inference		
	Trial 3 time*	0.239	0.005
Attention	CPT 3 (n = 136)		
	Hit Reaction Time raw	0.021	0.807
	Omission T score	0.076	0.380
	Commission T Score	0.118	0.170
	Trails Making Test		
	Trail A time	0.113	0.187
	Subj. Corrected Errors	-0.001	0.999
	Examiner Corrected Errors*	0.199	0.019

Table 10. Self-reported sleep problems on the PSQI by war-related exposures and GWI case status

	Exposed mean	Unexposed mean	P-value
<i>GWI Cases</i>			
Oil well fires	12.91	13.42	0.404
Chemical alarms*	13.72	12.42	0.025
Pesticide cream	13.21	12.95	0.677
Pesticide fog	13.80	12.84	0.163
PB pills	12.69	13.54	0.161
Biological weapons	13.22	12.96	0.660
<i>GWI Controls</i>			

Oil well fires	6.77	7.14	0.684
Chemical alarms	6.68	7.04	0.710
Pesticide cream	8.64	6.63	0.097
Pesticide fog	6.43	6.99	0.720
PB pills	7.34	6.70	0.462
Biological Weapons	7.72	6.77	0.447

Table 11. Self-reported pain on the McGill by war-related exposures and GWI case status

	Exposed <i>mean</i>	Unexposed <i>mean</i>	P-value
<i>GWI Cases</i>			
Oil well fires	33.35	31.97	0.534
Chemical alarms	34.48	21.42	0.159
Pesticide cream	34.33	31.41	0.188
Pesticide fog	34.84	32.21	0.290
PB pills*	30.97	35.34	0.049
Biological weapons	34.08	31.77	0.284
<i>GWI Controls</i>			
Oil well fires	17.47	12.66	0.272
Chemical alarms	12.91	16.74	0.370
Pesticide cream	13.11	16.02	0.573
Pesticide fog	17.58	15.30	0.750
PB pills*	22.29	12.22	0.010
Biological Weapons	8.00	17.26	0.147

7b. Perform machine learning and/or meta-analytic techniques on common data elements from prior study epidemiological, cognitive and brain imaging data

Common data elements (CDEs) are being compiled from the prior clinical datasets that have been shared and will then be combined with brain imaging outcomes for Dr. Koo to perform machine learning analyses. A review paper of cognitive outcomes was published in collaboration with NOVA Southeastern investigators while waiting for the common data elements to be available for planned meta-analysis studies (Jeffrey et al., 2019). CDEs were utilized to measure cognitive performance and self-report of health symptoms in multiple publications from Dr. Sullivan and BBRAIN investigators which have validated the current CDEs and made recommendations for further CDE additions for the GWI field (Keating et al., 2023; Kregel et al. in revision).

7c. Discussion of results and preparation of abstracts for meeting presentations and initial manuscript for publication

Study results have been prepared for abstracts and presented at meetings. Planned upcoming presentations are in progress as well as additional manuscripts for publication.

7d. Annual reports of progress will be written

Five annual progress reports have been written and submitted for review.

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 can tap into this talent pool by taking practicum and intern students in the fields of health communication and promotion, who as part of an academic program, will create a media and outreach program for the network. Doctoral students in related fields can also be brought on to aid in this project. Finally, as has been exhibited with the GWIC, the network will be financially continued through spin-off grants in GWI and similar fields through DOD, VA and NIH and by providing the tools for talented young researchers to aid us in tackling and solving the problem of GWI. For example, in summer of 2019 we had a student from Middlebury College, VT working with us a summer intern who helped develop the BBRAIN website. This year, we had MPH practicum students working with us to learn cognitive evaluations and to assist with subject recruitment efforts.

○ Participation in conferences, workshops, and seminars:

BBRAIN researchers will continue to attend conferences, workshops and seminars to present research findings.

- In February 2021, Dr. Sullivan and the BU research team presented on multiple findings from retrospective and prospective BBRAIN data at the International Neuropsychological Conference.
- On August 23rd, 2021, Dr. Sullivan was invited to present on the BBRAIN network to Congressional staffers at the Capitol Hill Gulf War Illness Forum hosted by Veterans for Common Sense.
- On February 25th, 2022, Dr. Sullivan was invited as a keynote speaker to present the BBRAIN network at the Gulf War, Operation Desert Shield virtual meeting that was co-led by CDMRP and VA.
- In July 2022, Dr. Sullivan and the BU research team presented on cognitive data and health symptom findings from retrospective and prospective BBRAIN data at the International Neuropsychological Conference.
- In November 2022, Dr. Sullivan and the BU research team presented on mood functioning and motor symptoms of Gulf War veterans at the American Public Health Association conference in Boston, MA.

- In February 2023, Dr. Sullivan and the BU research team presented on cognitive data in women veterans as well as MRI outcomes in veterans with GWI at the International Neuropsychology Conference in San Diego, CA.
- In August 2023, Dr. Sullivan and the BU research team presented on reproductive health effects of women GW veterans at the Military Research Systems Health Symposium.
- **How results will be disseminated to communities of interest:**
 Research staff will use some of the outreach structure already in place from the Boston GWI consortium. The 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. GWIC also has a Twitter account and study website that will be used to disseminate information to the community and refer to the BBRAIN social media sites. In addition, Boston University has a very proactive media team that produces text and video news stories that get widely shared. Research staff will also be aided by the Veteran Working Group in getting news out about the BBRAIN to fellow veterans and their communities as well as results. As the research team has done for GWIC, they will write professional publications that state what samples are available and who to contact. This was done for the stem cell repository that is now part of BBRAIN and was very well received in the research community. To date, a media story was written about BBRAIN in April 2019 at <https://www.bu.edu/sph/2019/04/08/professor-awarded-3-2m-to-establish-biorespository-network-for-gulf-war-illness-research/> and a new larger story about BBRAIN came out on Veterans Day 2019 at <https://www.bu.edu/sph/2019/11/13/the-brink-5/> to help with subject recruitment efforts and in letting investigators know about samples that will be shared for other relevant studies. An article highlighting the BBRAIN study 30 years after the Gulf War was published in November 2021: https://www.bu.edu/sph/news/articles/2021/30-years-after-gulf-war-veterans-still-battle-health-issues-caused-by-toxic-wounds/?fbclid=IwAR1_3yibizQ_PvH1Onlii4tIqlqvMsXB3tk0SmbiWneBY44jE02c_IvGH3s. Most recently, a media story highlighting the results of the BBRAIN cognitive retrospective manuscript was published in November 2023: <https://www.bu.edu/sph/news/articles/2023/gulf-war-veterans-continue-to-experience-cognitive-toxic-wounds/>
- **Plan to do during the next reporting period to accomplish the goals:** In the next reporting period, we plan to:
 - Continue to obtain Biorepository data for analysis and catalogue available biospecimens for sharing from Repository Resource Sites.
 - Continue to obtain imaging data from retrospective sites and perform post-processing of and machine learning analysis with combined common data elements.
 - Continue recruitment at the remaining prospective sites. We will continue to process, store and manage samples for a total of 280 new participants.
 - We believe that continuing our media stories and working with new web advertising companies will help us reach closer to our target enrollment by year 6.

4. IMPACT

- **Impact on the development of the principal discipline(s) of the project:**

- *Nothing to Report.*

- **Impact on other disciplines:**

- BBRAIN investigators have collaborated with researchers external to the consortium on 10 DoD, 4 VA applications and 8 additional study sites (Harvard University, Georgetown University, Roskamp Institute, Boston VA, Bronx VA, San Francisco VA, U-Alabama, Mass General Hospital). Each site has valuable and unique samples to share within and outside the repository network that would not otherwise be available to the research community. It is the goal of the BBRAIN to continue the expert infrastructure of scientific, laboratory and data management currently within the GWIC, include other expert scientist collaborators, and to hasten biomarker discovery and treatment development for GWI on multiple aspects of GWI disciplines (immune, neurological, respiratory, gastrointestinal etc) as well as other disciplines including comorbid conditions of ME/CFS, IBS and fibromyalgia. We also have investigators interested in studying the risk of toxicant induced Parkinson's disease and brain cancer and heavy metals exposure with our research network that we will continue to pursue.

- **Impact on technology transfer:**

- Data shared from the biorepository will allow more investigators across the U.S to perform more research in the area of Gulf War Illness and other related health conditions. Results from studies conducted with BBRAIN samples will also make it easier for investigators to secure funding for supplemental and diverse GWI related studies and to share them for public use. This has already been shown in Dr. Sullivan and Klimas contributing our biorepository knowledge and plans for BBRAIN with the National Academy of Science (NAS) Gulf War Respiratory Health Working Group meeting in October 2019. We have also created a stem cell line with organoid brain tissue available to other researchers that is the first in this field.

- **Impact on society beyond science and technology:**

- The demographic, exposure history, clinical data symptom assessment, diagnostic outcomes and other data gathered from prospective and retrospective sites will aid in the comprehensive understanding of the pathobiology of GWI. Results of our analysis may uncover and increase the diagnostic and treatment capability for GWI. The biorepository can also become a model for the establishment of other biorepositories in the field. Researchers may research out to us for questions regarding the setup and management of the biorepository. In fact, we have already spoken to CDC investigators studying ME/CFS and the NAS GW respiratory health working group for advice about biorepositories.

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**
 - **Journal publications.**

We have submitted two manuscripts for publication utilizing CDEs and retrospective cognitive data from the biorepository. We have also published a methods paper on BBRAIN study to share the knowledge of our resource with the GWI research community.

- Keating, D., Kregel, M., Dugas, J. *et al.* Cognitive decrements in 1991 Gulf War veterans: associations with Gulf War illness and neurotoxicant exposures in the Boston Biorepository, Recruitment, and Integrative Network (BBRAIN) cohorts. *Environ Health* **22**, 68 (2023). <https://doi.org/10.1186/s12940-023-01018-2>
- Kregel, M; Keating, D.; Chao, L.; Dugas, J.; Koo, B. B.; Heeren, T.; Quinn, E; Toomey, R.; Steele L; Klimas, N; Sullivan K. Characterizing 1991 Gulf War Women Veterans from the Boston Biorepository and Integrative Network for Gulf War Illness (BBRAIN): Demographics, Exposures, Neuroimaging and Cognitive Outcomes. *The Clinical Neuropsychologist* (In revision).
- Abdullah L, Nkiliza A, Niedospial D, Aldrich G, Bartenfelder G, Keegan A, Hoffmann M, Mullan M, Klimas N, Baraniuk J, Crawford F, Kregel M, Chao L, Sullivan K. Genetic association between the APOE ε4 allele, toxicant exposures and Gulf war illness diagnosis. *Environ Health*. 2023 Jul 6;22(1):51. doi: 10.1186/s12940-023-01002-w.
- 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.
- Yates PL, Case K, Sun X, Sullivan K, Baas PW, Qiang L. Veteran-derived cerebral organoids display multifaceted pathological defects in studies on Gulf War Illness. *Front Cell Neurosci*. 2022 Dec 23;16:979652. <https://doi:10.3389/fncel.2022.979652> eCollection 2022.
- Sullivan, K.; O'Callaghan, J.P. Advancing the Role of Neuroimmunity and Genetic Susceptibility in Gulf War Illness. *Brain Sci*. **2022**, *12*, 1068. <https://doi.org/10.3390/brainsci12081068>

- Friedman, A.; Janulewicz Lloyd, P.A.; Carlson, J.; Quinn, E.; Keating, D.; Toomey, R.; Heeren, T.; Coughlin, S.S.; Markenson, G.; Kregel, M.; Sullivan, K. Preliminary Findings from the Gulf War Women's Cohort: Reproductive and Children's Health Outcomes among Women Veterans. *Int. J. Environ. Res. Public Health* **2022**, *19*, 8483. <https://doi.org/10.3390/ijerph19148483>
- Oberlin S, Nkiliza A, Parks M, Evans JE, Klimas N, Keegan AP, Sullivan K, Kregel MH, Mullan M, Crawford F, Abdullah L. Sex-specific differences in plasma lipid profiles are associated with Gulf War Illness. *J Transl Med* **20**, 73 (2022). <https://doi.org/10.1186/s12967-022-03272-3>
- Bose D, Chatterjee S, Older E, Seth R, Janulewicz P, Saha P, Mondal A, Carlson JM, Decho AW, Sullivan K, Klimas N, Lasley S, Li J, Chatterjee S. Host gut resistome in Gulf War chronic multisymptom illness correlates with persistent inflammation. *Commun Biol.* 2022 Jun 7;5(1):552. doi:10.1038/s42003-022-03494-7.
- Yates PL, Patil A, Sun X, Niceforo A, Gill R, Callahan P, Beck W, Piermarini E, Terry AV, Sullivan KA, Baas PW, Qiang L. A cellular approach to understanding and treating Gulf War Illness. *Cell Mol Life Sci.* 2021 Nov;78(21-22):6941-6961. doi: 10.1007/s00018-021-03942-3. Epub 2021 Sep 27. PMID: 34580742
- Cohen, D.E., K.A. Sullivan, R.B. McNeil, N.G. Klimas, R. McNeil, W. Ashford, A. Bested, J. Bunker, A. Cheema, D. Cohen, D. Cook, J. Cournoyer, T. Craddock, J. Golier, A. Hardie, D. Helmer, J.B. Lindheimer, P.J. Lloyd, K. Kerr, M. Kregel, S. Nadkarni, S. Nugent, B. Paris, M. Reinhard, P. Rumm, A. Schneiderman, K.J. Sims, L. Steele, M. Turner, K. Sullivan, L. Abdullah, M. Abreu, M. Abu-Donia, K. Aenlle, J. Arocho, E. Balbin, J. Baraniuk, K. Block, M. Block, B. DeBeer, B. Engdahl, N. Filipov, M.A. Fletcher, V. Kalasinsky, E. Kokkotou, K. Lidie, D. Little, W. Loging, M. Morris, L. Nathanson, M.D. Nichols, G. Pasinetti, D. Shungu, P. Waziry, J. VanLeeuwen, J. Younger, and N. Klimas, A common language for Gulf War Illness (GWI) research studies: GWI common data elements. *Life Sci*, 2021.
- Cheng CH, Alshelh Z, Guan Y, Sullivan K, Loggia ML, Koo BB. Association of the tissue microstructural diffusivity and translocator protein PET in Gulf War Illness. *Brain Behav Immun Health.* 2021 Oct 6;18:100364. <https://doi.org/10.1016/j.bbih.2021.100364>. eCollection 2021 Dec. PMID: 34693367
- Keating D, Zundel CG, Abreu M, Kregel M, Aenlle K, Nichols MD, Toomey R, Chao LL, Golier J, Abdullah L, Quinn E, Heeren T, Groh JR, Koo BB, Killiany R, Loggia ML, Younger J, Baraniuk J, Janulewicz P, Ajama J, Quay M, Baas PW, Qiang L, Conboy L, Kokkotou E, O'Callaghan JP, Steele L, Klimas N, Sullivan K. Boston biorepository, recruitment and integrative network (BBRAIN): A resource for the Gulf War Illness scientific community. *Life Sci.* 2021 Nov 1;284:119903. doi: 10.1016/j.lfs.2021.119903. Epub 2021 Aug 26. PMID: 34453948. Link: <https://www.sciencedirect.com/science/article/pii/S0024320521008900?via%3DiHub>
- Guan Y, Cheng CH, Chen W, Zhang Y, Koo S, Kregel M, Janulewicz P,

Toomey R, Yang E, Bhadelia R, Steele L, Kim JH, Sullivan K, Koo BB. Neuroimaging Markers for Studying Gulf-War Illness: Single-Subject Level Analytical Method Based on Machine Learning. *Brain Sci.* 2020 Nov 20;10(11):884. doi: 10.3390/brainsci10110884. PMID: 33233672

- Joshi U, Pearson A, Evans JE, Langlois H, Saltiel N, Ojo J, Klimas N, Sullivan K, Keegan AP, Oberlin S, Darcey T, Cseresznye A, Raya B, Paris D, Hammock B, Vasylieva N, Hongsibsong S, Stern LJ, Crawford F, Mullan M, Abdullah L. A permethrin metabolite is associated with adaptive immune responses in Gulf War Illness. *Brain Behav Immun.* 2019 Oct;81:545-559. doi: 10.1016/j.bbi.2019.07.015. Epub 2019 Jul 17.
- Jeffrey MG, Krengel M, Kibler JL, Zundel C, Klimas NG, Sullivan K, Craddock TJA. Neuropsychological Findings in Gulf War Illness: A Review. *Front Psychol.* 2019 Sep 26;10:2088. doi: 10.3389/fpsyg.2019.02088. eCollection 2019. Review. PMID: 31616335. Link: <https://www.frontiersin.org/articles/10.3389/fpsyg.2019.02088/full>

▪ **Books or other non-periodical, one-time publications.**

Nothing to Report.

▪ **Other publications, conference papers, and presentations.**

Presentations:

- K. Sullivan. Military Biorepositories. National Academy of Science (NAS) Workshop on Gulf War Respiratory Health Committee (Invited Speaker), Washington, DC, October 3, 2019.
- K. Sullivan. Military Occupational Health and Toxicology. American Academy of Environmental Medicine annual meeting. (Invited keynote speaker), Louisville, KY, October 12, 2019.
- N. Klimas. Can We "Reboot" Human Homeostasis to Cure Chronic Illness? What We Are Learning from Gulf War Illness and ME. American Academy of Environmental Medicine annual meeting. (Invited keynote speaker), Louisville, KY, October 12, 2019.
- K. Sullivan. Boston, Biorepository and Integrative Network for GWI. GWI State of the Science 30th Anniversary of the Operation Desert Storm meeting, virtual, August 18, 2020.

Conference Posters:

- Dylan Keating, Emily Quinn, Steven Coughlin, Vahe Heboyan, Glenn Markenson, Patricia Janulewicz Lloyd, Maxine Krengel, Kimberly Sullivan. Effects of 1991 Gulf War-related toxicants on Women's Veterans Health During Pregnancy. Poster presented at the Military Health Systems Research Symposium (MHSRS), August 2023.
- Dylan Keating, Jenna Groh, Maxine Krengel, Rosemary Toomey, Linda Chao, Emily Quinn, Julianne Dugas, Kimberly Sullivan. Toxic Wounds are Associated with Cognitive

Decrements in Women Veterans of the 1991 Gulf War. Poster presented at International Neuropsychology Society conference (INS), February 2023.

- Dylan Keating, Jenna Groh, Maxine Kregel, Rosemary Toomey, Linda Chao, Emily Quinn, Julianne Dugas, Kimberly Sullivan. Neuropsychological Outcomes Vary by Sex in Neurotoxicant Exposed Veterans with Gulf War Illness. Poster presented at International Neuropsychology Society conference (INS), February 2023.
- Dylan Keating, Maxine Kregel, Rosemary Toomey, Emily Quinn, Rabindra Kadel, Kimberly Sullivan. Mood functioning in Gulf War veterans: findings for the Boston, Biorepository, Recruitment and Integrative Network for GWI (BBRAIN). Poster presented at the American Public Health Academy conference (APHA), November, 2022.
- Charlotte Jean Michel, Jenna Groh, Sarah O'Shea, Emily Sission, Julianne Dugas, Timothy Heeren, Rosemary Toomey, Maxine Kregel, Kimberly Sullivan. Non-motor symptoms of Parkinson's Disease Risk in 1991 Gulf War veterans. Poster presented at the American Public Health Academy conference (APHA), November 2022.
- Alexa Friedman, Dylan Keating, Jeff Carlson, Charlotte JeanMichel, Emily Sisson, Steve Coughlin, Vahe' Heboyen, Glenn Markenson, Lizette Mendez, Patricia Janulewicz Lloyd, Maxine Kregel, Kimberly Sullivan. *Reproductive and Developmental Outcomes Following Deployment: Gulf War and Gulf War-Era Women Veterans*. Poster presented at Military Health Systems Research Symposium (MHSRS), September 2022.
- Dylan Keating, Maxine Kregel, Rosemary Toomey, Linda Chao, Layla Abdullah, Lea Steele, Patricia Janulewicz, Emily Quinn, Julianne Dugas, Kimberly Sullivan. *Toxic wounds and cognition in US Gulf War veterans from the BBRAIN repository network*. Poster presented at International Neuropsychology conference (INS), July 2022.
- Charlotte JeanMichel, Alexa Friedman, Dylan Keating, Emily sisson, Julianne Dugas, Timothy Heeren, Rosemary Toomey, Lea Steele, Patricia Janulewicz, Maxine Kregel, Nancy Klimas, Kimberly Sullivan. *Self-reported sleep quality and cognitive outcomes in US veterans with Gulf War Illness*. Poster presented at International Neuropsychology conference (INS), July 2022.
- Dylan Keating, Alexa Friedman, Jenna Groh, Charlotte Jean Michel, Maria Abreu, PhD, Emily Quinn, Maxine Kregel, PhD, Kim Sullivan, PhD. *Neuropsychological Functioning and Interferon Gamma Levels in Veterans with Gulf War Illness from the Gulf War Illness Consortium (GWIC)*. Poster presented at International Neuropsychology Conference (INS), February 2022.
- **Website(s) or other Internet site(s)**
 - <http://sites.bu.edu/bbrain/>
 - https://wwwapp.bumc.bu.edu/BEDAC_BBRAINRetro
- **Technologies or techniques**
 - **REDCap:** This primary platform utilized in the BBRAIN study is a freely available platform used extensively for collaborative multi-site research. It can be used at all sites to collect existing data and assist with data mining strategies.

- **Laboratory Data Management System (LDMS):** Samples received by the biobank laboratory will be processed in LDMS. The LDMS (Frontier Science Foundation) system allows for the seamless management of bio-specimens from collection, processing, shipment, and storage processes. Specimens are barcoded and accessioned to ensure the integrity of the aliquot is maintained even over decades of storage.
- **Inventions, patent applications, and/or licenses**
Nothing to Report.
- **Other Products**
Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **Individuals that have worked on the project:**

Name: Kimberly Sullivan	Project Role:	<i>PI of Biorepository Network and Boston site</i>
	Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-7940-6123
	Nearest person month worked:	<i>12</i>
	Contribution to Project:	<i>Oversee the biorepository at BUSPH; oversee the recruitment and participation at BUSPH (200 participants); oversee data and sample coordination of all prospective and retrospective BBRAIN sites.</i>
	Funding Support:	<i>BBRAIN Award, W81XWH-18-1-0549</i>
Name: Nancy Klimas	Project Role	<i>Co-Investigator, PI of Miami Site</i>
	Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0003-1459-3268
	Nearest person month worked:	<i>12</i>
	Contribution to Project:	<i>Oversee the resource site at the Miami VA medical center; oversee recruitment and participation (100 participants).</i>
	Funding Support:	<i>BBRAIN Award, W81XWH-18-1-0549</i>
Name: Julia Golier	Project Role	<i>Co-Investigator, PI of Bronx VA Site</i>
	Researcher Identifier (e.g. ORCID ID)	

	ID):	
	Nearest person month worked:	12
	Contribution to Project:	<i>Oversee site at Bronx VA (100 participants); serve as neuroendocrine expert for BBRAIN; oversee cortisol study protocols, analyses, and outcomes.</i>
	Funding Support:	<i>BBRAIN Award, W81XWH-18-1-0549</i>
Name: Linda Chao	Project Role	<i>Co-Investigator, PI of San Francisco VA Site</i>
	Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-8593-2434
	Nearest person month worked:	12
	Contribution to Project:	<i>Serve as imaging expert for BBRAIN; oversee site in San Francisco (100 participants); share prior brain imaging data, saliva samples, and demographic, exposure, and survey data.</i>
	Funding Support:	<i>BBRAIN Award, W81XWH-18-1-0549</i>

- **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:** Harvard University and New England School of Acupuncture
 - **Location of Organization:** Boston, Massachusetts
 - **Partner's contribution to the project (identify one or more)**
 - **Financial support;** *Nothing to Report*
 - **In-kind support:** *Nothing to Report*
 - **Facilities:** *Nothing to Report*
 - **Collaboration:** Lisa Conboy, BBRAIN consultant affiliated with both listed institutions. Dr. Lisa Conboy is a social epidemiologist and is well-published in the area of Complementary and Alternative Medicine. She is an Instructor in Medicine at Harvard Medical School, and Research Director at the New England School of Acupuncture. Dr. Conboy has been involved in studies investigating diagnostic biomarkers and predictors of therapeutic responses in inflammatory bowel disease, irritable bowel syndrome (IBS) and GWI. Drs. Conboy have previously shared blood samples from their acupuncture study for a study of CNS autoantibodies in GWI in collaboration with Drs. Sullivan and colleagues. She has also

collaborated in the Boston area PI recruitment network sharing study flyers and recruitment information with their GW veteran cohort of other local studies. Dr. Conboy will continue to participate in the Boston PI Recruitment Network as well as contribute retrospective repository blood samples and data from her successful acupuncture trial with GW veterans.

- **Personnel exchanges:** *Nothing to Report*
- **Other.** *Nothing to Report*

1. **Organization Name:** Harvard University and Beth Israel Hospital
2. **Location of Organization:** Boston, Massachusetts
3. **Partner's contribution to the project** (*identify one or more*)

1. **Financial support;** *Nothing to Report*
2. **In-kind support:** *Nothing to Report*
3. **Facilities:** *Nothing to Report*
4. **Collaboration:** Drs. Efi Kokkotou, BBRAIN Consultant affiliated with both institutions. She has previously shared blood samples from her acupuncture study for a study of CNS autoantibodies in GWI in collaboration with Drs. Sullivan and colleagues. She has also collaborated in the Boston area PI recruitment network sharing study flyers and recruitment information with their GW veteran cohort of other local studies. Dr. Kokkotou will share samples from her large biorepository of IBS and GWI samples from DOD and NIH funded studies (W81XWH-09-0064 and K01AT004916).
5. **Personnel exchanges:** *Nothing to Report*
6. **Other.** *Nothing to Report*

1. **Organization Name:** VA Boston Healthcare System
2. **Location of Organization:** Boston, Massachusetts
3. **Partner's contribution to the project** (*identify one or more*)

1. **Financial support;** *Nothing to Report*
2. **In-kind support:** *Nothing to Report*
3. **Facilities:** *Nothing to Report*
4. **Collaboration:** Drs. Christopher Brady and Neil Kowall, BBRAIN Consultant and Co-Investigator, affiliated with this institution. The VA Biorepository Brain Bank (VABBB), Gulf War Veterans' Illnesses Biorepository (GWVIB) is a collaborative effort between multiple VAs (Boston-coordinating center/diagnostic neuropathology, Tucson-biorepository) and academic medical center affiliates (Harvard, BU and the University of Arizona). Dr. Brady has now retired and Dr. Russ Huber is now the VABBB PI. GWVIB VA Boston/BU/Harvard staff are professionals that bring essential skills to the project via their collaborative work on the GWVIB and other projects: the Boston

University Alzheimer's Disease Center (Dr. Kowall, Director); the Center for the Study of Chronic Traumatic Encephalopathy (Dr. Ann McKee, M.D., Director), and the VISN1 Neuropathology Laboratory (Dr. McKee, Director). Tissue is stored at the Southern Arizona VA Healthcare System (SAVAHCS) in Tucson, AZ. GWVIB Tucson/U. of Arizona staff is composed of biorepository storage, processing, management and tissue disbursement experts from the Pathology departments at SAVAHCS and the U. of Arizona. The VABBB has developed SOPs for tissue collection, processing, quality control, storage and disbursement, and developed a comprehensive tissue database annotated with relevant clinical and histopathological data. This collaboration will be leveraged for the BBRAIN and Drs. Kowall and Huber will advise Dr. Sullivan on best practices in multi-site biorepository management.

5. **Personnel exchanges:** *Nothing to Report*

6. **Other.** *Nothing to Report*

1. **Organization Name:** Boston University Medical Campus

2. **Location of Organization:** Boston, Massachusetts

3. **Partner's contribution to the project** (*identify one or more*)

1. **Financial support:** *Nothing to Report*

2. **In-kind support:** *Nothing to Report*

3. **Facilities:** *Nothing to Report*

4. **Collaboration:** Drs. Bang Bon Koo and Maxine Krengel, BBRAIN Co-Investigators affiliated with Boston University Medical Campus. Dr. Bang-Bon Koo, a clinical faculty member in the Anatomy and Neurobiology department at BUMC will assist with machine-learning analyses in GWI biomarker and diagnostic analyses. Dr. Bang-Bon Koo, a clinical faculty member in the Anatomy and Neurobiology department at BUMC will also be available to assist with machine-learning analyses in GWI biomarker and diagnostic analyses. Dr. Maxine Krengel currently leads the Ft. Devens cohort research effort and has published several recent publications in collaboration with Drs. Sullivan and Patricia Janulewicz on long-term health outcomes in GW veterans from the DOD funded Time 5 survey (GW100046).

5. **Personnel exchanges:** *Nothing to Report*

6. **Other.** *Nothing to Report*

1. **Organization Name:** CDC NIOSH

2. **Location of Organization:** Morgantown, West Virginia

3. **Partner's contribution to the project** (*identify one or more*)

1. **Financial support:** *Nothing to Report*

2. In-kind support: *Nothing to Report*

3. Facilities: *Nothing to Report*

4. Collaboration: Dr. Jim O’Callaghan, BBRAIN Co-Investigator affiliated with CDC NIOSH. Dr. James O’Callaghan serves as Distinguished Consultant, Centers for Disease Control (CDC) and Prevention, and Head of the Molecular Neurotoxicology Laboratory in the Toxicology and Molecular Biology Branch of the Health Effects Laboratory Division at the CDC-NIOSH. He is an expert neurotoxicologist who is a member of the Boston GWIC (GW120037) and formerly served on the VA Research Advisory Committee on Gulf War Veterans’ Illnesses (RAC-GWVI). He has a very active and well-funded animal GWI research program aimed at establishing the relationship among toxicant-induced changes in gene expression, growth-associated phosphorylation cascades and signaling events associated with astrocytic hypertrophy and microglial activation. He will share mouse brain samples from his GWI model for the BBRAIN studies and repository.

5. Personnel exchanges: *Nothing to Report*

6. Other. *Nothing to Report*

1. **Organization Name:** Roskamp Institute/ Tampa VAMC

2. **Location of Organization:** Sarasota, Florida

3. **Partner's contribution to the project** (*identify one or more*)

1. Financial support: *Nothing to Report*

2. In-kind support: *Nothing to Report*

3. Facilities: *Nothing to Report*

4. Collaboration: Drs. Laila Abdullah and Ghania Ait Ghezalia, BBRAIN Consultants affiliated with Roskamp Institute/ TAMPA VAMC. Dr. Laila Abdullah is a senior neuroscientist at the Roskamp Institute, a non-profit Research Center. Dr. Abdullah has identified novel therapies and biomarkers for GWI by applying omics (proteomics, lipidomics, and metabolomics) technology to target lipid metabolism. Dr. Abdullah has been working on identifying lipid metabolism pathways associated with immune/inflammation and metabolic disturbances in the brains of GWI mouse models. In collaboration with Drs. Sullivan, Klimas, and Ait-Ghezala, Dr. Abdullah has been evaluating blood lipids as potential biomarkers of GWI and has successfully validated some aspects of lipid disturbances in the GWI mouse model against lipid disturbances observed in blood from veterans with GWI using Boston GWIC repository samples. Dr. Abdullah will contribute her expertise in ‘omics’ research, share omics data and biological samples from animal and clinical studies of GWI. Samples and data will be shared for BBRAIN through CDMRP awards (GW130045, GW150056) and a VA Merit award (RX002260-01A1).

5. Personnel exchanges: *Nothing to Report*

6. Other. *Nothing to Report*

1. **Organization Name:** Drexel University
2. **Location of Organization:** Philadelphia, Pennsylvania
3. **Partner's contribution to the project** (*identify one or more*)

1. **Financial support:** *Nothing to Report*

2. **In-kind support:** *Nothing to Report*

3. **Facilities:** *Nothing to Report*

4. **Collaboration:** Drs. Peter Baas and Liang Qiang, BBRAIN Co-Investigators affiliated with Drexel University. Drs. Peter Baas and Liang Qiang also Boston GWIC members from Drexel University are axonal transport, neuronal microtubule and stem cell experts. They have most recently developed a cutting-edge bank of human induced pluripotent (hiPSC) stem cells from GW veterans, in order to conduct mechanistic studies and high-throughput testing of potential therapies. The stem cell biorepository will be shared with BBRAIN and outside collaborators. Drexel and BUSPH researchers have established a repository of hiPSCs from peripheral blood mononuclear cells (PBMCs) directly from GW veterans. They have utilized contemporary stem cell technology to convert somatic cells from GW veterans into pluripotent cell lines that can be differentiated into various cell types, including neurons, glia, muscle, or other relevant cell types. They have generated hiPSCs from 2 groups of age-matched individuals: GW veterans with GWI versus those without GWI. The sets were validated for comparable and appropriate hiPSC induction, neuronal and glial differentiation, neural cell survival over time, and basic electrophysiological properties of the terminally differentiated cells. Two high profile papers have been published about this hiPSC repository. These cell lines are immortal and will be a resource for GWI researchers to pursue mechanistic hypotheses and therapies for GWI as a result of DOD funding (GW140086) to Drexel and BUSPH investigators.

5. **Personnel exchanges:** *Nothing to Report*

6. **Other.** *Nothing to Report*

1. **Organization Name:** Mass General Hospital
2. **Location of Organization:** Boston, Massachusetts
3. **Partner's contribution to the project** (*identify one or more*)

1. **Financial support:** *Nothing to Report*

2. **In-kind support:** *Nothing to Report*

3. **Facilities:** *Nothing to Report*

4. **Collaboration:** Dr. Marco Loggia, BBRAIN Consultant affiliated with Mass General Hospital and Harvard University. Dr. Marco Loggia is an Assistant Professor of Radiology at Harvard Medical School, the Associate Director of the Center for Integrative Pain NeuroImaging (CiPNI) at Massachusetts General

Hospital, and Faculty in the MGH/HSY Athinoula A. Martinos Center for Biomedical Imaging. He is a pain expert and has been funded for a ground-breaking study of positron emission tomography (PET) markers of glial activation in GWI working in collaboration with GWIC and other Boston area investigators (GW130100). Dr. Loggia will share blood samples and imaging, cognitive and demographic data from his DOD funded [11C]PBR28 PET/MR study of glial activation in GWI and BBRAIN will analyze his stored blood samples for cytokine markers of microglial activation to compare with his PET study outcomes.

5. Personnel exchanges: *Nothing to Report*

6. Other. *Nothing to Report*

- 1. Organization Name:** Georgetown University
- 2. Location of Organization:** Washington, DC
- 3. Partner's contribution to the project** (*identify one or more*)

1. Financial support: *Nothing to Report*

2. In-kind support: *Nothing to Report*

3. Facilities: *Nothing to Report*

4. Collaboration: Drs. James Baraniuk, BBRAIN Consultant is affiliated with Georgetown University. Dr. James Baraniuk is Professor of Medicine at Georgetown University and a practicing rheumatologist. He specializes in fatigue and pain management in patients with GWI, fibromyalgia and chronic fatigue syndrome (CFS)⁹. Dr. Baraniuk has been funded for multiple DOD studies of biomarkers of GWI including plasma, cerebrospinal fluid and brain imaging markers pre-and-post exercise challenge and treatment trials of GWI. Dr. Baraniuk and the GWIC are the only DOD funded sites that we are aware of that have highly valuable CSF samples in repositories. Dr. Baraniuk will share CSF, blood and brain imaging data from GWI, CFS and healthy control groups from DOD and NIH funded studies (*CDMRP* GW080053; GW140064; GW060044). Collaborative studies utilizing both Dr. Baraniuk's and Sullivan's CSF samples will be planned.

5. Personnel exchanges: *Nothing to Report*

6. Other. *Nothing to Report*

- 1. Organization Name:** University of Alabama
- 2. Location of Organization:** Birmingham, Alabama
- 3. Partner's contribution to the project** (*identify one or more*)

1. Financial support: *Nothing to Report*

2. In-kind support: *Nothing to Report*

3. Facilities: *Nothing to Report*

4. Collaboration: Dr. Jared Younger, BBRAIN Consultant is affiliated with University of Alabama. Dr. Jarred Younger is Associate Professor at the University of Alabama at Birmingham (UAB), and Director of the Neuroinflammation, Pain and Fatigue Laboratory. Dr. Younger's group specializes in intensive longitudinal studies where biosamples are collected daily. This unique dataset provides measurement of self-reported symptom severity over time and biomarkers in blood. These data and samples will be shared with BBRAIN researchers. Dr. Younger maintains sera and plasma stored in 0.5mL aliquots. Crosssectional studies and 150 intensive longitudinal (daily) samples are available. Half of the samples are plasma and half sera from 6,000 GWI samples, 7,200 Healthy Controls samples, 11,200 Chronic Fatigue Syndrome samples and 4,800 Fibromyalgia samples will be shared as well as several hundred brain scans from healthy control, fibromyalgia and CFS groups.

5. Personnel exchanges: *Nothing to Report*

6. Other. *Nothing to Report*

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:**

Nothing to report

- **QUAD CHARTS:**

9. APPENDICES:

Appendix:

Please see attached publications:

Keating, D., Kregel, M., Dugas, J. *et al.* Cognitive decrements in 1991 Gulf War veterans: associations with Gulf War illness and neurotoxicant exposures in the Boston Biorepository, Recruitment, and Integrative Network (BBRAIN) cohorts. *Environ Health* **22**, 68 (2023). <https://doi.org/10.1186/s12940-023-01018-2>

Abdullah L, Nkiliza A, Niedospial D, Aldrich G, Bartenfelder G, Keegan A, Hoffmann M, Mullan M, Klimas N, Baraniuk J, Crawford F, Kregel M, Chao L, Sullivan K. Genetic association between the APOE ε4 allele, toxicant exposures and Gulf war illness diagnosis. *Environ Health*. 2023 Jul 6;22(1):51. doi: 10.1186/s12940-023-01002-w.

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.

RESEARCH

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Cognitive decrements in 1991 Gulf War veterans: associations with Gulf War illness and neurotoxicant exposures in the Boston Biorepository, Recruitment, and Integrative Network (BBRAIN) cohorts

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Abstract

Background During deployment, veterans of the 1991 Gulf War (GW) were exposed to multiple war-related toxicants. Roughly a third of these veterans continue to exhibit neurotoxicant induced symptoms of Gulf War Illness (GWI), a multi-faceted condition that includes fatigue, pain and cognitive decrements. When studied empirically, both deployed veterans with exposures and those who meet the criteria for GWI are more likely to show deficits in the area of neuropsychological functioning. Although studies have shown cognitive impairments in small sample sizes, it is necessary to revisit these findings with larger samples and newer cohorts to see if other areas of deficit emerge with more power to detect such differences. A group of researchers and clinicians with expertise in the area of GWI have identified common data elements (CDE) for use in research samples to compare data sets. At the same time, a subgroup of researchers created a new repository to share these cognitive data and biospecimens within the GWI research community.

Methods The present study aimed to compare cognitive measures of attention, executive functioning, and verbal memory in a large sample of GWI cases and healthy GW veteran controls using neuropsychological tests recommended in the CDEs. We additionally subdivided samples based on the specific neurotoxicant exposures related to cognitive deficits and compared exposed versus non-exposed veterans regardless of case criteria status. The total sample utilized cognitive testing outcomes from the newly collated Boston, Biorepository, Recruitment, and Integrative Network (BBRAIN) for GWI.

Results Participants included 411 GW veterans, 312 GWI (cases) and 99 healthy veterans (controls). Veterans with GWI showed significantly poorer attention, executive functioning, learning, and short-and-long term verbal memory than those without GWI. Further, GW veterans with exposures to acetylcholinesterase inhibiting pesticides and nerve gas agents, had worse performance on executive function tasks. Veterans with exposure to oil well fires had worse performance on verbal memory and those with pyridostigmine bromide anti-nerve gas pill exposures had better verbal memory and worse performance on an attention task compared to unexposed veterans.

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Conclusions This study replicates prior results regarding the utility of the currently recommended CDEs in determining impairments in cognitive functioning in veterans with GWI in a new widely-available repository cohort and provides further evidence of cognitive decrements in GW veterans related to war-related neurotoxicant exposures.

Keywords Gulf war illness, Neurotoxicant, Veterans, Gulf war, common data elements, repository, cognitive

Background

One third of veterans who served in the 1990–1991 Gulf War (GW) have experienced chronic health symptoms including debilitating fatigue, chronic pain, and cognitive impairments [1–5]. More than thirty years after the war, veterans are still suffering from these health consequences classified as Gulf War Illness (GWI). Cognitive impairment has been noted as one of the most distressing symptoms reported by GW veterans since shortly after the war [6, 7]. The two most widely used GWI case definitions to classify the condition, Centers for Disease Control (CDC) and Kansas, both include neurocognitive impairment as one of the categories and it remains one of the most commonly reported symptom [6, 8, 9]. Prior individual studies of neuropsychological outcomes and meta-analyses of cognitive outcomes have shown cognitive decrements in veterans with GWI when compared to healthy GW veterans in the domains of attention and executive functioning, learning and memory and visuospatial functions but specific test outcomes within the domains varied by study [5, 10]. Further, environmental exposures including pesticides, pyridostigmine bromide (PB) anti-nerve gas pills and sarin nerve agent during the GW have been correlated with neuropsychological decrements, including reduced processing speed, attention and memory functioning [3, 4, 11–16]. For the past three decades, GWI researchers have attempted to gain a clearer picture of the association between toxicant exposures during the war and neurocognitive decrements in veterans with GWI, regardless of exposure status and in those exposed regardless of GWI case status. Several studies with smaller sample sizes showed neuropsychological deficits in exposed veterans, compared to unexposed veterans, while other studies clearly lacked the power to assess even moderate differences between groups [5, 10]. Even for those with adequate sample sizes in individual cohorts, results were not always consistent [5, 10]. In addition, studies were not consistent in the cognitive test measures that were used making it difficult to compare and contrast specific areas of impairment across studies. This also hindered the ability to choose the most sensitive and specific cognitive measures for use in GWI biomarker and treatment development studies. The major concern was that if the primary outcome measures were not sensitive to GWI, then it would be almost impossible to assess biomarker and treatment study outcomes

appropriately. Consensus-driven common data elements (CDEs) for cognitive outcomes spearheaded by the GWI programs of the United States Department of Veteran Affairs and the Department of Defense Congressionally Directed Medical Research Program (CDMRP) were designed to encourage collaboration, data sharing and far-reaching analysis. These CDEs included tests from the cognitive domains of attention, executive function, memory, language, visuospatial, motor and mood and included 16 individual test measures [17]. Leaders in the fields of neuropsychology and cognitive science provided expertise to create the CDEs for GWI neuropsychological testing but until now they remained to be replicated in larger study samples from different geographically represented cohorts [17]. Therefore, it was necessary to compare exposures and cognitive outcomes in a larger, more geographically representative sample with adequate power to detect differences [14]. The Boston Biorepository, Recruitment, and Integrative Network (BBRAIN) for Gulf War Illness was designed to collect new and existing data sets that could be combined and shared for future analyses. One main aim was to provide larger combined datasets for confirmation of the utility of the CDEs [18]. The current analyses were performed to replicate previous findings in a larger cohort and to further examine the relationship between toxicant exposures and neurocognitive effects in ailing GW veterans.

Data were shared from individual studies where standard cognitive testing measures including qualitative and quantitative outcomes (error types, time to task completion etc.) recommended in the GWI CDEs were used as outcomes. New and previously published BBRAIN cognitive data were combined to produce larger datasets to provide the power needed to assess the currently recommended GWI CDEs for cognitive outcomes [5, 17]. This analysis is one of the first to utilize and replicate these cognitive CDEs on a large scale to share and interpret important GWI findings. As CDEs are working recommendations, it is necessary to replicate or amend outcome measures as new evidence emerges on their current sensitivity. In addition, this combined data study sought to examine and replicate prior reported differences in neuropsychological outcomes in relation to toxicant exposures during the war with larger more representative study samples of GW veterans from the BBRAIN repository.

Methods

Study participants

Retrospective cognitive data from the BBRAIN repository was compiled from the Gulf War Illness Consortium (GWIC) study ($n=269$) with 223 GWI cases and 46 controls and from participants of the University of California San Francisco and San Francisco VA (SFVA) cohorts ($n=142$) with 89 cases and 53 controls [15, 16, 19]. These cohorts have been previously described and have been added to the BBRAIN repository for use in future studies [15, 16, 19]. The GWIC cognitive findings have not been previously published and provides new data for comparison of CDE outcomes. The SFVA cohort cognitive data have been previously published [15, 16, 19]. Total participants included 411 GW veterans, who were deployed to the Persian Gulf Theater between August 1990 and July 1991, including 312 with GWI and 99 healthy GW veteran controls as measured by the Kansas criteria [8]. All participants signed informed consents to share data for future studies (CDMRP/GWIRP GW170055, IRB # H-37,828).

Case status

The Kansas Symptom Criteria was utilized to determine GWI case status as recommended in the GWI Common Data Elements [8, 17]. Participants were categorized as GWI cases if they endorsed multiple mild or moderate-to-severe chronic symptoms in at least three of the six statistically defined symptom domains: fatigue/sleep problems, somatic pain, neurological/cognitive/mood symptoms, gastrointestinal symptoms, respiratory symptoms, and skin abnormalities. Veterans were excluded from the study if they reported a diagnosis included in the Kansas Exclusionary Criteria. Exclusionary diagnoses included uncontrolled diabetes, heart disease other than hypertension, stroke, lupus, multiple sclerosis, cancer in the previous three years, liver disease, kidney disease, or chronic infection [8]. Veterans were also excluded if they reported a diagnosis of schizophrenia or bipolar disorder or have been hospitalized in the past two years for alcohol/drug dependence, depression, or PTSD. GW deployed veterans who did not meet case criteria or exclusionary criteria served as controls.

Cognitive common data elements and current neuropsychological test battery

CDEs include criteria and specific tests recommended to standardize and systematically obtain, and utilize shared data across the GWI research community [17]. A working group of GWI stakeholders including researchers, clinicians and veteran advocates met to determine a consensus list of reliable instruments for GWI research. These recommended CDEs were determined in 2019

with the understanding that they may need to be updated as GWI research progresses. As part of the CDE process, specific CDEs were recommended for use in neuropsychological studies of GWI. Tests from each cognitive domain were chosen by a working group of GWI neuropsychological experts based on the criteria that each test had shown significant differences in at least three prior studies [17]. Each domain resulted in multiple tests that appeared sensitive and specific enough to be recommended in future biomarker and treatment studies. The CDE recommendations included tests of attention, executive function, memory, and processing speed as described in Table 1 [5].

From the recommended CDE list, the following neuropsychological test variables used for analyses in the current study were selected based on commonality from multiple studies within the BBRAIN repository and on sensitivity found in clinical examinations of our prior GWI cohorts (Table 2). Overlapping cognitive tests from the neuropsychological batteries in the two cohorts included the California Verbal Learning Test Second Edition (CVLT-II) [20], Delis Kaplan Executive Function System Color-Word Interference Test (D-KEFS) [21], Trail Making Test (TMT) [22], and the Conners Continuous Performance Test Third Edition (CPT3) [23]. All tests were administered by a trained neuropsychological test administrator. All cognitive measures compared in this study are included in the current list of CDEs for GWI as reported by Cohen et al. and Jeffrey et al. and reported in Tables 1 and 2 [5, 17].

War-related exposures

Environmental exposures were collected from the Kansas Gulf War Experiences and Exposures Questionnaire, a self-reported survey about exposures to chemical weapons, pesticides, and anti-nerve gas pills during the 1991 Gulf War which is part of the current CDE for GWI exposures [8, 17]. Chemical weapon exposure was determined by reporting hearing chemical alarms, while seeing smoke from oil well fires was used to determine particulate matter exposure. Pesticide exposure was determined if participants reported using pesticide cream or spray on their skin or seeing the area in which they lived sprayed or fogged with pesticides. Anti-nerve gas pill exposure was determined if the veteran reported taking pyridostigmine bromide (PB) pills [3]. For analysis purposes, GW veterans were categorized as having a respective exposure if they reported seven or more days of experiencing the area in which they lived fogged with pesticides, seeing smoke from oil well fires, hearing chemical alarms and taking PB pills. They were categorized as unexposed for these exposures if they reported less than seven days of the exposure. These exposure duration periods were

Table 1 Cognitive common data elements recommendations for Gulf War illness research

Cognitive Domain	Neuropsychological Test
Premorbid Function	Word Reading Subtest of the Wide Range Achievement Test (WRAT-4)
Attention	Continuous Performance Test – 3 (CPT3)
Executive Function	Wechsler Adult Intelligence Scale-IV (WAIS-IV) Recommended tests: Digit Spans Delis-Kaplan Executive Function System (D-KEFS) Recommended modules: Color-Word-Interference Test, Trail Making Test, Verbal Fluency Wechsler Adult Intelligence Scale-IV (WAIS-IV) Recommended tests: Digit Spans Continuous Performance Test – 3 (CPT3)
Verbal Memory	California Verbal Learning Test - Second Edition (CVLT-II) Hopkins Verbal Learning Test (HVLIT)
Visual Memory	Rey-Osterrieth Complex Figure Test (ROCF) Brief Visual Memory Test (BVMIT)
Motor Function	Finger Tapping Test Grooved Pegboard Test
Mood	Profile of Mood States (POMS) Davidson Trauma Scale (DTS) - PTSD PTSD Checklist for DSM-V (PCL-5) Center for Epidemiological Studies Depression Scale (CES-D) Clinician Administered PTSD Scale (CAPS-5) Structured Clinical Interview for DSM-V (SCID-5)

Table 2 BBRAIN repository neuropsychological tests by cognitive domain

Neuropsychological Test	Task	Outcome
I. Attention/Processing Speed		
Conner's Continuous Performance Test Third Edition (CPT3) [23]	Target letter embedded in series of distractors; to assess sustained attention and reaction time	Reaction Time (seconds) Total Omission Errors (T score) Commission Errors (T score)
D-KEFS Color-Word Interference Test [21]: Trial 1 Trial 2	Trials 1 and 2 measure processing speed of verbal (words) and nonverbal (colors) stimuli via a timed response	Total time (seconds) Self-corrected errors (number of errors) Uncorrected errors (number of errors)
Trail Making Test [22]: Trails A	Timed connect-a-dot task to assess attention and motor control requiring number sequencing	Time to Completion (seconds)
II. Verbal Memory		
California Verbal Learning Test: Second Edition [20]	List of 16 nouns from 4 categories presented over multiple learning trials with recall after interference; assesses memory and learning strategies	Learning Trials 1–5 (number correct) Short Delay Recall (number correct) Long Delay Recall (number correct)
III. Executive Functioning		
Trail-making Test [22]: Trails B	Timed connect-a-dot task to assess alternating sequences of letters and numbers	Time to Completion (seconds)
D-KEFS Color-Word Interference [21] Test: Trial 3 Trial 4	Trials 3 and 4 measure inhibition and inhibition switching	Total time (seconds) Self-corrected errors (number of errors) Uncorrected errors (number of errors)

based on previous research. Wolfe et al., showed that at least 7 days of exposure to 3 blister packs of PB pills per day were associated with worsened health symptoms compared to those who took less than 21 PB pills [24]. Exposure to pesticide cream on skin was a proxy for DEET exposure and was categorized as exposed if the

veteran reported 31 or more days of use and unexposed if they reported less than 31 days of use. This exposure definition was based on prior exposure modeling from the RAND report and the DOD Environmental Exposure Report – Pesticides which showed that 31 or more days of exposures was the 50th percentile of exposure for

DEET [25, 26]. In addition, our prior studies with pesticide applicators from the Gulf War who reported 31 or more days of exposure to pesticide cream on skin were more likely to have poorer cognitive functioning [3].

Toxicant exposures during the Gulf War were classified into exposed and unexposed for chemical weapons, oil well fires, pesticide fog and PB pills: 0–6 days of reported exposure (unexposed) and 7 or more days of reported exposure (exposed). Pesticide cream on skin was classified into unexposed if the veteran reported less than 31 days of exposure and exposed if they reported 31 or more days of exposure.

Statistical analyses

Demographic characteristics of cases and controls are described through means and standard deviations (SD) and compared through the Wilcoxon rank sum test for continuous variables (age, years of education), and described through n's and percentages and compared through chi-square tests for categorical variables (sex, race/ethnicity, study site). Specifically, these variables were dichotomized as male or female, White non-Hispanic or Other race/ethnicity and GWIC or UCSF study sites. Further analyses controlled for sex, race/ethnicity and study site as variables of a-priori interest, and potential confounding variables including age and education identified as differing between cases and controls. While groups were compared on employment status, unemployment could in part be a consequence of GWI and so occupation (unemployment) was not considered as a potential confounder. Differences in cognitive outcomes between cases and controls were investigated through regression models, controlling for potential confounding variables of age, sex, race/ethnicity, study site and education, in order to estimate adjusted means, standard errors of the mean (SE), 95% confidence intervals (CI), and β -coefficients. Associations between environmental exposures and cognitive outcomes were also investigated through multiple linear regression models controlling for sex, age, education, study site and other exposures. These analyses were conducted for exposed vs. unexposed regardless of GWI status. These analyses were also repeated on the subset of GWI cases, and the subset of GW veteran controls for comparison purposes (Additional file 1: Appendix A and B). We performed all analyses using SAS 9.4 (Cary, NC).

Results

Demographics

Demographics of the combined study sample are presented in Table 3. Of the 411 study participants, 312 met criteria for GWI case status and 99 were considered controls. The study population was comprised of mostly

white, non-Hispanic males approximately 52 years old with some schooling post-high school. The study population included 16% women and 19% non-white participants. The differences in age, occupation, and years of education between cases and controls were found to be statistically significant ($p < 0.01$). Overall, controls were slightly older, more highly educated, and less likely to be unemployed than GWI cases (Table 3).

Gulf War illness cases status and cognitive outcomes

After adjusting for age, gender, race/ethnicity, study site and years of education, veterans with GWI had significantly slower D-KEFS Color-word times on all trials compared to controls ($p < 0.05$). In addition, significantly higher mean T-scores on the Conners CPT3 commission errors were found in GWI cases compared to controls ($p < 0.05$). GWI cases also had significantly fewer words recalled in CVLT-II learning trials 1–5, and short-and-long-delayed recall than controls ($p < 0.05$, Fig. 1; Table 4). Findings suggest differences in the attention, executive and verbal memory domains.

Environmental exposures and cognitive outcomes

Multiple linear regression modeling was used to compare mean neuropsychological measures in the unexposed versus exposed groups. All analyses were adjusted for age, gender, years of education, study site and the other exposures listed (Table 5, Additional file 1: Appendix A). For example, hearing chemical alarms was controlled for seeing smoke from oil well fires, using pesticide cream or spray on skin, seeing the area in which you lived fogged or sprayed with pesticides, and taking PB pills.

Chemical weapons

Among all veterans, exposure to chemical weapons was significantly associated with slower times on the Trail Making Test Trails B ($p < 0.005$, Table 5) suggesting differences in the executive domain. When performing the same analysis among only veterans with GWI, exposed GWI veterans had significantly slower times to completion on Trail Making Test Trails B ($p < 0.005$, Additional file 1: Appendix A). When performing the same analyses among only GW veteran controls, exposed veterans had significantly higher Conners CPT3 omission scores and slower times on the Trail Making Test Trails B and slower speed on the D-KEFS Color-Word Interference Trial 3 ($p < 0.05$, Additional file 1: Appendix B).

Smoke from oil well fires

Exposure to smoke from oil well fires was significantly associated with fewer correct words in both the CVLT-II short and long delay recall in all GW veterans ($p < 0.05$, Table 5) suggesting differences in verbal memory

Table 3 Descriptive table of demographics of the sample

		Overall (N=411) Mean (SD)	Cases (N=312)	Control (N=99)
Age*	---	52.6 (6.4) N (%)	52.0 (6.1)	54.7 (7.1)
Race	Black/African American	44 (11.3%)	37 (12.5%)	7 (7.4%)
	White/Caucasian	317 (81.3%)	235 (79.7%)	82 (86.3%)
	Asian/Pacific Islander	3 (0.8%)	2 (0.7%)	1 (1.1%)
	Aleutian Eskimo, or American Indian	3 (0.8%)	3 (1.0%)	0 (0.0%)
	Other/Multiracial	23 (5.9%)	18 (6.1%)	5 (5.3%)
Sex	Male	344 (83.7%)	257 (82.4%)	87 (87.9%)
	Female	67 (16.3%)	55 (17.6%)	12 (12.1%)
Hispanic or Latino ethnicity	Yes	22 (8.4%)	20 (9.2%)	2 (4.3%)
	No	241 (91.6%)	197 (90.8%)	44 (95.7%)
Employment Status*	disabled/unemployed for health reasons	10 (2.5%)	9 (3.0%)	1 (1.0%)
	employed (part-time or full-time)	304 (75.4%)	226 (74.3%)	78 (78.8%)
	retired	25 (6.2%)	12 (3.9%)	13 (13.1%)
	student	6 (1.5%)	4 (1.3%)	2 (2.0%)
	unemployed/seeking employment	58 (14.4%)	53 (17.4%)	5 (5.1%)
Current marital status	single	24 (6.0%)	15 (4.9%)	9 (9.1%)
	married/living with significant other	284 (70.5%)	209 (68.8%)	75 (75.8%)
	divorced/separated	89 (22.1%)	74 (24.3%)	15 (15.2%)
	widowed	6 (1.5%)	6 (2.0%)	0 (0.0%)
Years of education*	Mean years of education (SD)	15.4 (2.3)	14.5 (2.3)	16.3 (2.5)
Branch of Service	Army	255 (62.04%)	195 (62.5%)	60 (60.6%)
	Air Force	32 (7.79%)	23 (7.4%)	9 (9.1%)
	Marines	64 (15.57%)	50 (16.0%)	14 (14.2%)
	Navy	42 (10.22%)	26 (9.3%)	16 (16.2%)

* $p < 0.01$

domain. Among GWI cases, those exposed to smoke from oil well fires had significantly fewer words recalled on the CVLT-II short delay recall and slower times on the D-KEFS Color Word Interference trial 2 ($p < 0.05$, Additional file 1: Appendix A). Among GW controls, those exposed to smoke from oil well fires had no significant differences when compared with those with no oil well fire exposures.

Pesticide exposure

In all GW veterans, reporting using pesticide cream or spray on skin (a proxy for DEET exposure) was not significantly associated with any cognitive differences. In GWI cases only, there were no significant associations found between exposure and neuropsychological test scores, (Additional file 1: Appendix A). Among GW controls, total time on D-KEFS trial 3 and time to completion on the Conners CPT3 were significantly slower with pesticide cream or spray exposure (Additional file 1: Appendix

B). All GW veterans who reported seeing the area in which they lived fogged or sprayed with pesticides (proxy for organophosphate and carbamate pesticides) for more than 7 days had significantly slower time on the D-KEFS Color-Word Interference trial 1 and more self-corrected errors on trial 4 ($p < 0.05$, Table 5) suggesting attention and executive functioning differences. Among those with GWI, the same measures were found to significantly differ between groups ($p < 0.05$, Additional file 1: Appendix A). Among GW controls, those exposed to pesticide sprays and fogs, had significantly more errors on the D-KEFS Color-Word Interference Trial 3 and slower time on Trail Making Test Trails B ($p < 0.05$, Additional file 1: Appendix B).

PB anti-nerve gas pills

In all GW veterans, exposure to PB pills was significantly associated with more self-corrected errors on D-KEFS Color Word trial 1 and more words recalled in

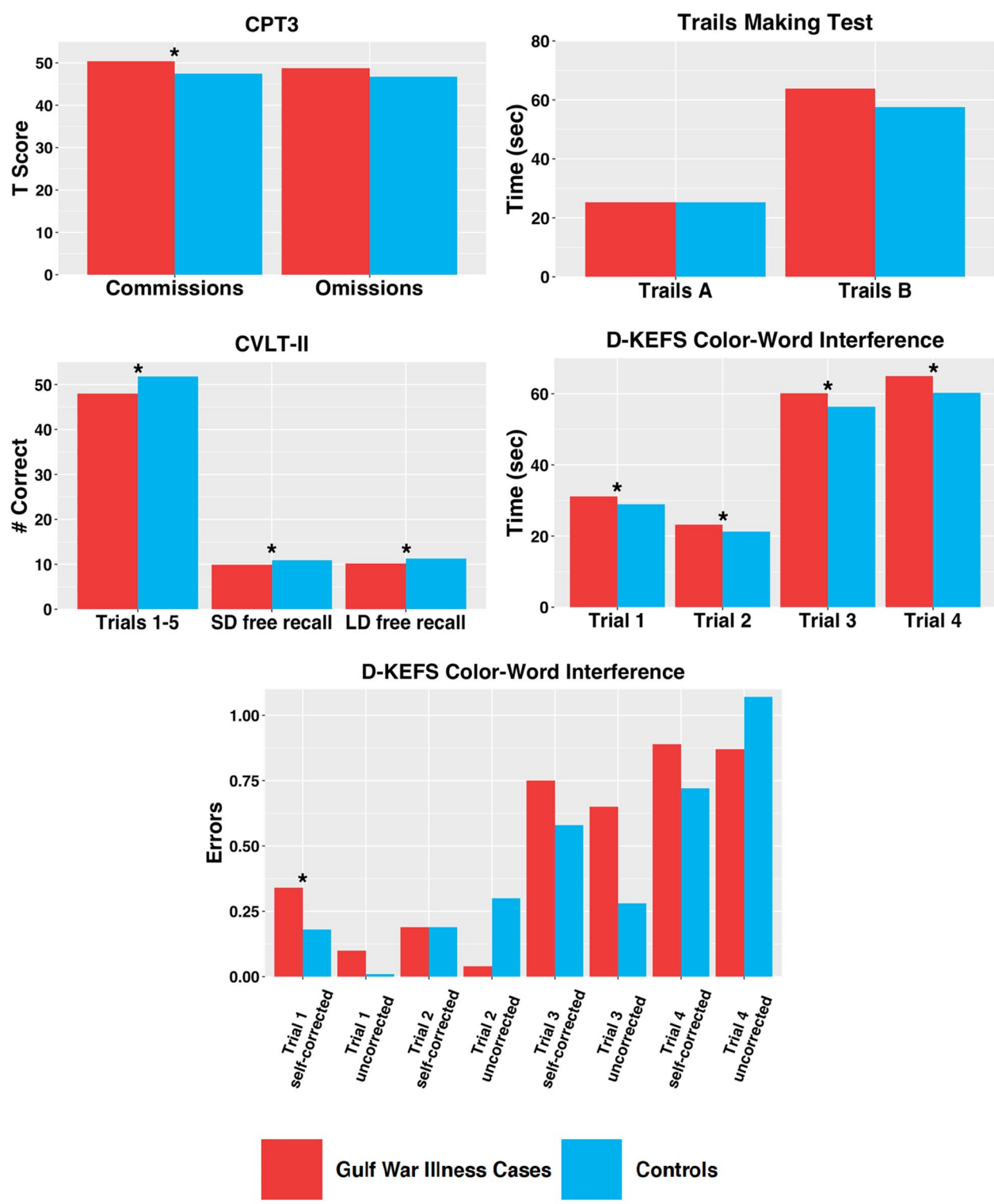


Fig. 1 Neuropsychological mean outcome measures by Gulf War Illness status

Table 4 Neuropsychological measures by Gulf War illness status

		GWI Cases (n = 312)			Controls (n = 99)			p-value	
		Unadjusted Mean (SD)	Adjusted Mean (SE)	95% CI	Unadjusted Mean (SD)	Adjusted Mean (SE)	95% CI	β	
Attention/processing speed	CPT 3 Omissions	49.76(10.07)	48.56(0.76)	47.07, 50.05	47.27(9.36)	46.73(1.15)	44.46, 48.99	1.83	0.1349
	CPT 3 Commissions*	53.38(10.52)	50.37(0.77)	48.85, 51.88	47.78(9.93)	47.45(1.17)	45.15, 49.75	2.92	0.0193
	CPT 3 Hit Reaction Time Raw Score	421.44(81.76)	425.09(6.32)	412.66, 437.52	431.81(74.97)	425.56(9.60)	406.68, 444.45	-0.48	0.9628
	D-KEFS trial 1 time (sec)*	32.6(7.82)	31.15(0.55)	30.08, 32.22	29.67(6.55)	28.98(0.85)	27.32, 30.65	2.17	0.0151
	D-KEFS trial 1 self-corrected errors*	0.36(0.71)	0.34(0.05)	0.25, 0.44	0.17(0.41)	0.18(0.08)	0.04, 0.33	0.16	0.0427
	D-KEFS trial 1 uncorrected errors	0.16(0.58)	0.10(0.04)	0.02, 0.18	0.07(0.46)	0.01(0.06)	-0.12, 0.14	0.09	0.1944
	D-KEFS trial 2 time (sec)*	24.17(5.65)	23.21(0.39)	22.43, 23.98	21.57(4.40)	21.19(0.61)	20.00, 22.39	2.01	0.0017
	D-KEFS trial 2 self-corrected errors	0.17(0.45)	0.19(0.03)	0.12, 0.25	0.17(0.43)	0.19(0.05)	0.09, 0.29	-0.01	0.9266
	D-KEFS trial 2 uncorrected errors	0.05(0.26)	0.04(0.19)	-0.33, 0.41	0.52(5.03)	0.30(0.29)	-0.27, 0.88	-0.26	0.3890
	TMT Trail A: Time (sec)	32.00(13.58)	25.30(0.98)	23.36, 27.25	27.46(10.02)	25.21(1.30)	22.63, 27.79	0.09	0.9494
Verbal Memory	CVLT-II Correct in Trials 1–5*	46.43(10.28)	47.97(0.73)	46.53, 49.41	50.52(9.79)	51.77(1.13)	49.54, 54.00	-3.8	0.0015
	CVLT-II Correct in short delay free recall*	9.39(3.28)	9.88(0.23)	9.42, 10.33	10.56(3.04)	10.89(0.36)	10.18, 11.60	-1.0	0.0077
	CVLT-II Correct in long delay free recall*	9.72(3.41)	10.22(0.25)	9.73, 10.70	10.93(3.17)	11.27(0.38)	10.51, 12.02	-1.1	0.0095
Executive Functioning	D-KEFS trial 3 time (sec)*	62.01(16.23)	60.11(1.14)	57.88, 62.35	57.71(14.88)	56.34(1.76)	52.88, 59.81	3.77	0.0420
	D-KEFS trial 3 self-corrected errors	0.82(1.39)	0.75(0.10)	0.56, 0.94	0.59(1.00)	0.58(0.15)	0.28, 0.87	0.18	0.2696
	D-KEFS trial 3 uncorrected errors	0.75(3.14)	0.65(0.21)	0.24, 1.07	0.37(1.13)	0.28(0.33)	-0.37, 0.93	0.37	0.2817
	D-KEFS trial 4 time (sec)*	67.53(19.31)	65.02(1.35)	62.37, 67.67	60.68(15.80)	60.24(2.09)	56.13, 64.35	4.78	0.0300
	D-KEFS trial 4 self-corrected errors	0.85(1.13)	0.89(0.08)	0.73, 1.04	0.63(0.85)	0.72(0.12)	0.47, 0.96	0.17	0.1995
	D-KEFS trial 4 uncorrected errors	1.06(1.77)	0.87(0.22)	0.44, 1.31	1.14(5.05)	1.07(0.34)	0.39, 1.74	-0.20	0.5868
	TMT Trail B: Time (sec)	73.50(29.44)	63.88(3.32)	57.32, 70.44	64.74(31.64)	57.55(4.40)	48.84, 66.25	6.33	0.2007

Multiple Logistic Regression modeling was used for analysis in the table adjusting for age, gender, years of education, race/ethnicity and study site. Beta is the regression parameter representing the difference in adjusted means. p-value testing whether beta = 0, or equivalently a difference in adjusted means. Abbreviations: SD (standard deviation), SE (standard error of the mean), CI (confidence interval). CIs are shown for adjusted means

* $p < 0.05$

** $p < 0.001$

Table 5 Neuropsychological measures by toxicant exposure in all GW veterans controlling for age, gender, years of education, site and other exposures

			Exposed	Unexposed	β	<i>p</i> -value
			Adjusted Mean <i>N</i> = 152	Adjusted Mean <i>N</i> = 214		
Chemical Weapons (sarin/cyclo-sarin)	<i>Attention/processing speed</i>	CPT 3 Omissions	48.03	48.59	0.57	0.604
		CPT 3 Commissions	50.30	48.41	-1.89	0.092
		CPT 3 Hit Reaction Time Raw Score	420.24	434.92	14.68	0.111
		D-KEFS trial 1 time (sec)	31.17	31.11	-0.05	0.948
		D-KEFS trial 1 self-corrected errors	0.31	0.37	0.06	0.384
		D-KEFS trial 2 time (sec)	23.10	22.44	-0.66	0.251
		D-KEFS trial 2 self-corrected errors	0.14	0.18	0.04	0.458
		TMT Trail A: Time (sec)	26.80	25.91	-0.90	0.562
	<i>Verbal Memory</i>	CVLT-II Correct in Trials 1–5	47.83	49.06	1.23	0.247
		CVLT-II Correct in short delay free recall	10.04	10.19	0.15	0.659
	<i>Executive Functioning</i>	CVLT-II Correct in long delay free recall	10.14	10.76	0.62	0.083
		D-KEFS trial 3 time (sec)	61.17	57.96	-3.21	0.053
		D-KEFS trial 3 self-corrected errors	0.77	0.65	-0.12	0.390
		D-KEFS trial 4 time (sec)	65.78	62.92	-2.86	0.152
		D-KEFS trial 4 self-corrected errors	0.95	0.94	-0.01	0.915
		TMT Trail B: Time (sec)*	72.72	59.14	-13.60	0.009
Smoke from oil well fires	<i>Attention/processing speed</i>		<i>N</i> = 229	<i>N</i> = 137		
		CPT3 Omissions	48.81	47.80	-1.01	0.348
		CPT3 Commissions	49.99	48.72	-1.27	0.250
		CPT3 Hit Reaction Time Raw Score	429.33	425.83	-3.49	0.700
		D-KEFS trial 1 time (sec)	31.73	30.55	-1.18	0.140
		D-KEFS trial 1 self-corrected errors	0.36	0.33	-0.03	0.642
		D-KEFS trial 2 time (sec)*	23.40	22.14	-1.27	0.028
		D-KEFS trial 2 self-corrected errors	0.18	0.14	-0.04	0.473
	<i>Verbal Memory</i>	TMT Trail A: Time (sec)	26.64	26.07	-0.57	0.704
		CVLT-II Correct in Trials 1–5	47.49	49.41	1.92	0.072
		CVLT-II Correct in short delay recall*	9.64	10.58	0.94	0.005
	<i>Executive Functioning</i>	CVLT-II Correct in long delay recall*	10.10	10.81	0.71	0.049
		D-KEFS trial 3 time (sec)	60.42	58.70	-1.72	0.301
		D-KEFS trial 3 self-corrected errors	0.77	0.65	-0.12	0.481
		D-KEFS trial 4 time (sec)	65.54	63.16	-2.39	0.234
		D-KEFS trial 4 self-corrected errors	1.04	0.85	-0.19	0.107
		TMT Trail B: Time (sec)	64.12	67.74	3.62	0.465
			<i>N</i> = 150	<i>N</i> = 216		

Table 5 (continued)

			Exposed Adjusted Mean N= 152	Unexposed Adjusted Mean N= 214	β	p-value
Pesticide cream or spray on skin (DEET)	<i>Attention/processing speed</i>	CPT3 Omissions	48.43	48.19	-0.23	0.834
		CPT3 Commissions	48.78	49.93	1.15	0.317
		CPT3 Hit Reaction Time Raw Score	432.61	422.55	-10.1	0.286
		D-KEFS trial 1 time (sec)	31.58	30.71	-0.87	0.297
		D-KEFS trial 1 self-corrected errors	0.38	0.30	-0.08	0.262
		D-KEFS trial 2 time (sec)	22.76	22.78	0.02	0.977
		D-KEFS trial 2 self-corrected errors	0.12	0.20	0.08	0.122
		TMT Trail A: Time (sec)	27.22	25.49	-1.74	0.276
	<i>Verbal Memory</i>	CVLT-II Correct in Trials 1–5	47.83	49.07	1.24	0.260
		CVLT-II Correct in short delay recall	9.89	10.34	0.46	0.189
		CVLT-II Correct in long delay recall	10.41	10.49	0.08	0.830
	<i>Executive Functioning</i>	D-KEFS trial 3 time (sec)	59.71	59.41	-0.30	0.192
		D-KEFS trial 3 self-corrected errors	0.68	0.74	0.06	0.670
		D-KEFS trial 4 time (sec)	63.66	65.04	1.38	0.507
		D-KEFS trail 4 self-corrected errors	0.88	1.01	0.13	0.276
		TMT Trail B: Time (sec)	69.06	62.80	-6.25	0.240
Pesticide fog (organophosphate/carbamate)	<i>Attention/processing speed</i>		N= 68	N= 298		
		CPT3 Omissions	49.00	47.62	-1.38	0.310
		CPT3 Commissions	49.39	49.32	-0.07	0.962
		CPT3 Hit Reaction Time Raw Score	430.57	424.59	-5.97	0.601
		D-KEFS trial 1 time (sec)*	32.15	30.13	-2.02	0.046
		D-KEFS trial 1 self-corrected errors	0.39	0.29	-0.10	0.279
		D-KEFS trial 2 time (sec)	23.06	22.45	-0.65	0.375
		D-KEFS trial 2 self-corrected errors	0.13	0.19	0.05	0.409
	<i>Verbal Memory</i>	TMT Trail A: Time (sec)	27.34	25.38	-1.96	0.375
		CVLT-II Correct in Trials 1–5	47.93	48.96	1.03	0.443
		CVLT-II Correct in short delay recall	10.02	10.20	0.18	0.671
	<i>Executive Functioning</i>	CVLT-II Correct in long delay recall	10.36	10.55	0.18	0.683
		D-KEFS trial 3 time (sec)	60.68	58.44	-2.25	0.283
		D-KEFS trial 3 self-corrected errors	0.72	0.70	-0.02	0.924
		D-KEFS trial 4 time (sec)	65.36	63.34	-2.02	0.423
		D-KEFS trial 4 self-corrected errors *	1.14	0.75	-0.39	0.008
		TMT Trail B: Time (sec)	66.51	65.35	-1.15	0.875
			N= 184	N= 182		

Table 5 (continued)

			Exposed	Unexposed	β	<i>p</i> -value
			Adjusted Mean			
			<i>N</i> = 152	<i>N</i> = 214		
PB Pills	<i>Attention/processing speed</i>	CPT3 Omissions	48.36	48.26	-0.11	0.921
		CPT3 Commissions	48.88	49.83	0.95	0.390
		CPT3 Hit Reaction time raw score	428.62	426.54	-2.08	0.819
		D-KEFS trial 1 time (sec)	31.21	31.07	-0.14	0.858
		D-KEFS trial 1 self-corrected errors*	0.42	0.27	-0.15	0.036
		D-KEFS trial 2 time (sec)	22.73	22.81	0.08	0.889
		D-KEFS trial 2 self-corrected errors	0.19	0.13	-0.07	0.187
		TMT Trail A: Time (sec)	25.64	27.08	1.44	0.350
	<i>Verbal Memory</i>	CVLT-II Correct in Trials 1–5*	49.69	47.21	-2.47	0.023
		CVLT-II Correct in short delay recall*	10.58	9.65	-0.92	0.006
		CVLT-II Correct in long delay recall*	10.97	9.94	-1.03	0.004
	<i>Executive Functioning</i>	D-KEFS trial 3 time (sec)	59.55	59.57	0.02	0.992
		D-KEFS trial 3 self-corrected errors	0.72	0.69	-0.03	0.845
		D-KEFS trial 4 time (sec)	63.97	64.73	0.705	0.442
		D-KEFS trail 4 self-corrected errors	0.95	0.94	-0.01	0.909
		TMT Trail B: Time (sec)	62.78	69.08	6.30	0.217

**p* < 0.05

Multiple Regression analysis was used for analysis in the table adjusting for age, gender, education, study site and other exposures

the CVLT-II learning trials 1–5 as well as more words recalled on the short-and-long delay recall ($p < 0.05$, Table 5) suggesting mixed differences in attention and memory domains. Among only those with GWI, exposed veterans showed the same pattern of significant differences as the overall GW veteran group ($p < 0.05$, Additional file 1: Appendix A). Among GW controls, exposed veterans had no significant differences compared with the unexposed group (Additional file 1: Appendix B).

Discussion

Neuropsychological tests are proxies for central nervous system (CNS) function; it has long been known that GW veterans have had difficulty with CNS and specifically cognitive functioning since their return from deployment [2, 3, 5, 10, 11, 14–16]. However, different neuropsychological tests were used in prior studies making comparisons across studies challenging. This is particularly important now that substantial progress has been made with regard to biomarker and treatment development for GWI and using cognitive outcomes measures that differentiate cases of the disorder are critical to comparatively assess study outcomes. An initial approach to deal with this problem was a meta-analysis of cognitive

outcomes used in the GWI field and from this cognitive common data elements (CDEs) were recommended for the field based on these findings [5, 10]. Several differences in neuropsychological function have been observed between GW veterans and healthy controls and have also been associated with neurotoxicant exposures during the war [5]. A meta-analysis of neuropsychological characteristics of GWI published in 2017 identified significantly decreased performance in the functional domains of executive function, visuospatial skills, and learning and memory across 16 studies [10]. High mixed exposure to pesticides and PB anti-nerve gas pills has also been associated with significantly slowed information processing speed, increased attentional errors, poor visual memory functioning, and increased mood complaints [3]. Conversely, PB pill usage without high pesticide exposure during the war associated with better verbal memory functioning [3]. The cognitive CDEs that were recommended for the GWI field in 2019 by a working group of experts including VA and DOD investigators included 16 tests across 7 cognitive domains (attention, executive function, language, memory, visuospatial, motor and mood) [17]. However, these CDEs still remained to be evaluated across multiple GW veteran cohorts

representing different parts of the country and branches of service in studies that were conducted more recently to replicate findings from prior studies. This study assessed cognitive CDE outcomes in a newly combined cohort of veterans from the BBRAIN repository. The BBRAIN repository is being shared for multiple ongoing and planned studies of GWI and therefore, ensuring the reproducibility of prior findings in the field within this new combined cohort is essential to establishing the feasibility of use of the repository for future biomarker and treatment development studies. This is particularly relevant because BBRAIN includes previously unpublished as well as prior published cognitive testing results. Specifically, our current results showed differences in measures of attention, processing speed, verbal memory and executive functioning in veterans with GWI compared to those without the disorder. These results replicate the findings of our prior meta-analysis of 16 publications from studies that had been conducted between 1992 and 2015, where potential confounders could not be controlled for at the individual level [10]. The current study was able to control for multiple potential confounders and still replicated many of the prior meta-analytic findings therefore strengthening the body of evidence supporting utility of these specific cognitive test measures in GWI research. These results suggest that CPT, Trail Making Test, CVLT and D-KEFS Color Word Interference test should be used in future studies of GWI. We have found that not only summary scores but also error types and qualitative outcome scores also differ among cases and controls and among exposed vs. unexposed groups. Our recommendation is therefore, to utilize tests and specific test outcomes as listed in Table 2 for future biomarker and treatment studies of veterans with GWI.

In addition, we showed neurotoxicant exposures including chemical weapons, pesticides, PB pills and oil well fires are associated with cognitive decrements in attention, executive function and verbal memory. Although no clear patterns appeared with regard to cognitive test outcomes and exposures, it is worth noting that exposures related to acetylcholinesterase inhibition (chemical weapons, pesticide creams, pesticide sprays/fogs) were associated with executive system decrements as noted on Trails B time to completion, D-KEFS Color-Word Interference Test Trial 3 and Trial 1 time to completion and Trial 4 increased self-corrected errors. These results do correspond with executive system function decrements reported in other pesticide and sarin exposed groups [3, 13, 14]. In addition, smoke from oil well fires was associated with poorer verbal memory recall on CVLT-II. To our knowledge, this is the first time that verbal memory has been associated with smoke from oil wells in GWI. In addition, exposure to oil well smoke was

recently associated with increased risk of GWI in APOE4 carrier veterans [27]. Conversely, PB alone was associated with better performance on verbal memory outcomes. This replicates prior reports of better verbal memory from a different cohort of GW veterans reporting high PB exposure but few other exposures during the war [5]. Therefore, this combined data study from the BBRAIN repository network now reproduces these CDEs for cognitive outcomes for GWI and adds to the literature for those with neurotoxicant exposures during the war.

Specifically, it was found that veterans who met criteria for GWI relative to healthy veteran controls without GWI showed impairments in the domains of attention and memory with short-and-long delayed recall on a verbal list learning task on the CVLT-II suggesting clear differences between cases and controls. Further, GW veterans who reported neurotoxicant exposures showed deficits in cognitive domains relative to those without such exposures regardless of case status. However, exposure-cognitive associations in the full combined cohort appeared to be largely driven by associations in the GWI cases. A number of associations were identified only in controls, however, suggesting possible CNS effects of GW exposures that are not limited to veterans who meet criteria for GWI. Specifically, cognitive testing results suggested a relationship between exposure to chemical weapons (sarin/cyclosarin) and diminished executive control and slower processing speed on a task of alternating letter and number sequencing. Exposure to smoke from oil well fires was significantly associated with poorer immediate and sustained verbal memory. Skin pesticide exposure was used as a proxy for DEET exposure during the war and was significantly associated with poor executive control and slower processing speed on two measures in GW controls only. While pesticide sprays (organophosphate and carbamates) was associated with attention and executive function on a test of inhibition switching. It should be noted that DEET concentration used during this deployment was up to 75% active ingredient, much higher than current standards. In addition, exposure to anti-nerve gas (PB) pills appeared to be associated with both protective and detrimental effects in relation to attention and memory outcomes. PB pill usage was associated with more self-corrected errors on the D-KEFS Color Word Interference test compared with unexposed veterans. Use of PB pills had strong relationships with memory functioning specifically in verbal learning as well as verbal long and short delay measures and was protective. This corresponds with prior studies with preventive health military personnel with only PB exposure [3]. However, this work also reported that exposure to both PB and pesticides was detrimental to verbal memory outcomes. When these environmental

exposures were examined among veterans who met the definition for GWI case status, the cognitive outcomes differed slightly and had somewhat fewer differences but the results generally indicate that the exposure-cognitive associations are largely driven by associations in GWI cases. There were however, associations identified only in controls suggesting possible CNS effects of GW exposures that are not limited to veterans with GWI. This suggests that exposures including to acetylcholinesterase (AChE) inhibitors and oil well fires regardless of case status may also be important to monitor with regard to chronic cognitive outcomes but more confirmatory research is needed in this area.

This study documents and replicates reduced performance on tasks of sustained and divided attention, executive tasks including impulsivity and inattentiveness and verbal learning and recall memory in veterans with GWI from the currently recommended CDEs for cognitive outcomes [17]. It also documents the association of these decrements with environmental toxicant exposures during the war. Replicating these CDE recommended tests for future studies in current GW veteran cohorts including the now widely available BBRAIN repository is critical for determining which tests will be sensitive for use in ongoing biomarker and treatment studies within the field. It is important to note verbal memory decrements as this may indicate the need for future follow up as these veterans continue to age and may be more vulnerable to neurodegenerative disorders if they have GWI and/or prior neurotoxicant exposures [12, 28]. It is also important to note that not all neurotoxicant exposures have resulted in the same cognitive decrements even when they may have been in the same chemical class (i.e. AChE inhibitors). This suggests that specific exposures to repellents or organophosphate and carbamate pesticides may have individual and combined effects on cognitive outcomes for exposed veterans [29, 30]. Future studies should utilize the cognitive CDEs supported by findings from this study and may also benefit from comparing more sensitive computerized and screening measures as well as those that assess real-world subjective impacts of cognitive changes as Gulf War veterans age [31, 32]. However, the CDE tests utilized in this report could be used as minimum data elements for all future studies evaluating cognitive outcomes in veterans with GWI.

Despite replicating many of the CDEs for neuropsychological testing, our study is limited to the most commonly used neuropsychological tests and cognitive domains evaluated for the multiple study datasets shared in the BBRAIN repository including the CVLT-II, CPT3, D-KEFS Color-Word Interference Test and Trail Making Test. Due to the heterogeneity of prior studies, it was not possible to assess all neuropsychological tests within the

currently recommended CDEs. This included a lack of visuospatial tests across our repository studies. In addition, further insight into cognitive deficits may be gained with a wider array and fuller battery of tests including newer computerized and screening instruments from the CDEs [31]. Testing results from CDE measures also largely remain to be correlated with subjective cognitive complaints to assess their real-world implications [32]. Another limitation of our study as with almost all of the GW studies was measuring exposures using self-report and recall from events from many years ago. However, due to the lack of official notification of most exposures; self-report is the best way to capture exposures during the Gulf War.

These findings suggest that GW veterans, especially those who suffer from GWI, have sustained neurotoxic wounds including impaired cognition relative to controls who do not meet the Kansas criteria. This work substantiates the need for more refined exposure-based CDEs as well. As GW veterans age, neurocognitive deficits that may already exist as a result of toxic wounds have the potential to become more debilitating and overtax available cognitive reserves and potentially lead to increased risk for neurodegenerative disorders [33]. An increase in attention and care is needed for this GW veteran population to mitigate these cognitive deficits in their daily lives. Identification of the most sensitive and specific neuropsychological measure CDEs is integral for assessing treatment trial efficacy and biomarker sensitivity: two areas critical for the GWI field.

Conclusion

This study documents and replicates reduced performance on tasks of sustained and divided attention, executive function and verbal memory in veterans with GWI from the currently recommended common data elements for cognitive outcomes [17]. It also documents the association of some of these decrements with environmental toxicant exposures during the war. Specifically, CPT3, D-KEFS Color-word Interference Test, CVLT-II and Trail Making Test should be used in future studies of veterans with GWI. It would also be helpful to compare these cognitive outcomes with subjective cognitive complaints to further document the daily impact of these decrements.

Abbreviations

AChE	Acetylcholinesterase
BBRAIN	Boston, Biorepository, Recruitment and Integrative Network for Gulf War Illness
CDC	Center for Disease Control
CDE	Common Data Elements
CDMRP	Congressionally Directed Medical Research Program
CNS	Central Nervous System
CPT3	Conners Performance Test Third Edition
CVLT-II	California Verbal Language Test Second Edition

D-KEFS Delis-Kaplan Executive Function System
 GW Gulf War
 GWI Gulf War Illness
 PB Pyridostigmine bromide
 PTSD Post Traumatic Stress Disorder
 TMT Trail Making Test
 VA Veterans Affairs

Supplementary Information

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Additional file 1.

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Authors' contributions

DK, KS, MK, RT – Conceptualization, Methodology, Investigation, Writing – Original Draft, Writing-Review and Editing, Visualization, Supervision, Project Administration; TH, LC – Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing-Original Draft, Writing-Review and Editing, Visualization, Project Administration; EQ, JD – Data Curation, Formal Analysis, Writing-Review and Editing; PAJ, LS, NK – Writing-Review and Editing. The author(s) read and approved the final manuscript.

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Availability of data and materials

The dataset(s) supporting the conclusions of this article is(are) available in the BBRAIN repository, https://www.wapp.bumc.bu.edu/BEDAC_BBRAINRetro.

Declarations

Ethics approval and consent to participate

All participants gave their informed consent before they participated in each separate study including the Boston Gulf War Illness Consortium and the University of California at San Francisco (UCSF) studies and consented to share their data for future studies. Institutional review board approvals were obtained from Boston University and UCSF prior to initiating the studies. Consent for publication - Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. RAC-GWVI. Gulf war illness and the health of Gulf war veterans: scientific findings and recommendations, Research Advisory Committee Report on Gulf War Illness and Health of Gulf War Veterans. Dept of Veterans Affairs. Washinton, DC: US Government Printing Office; 2008.
2. White RF, Steele L, O'Callaghan JP, Sullivan K, Binns JH, Golomb BA, et al. Recent research on Gulf War illness and other health problems in veterans of the 1991 Gulf War: Effects of toxicant exposures during deployment. *Cortex*. 2016;74:449–75.
3. Sullivan K, Krengel M, Bradford W, Stone C, Thompson TA, Heeren T, et al. Neuropsychological functioning in military pesticide applicators from the Gulf War: Effects on information processing speed, attention and visual memory. *Neurotoxicol Teratol*. 2018;65:1–13.
4. Krengel MH, Zundel CG, Heeren T, Yee M, Spiro A, Proctor SP, et al. Health symptom trajectories and neurotoxicant exposures in Gulf War veterans: the Ft. Devens cohort. *Environ Health*. 2022;21(1):7.
5. Jeffrey MG, Krengel M, Kibler JL, Zundel C, Klimas NG, Sullivan K, et al. Neuropsychological findings in Gulf War Illness: a review. *Front Psychol*. 2019;10:2088.
6. Smith BN, Wang JM, Vogt D, Vickers K, King DW, King LA. Gulf war illness: symptomatology among veterans 10 years after deployment. *J Occup Environ Med*. 2013;55(1):104–10.
7. Maule AL, Janulewicz PA, Sullivan KA, Krengel MH, Yee MK, McClean M, et al. Meta-analysis of self-reported health symptoms in 1990–1991 Gulf War and Gulf War-era veterans. *BMJ Open*. 2018;8(2):e016086.
8. Steele L. Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. *Am J Epidemiol*. 2000;152(10):992–1002.
9. Fukuda K, Nisenbaum R, Stewart G, Thompson WW, Robin L, Washko RM, et al. Chronic multisymptom illness affecting Air Force veterans of the Gulf War. *JAMA*. 1998;280(11):981–8.
10. Janulewicz PA, Krengel MH, Maule A, White RF, Cirillo J, Sisson E, et al. Neuropsychological characteristics of Gulf War illness: a meta-analysis. *PLoS ONE*. 2017;12(5):e0177121.
11. Sullivan K, Krengel M, Proctor SP, Devine S, Heeren T, White RF. Cognitive functioning in treatment-seeking Gulf War Veterans: Pyridostigmine Bromide Use and PTSD. *J Psychopathol Behav Assess*. 2003;25(2):95–103.
12. Zundel CG, Heeren T, Grasso CM, Spiro A 3rd, Proctor SP, Sullivan K, et al. Changes in Health Status in the Ft. Devens Gulf War Veterans Cohort: 1997–2017. *Neurosci Insights*. 2020;15:2633105520952675.
13. Proctor SP, Heaton KJ, White RF, Wolfe J. Chemical sensitivity and chronic fatigue in Gulf War veterans: a brief report. *J Occup Environ Med*. 2001;43(3):259–64.
14. Toomey R, Alpern R, Vasterling JJ, Baker DG, Reda DJ, Lyons MJ, et al. Neuropsychological functioning of U.S. Gulf War veterans 10 years after the war. *J Int Neuropsychol Soc*. 2009;15(5):717–29.
15. Chao LL, Rothlind JC, Cardenas VA, Meyerhoff DJ, Weiner MW. Effects of low-level exposure to sarin and cyclosarin during the 1991 Gulf War on brain function and brain structure in US veterans. *Neurotoxicology*. 2010;31(5):493–501.
16. Chao LL, Abadjian L, Hlavín J, Meyerhoff DJ, Weiner MW. Effects of low-level sarin and cyclosarin exposure and Gulf War Illness on brain structure and function: a study at 4T. *Neurotoxicology*. 2011;32(6):814–22.
17. Cohen DE, Sullivan KA, McNeil RB, Gulf War Illness Common Data Elements Working G, Symptoms Assessment Working G, McNeil RB, et al. A common language for Gulf War Illness (GWI) research studies: GWI common data elements. *Life Sci*. 2022;290:119818.

18. Keating D, Zundel CG, Abreu M, Krengel M, Aenlle K, Nichols MD, et al. Boston biorepository, recruitment and integrative network (BBRAIN): a resource for the Gulf War Illness scientific community. *Life Sci*. 2021;284:119903.
19. Steele L, Klimas N, Krengel M, Quinn E, Toomey R, Little D, et al. Brain-Immune interactions as the basis of Gulf War Illness: Clinical Assessment and Deployment Profile of 1990–1991 Gulf War Veterans in the Gulf War Illness Consortium (GWIC) Multisite Case-Control Study. *Brain Sci*. 2021;11(9):1132.
20. Delis DC, Kramer JH, Kaplan E, Ober BA. California Verbal Learning Test–Second Edition (CVLT –II). San Antonio: The Psychological Corporation; 2000.
21. Delis DC, Kaplan E, Kramer JH. Delis-Kaplan executive function system: Examiner's manual. San Antonio: The Psychological Corporation; 2001a.
22. Reitan RM, Wolfson D. The Halstead–Reitan Neuropsychological Test Battery: Therapy and clinical interpretation. Tucson, AZ: Neuropsychological Press; 1985.
23. Conners CK. Conners continuous performance test 3rd edition manual. Toronto: Multi-Health Systems; 2014.
24. Wolfe J, Proctor SP, Erickson DJ, Hu H. Risk factors for multisymptom illness in US Army veterans of the Gulf War. *J Occup Environ Med*. 2002;44(3):271–81.
25. Fricker RD, Elaine Reardon DM, Spektor SK, Cotton J, Hawes-Dawson JE, Pace D. Hoses. Pesticide Use During the Gulf War: A Survey of Gulf War Veterans. Santa Monica: RAND Corporation; 2000.
26. Winkenwerder, Environmental Exposure Report. Pesticides Final Report U.S. Department of Defense, Office of the Special Assistant to the Undersecretary of Defense (Personnel and Readiness) from Gulf War Illnesses Medical Readiness and Military deployments. WASHINGTON, DC: US GOVERNMENT PRINTING OFFICE; 2003.
27. Abdullah L, Nkiliza A, Niedospial D, Aldrich G, Bartenfelder G, Keegan A, Hoffmann M, Mullan M, Klimas N, Baraniuk J, Crawford F, Krengel M, Chao L, Sullivan K. Genetic association between the APOE ε4 allele, toxicant exposures and Gulf war illness diagnosis. *Environ Health*. 2023;22(1):51–58. <https://doi.org/10.1186/s12940-023-01002-w>.
28. Zundel CG, Krengel MH, Heeren T, Yee MK, Grasso CM, Janulewicz Lloyd PA et al. Rates of Chronic Medical Conditions in 1991 Gulf War Veterans compared to the General Population. *Int J Environ Res Public Health*. 2019;16(6).
29. Golomb BA. Acetylcholinesterase inhibitors and Gulf War illnesses. *Proc Natl Acad Sci U S A*. 2008;105(11):4295–300.
30. Michalovic LT, Kelly KA, Sullivan K, O'Callaghan JP. Acetylcholinesterase inhibitor exposures as an initiating factor in the development of Gulf War Illness, a chronic neuroimmune disorder in deployed veterans. *Neuropharmacology*. 2020;171:108073.
31. Reinhard MJAN, Crock LE, McCarron KK, Veltkamp GM, Brewster RC. Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) Clinical Normative Data for Gulf War Veterans. *J Occup Environ Med*. 2022 Dec(1;64(12):e799–e804. <https://doi.org/10.1097/JOM.0000000000000706>. Epub 2022 Oct 3. PMID: 36190917).
32. Chao LL. Evidence of Objective Memory Impairments in Deployed Gulf War Veterans with subjective memory complaints. *Mil Med*. 2017;182(5):e1625–1631. <https://doi.org/10.7205/MILMED-D-16-00309>.
33. Janulewicz P, Krengel M, Quinn E, Heeren T, Toomey R, Killiany R et al. The multiple hit hypothesis for Gulf War Illness: self-reported Chemical/Biological Weapons exposure and mild traumatic brain injury. *Brain Sci*. 2018;8(11).

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Genetic association between the APOE ϵ 4 allele, toxicant exposures and Gulf war illness diagnosis

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Abstract

Introduction Exposure to nerve agents, pyridostigmine bromide (PB), pesticides, and oil-well fires during the 1991 Gulf War (GW) are major contributors to the etiology of Gulf War Illness (GWI). Since the apolipoprotein E (APOE) ϵ 4 allele is associated with the risk of cognitive decline with age, particularly in the presence of environmental exposures, and cognitive impairment is one of the most common symptoms experienced by veterans with GWI, we examined whether the ϵ 4 allele was associated with GWI.

Methods Using a case-control design, we obtained data on APOE genotypes, demographics, and self-reported GW exposures and symptoms that were deposited in the Boston Biorepository and Integrative Network (BBRAIN) for veterans diagnosed with GWI ($n = 220$) and healthy GW control veterans ($n = 131$). Diagnosis of GWI was performed using the Kansas and/or Center for Disease Control (CDC) criteria.

Results Age- and sex-adjusted analyses showed a significantly higher odds ratio for meeting the GWI case criteria in the presence of the ϵ 4 allele (Odds ratio [OR] = 1.84, 95% confidence interval [CI] = 1.07–3.15, $p \leq 0.05$) and with two copies of the ϵ 4 allele (OR = 1.99, 95% CI [1.23–3.21], $p \leq 0.01$). Combined exposure to pesticides and PB pills (OR = 4.10 [2.12–7.91], $p \leq 0.05$) as well as chemical alarms and PB pills (OR = 3.30 [1.56–6.97] $p \leq 0.05$) during the war were also associated with a higher odds ratio for meeting GWI case criteria. There was also an interaction between the ϵ 4 allele and exposure to oil well fires (OR = 2.46, 95% CI [1.07–5.62], $p \leq 0.05$) among those who met the GWI case criteria.

Conclusion These findings suggest that the presence of the ϵ 4 allele was associated with meeting the GWI case criteria. Gulf War veterans who reported exposure to oil well fires and have an ϵ 4 allele were more likely to meet GWI case criteria. Long-term surveillance of veterans with GWI, particularly those with oil well fire exposure, is required to better assess the future risk of cognitive decline among this vulnerable population.

Keywords Gulf War Illness, Apolipoprotein E, Pesticides, Pyridostigmine bromide and oil well fires

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Introduction

The 1990–1991 Gulf War (GW) was fought by a coalition of 30 countries that included approximately 700,000 U.S. troops, as well as soldiers from Canada, the United Kingdom, Australia, France, and the Arab nations [1]. Although the air and ground war itself was brief, nearly 30% of returning soldiers developed Gulf War Illness (GWI) which consists of symptoms such as memory impairment, debilitating fatigue, and widespread pain [1]. Cognitive deficits persist as the most common complaint [2, 3]. During this conflict, soldiers were exposed to a variety of toxic substances and pharmaceuticals, including smoke and combustion products from oil well fires, multiple vaccines, pesticides, prophylactic pyridostigmine bromide (PB) pills, and nerve agents, such as sarin gas [1]. There is now considerable support in the literature that these toxic chemicals have played a causal role in the pathogenesis of GWI. However, a key question remains regarding the role of genetic predisposition in the development of GWI, especially after chemical and airborne hazard exposure.

The apolipoprotein E (APOE) $\epsilon 4$ allele is one of the largest genetic risk factors for cardiovascular and neurological illnesses [4]. Current research provides substantial evidence that highlights how interactions of environmental risk factors with the $\epsilon 4$ allele enhance the risk of developing cognitive dysfunction [5]. Furthermore, carriers of the $\epsilon 4$ allele experience significant cognitive decline with age [6–8] and after a traumatic brain injury [9–11]. Studies have also shown that among individuals who took part in the search and rescue efforts at the World Trade Center (WTC) following the 9/11/2001 terrorist attack and consequently inhaled dust and smoke-containing particulate matter and other toxic chemical pollutants, an increased diagnosis of cognitive impairment exists among $\epsilon 4$ carriers compared to non-carriers [12]. Other studies on air pollution and cognition show that $\epsilon 4$ carriers who were service workers in a large aluminum factory in the Shanxi Province of China experience cognitive impairment with increasing levels of aluminum exposure [13]. Similarly, the risk of cognitive impairment was elevated among $\epsilon 4$ carriers who experienced poor air quality in Northern Manhattan [14]. Hence, the $\epsilon 4$ allele may play a critical role in modifying the risk of central nervous system (CNS) disorders when combined with exposure to environmental pollutants and/or a traumatic brain injury (TBI) in a gene-by-exposure (GxE) relationship.

Cognitively healthy $\epsilon 4$ carriers have exhibited a slower processing speed as they age [15, 16], while veterans with GWI exhibit a similar pattern of cognitive impairment; this deficit remains their most common complaint [17–19]. During neuropsychological testing, veterans with GWI present significant problems with attention, executive function, visuospatial skills, learning, memory, and

recognition [18, 20–22]. More specifically, they exhibit a loss of short-term memory [21], which is present earlier among $\epsilon 4$ carriers compared to non- $\epsilon 4$ carriers [23, 24]. Since the $\epsilon 4$ allele is a risk factor for long-term cognitive decline, both in the context of aging and following environmental exposures, we hypothesized that the $\epsilon 4$ allele frequency would be elevated among veterans with GWI compared to healthy GW veterans. As such, the current case-control study was designed to examine the association of the APOE $\epsilon 4$ allele with meeting GWI case criteria. The study also focused on the interaction between the $\epsilon 4$ allele and self-reported GW exposures that contributed to increased odds of meeting GWI case status.

Materials and methods

Cohort characteristics

All protocols were conducted in compliance with the relevant guidelines and regulations and approved by institutional review boards (IRB) at each institution. Informed consent was obtained from all participants. These secondary data analyses were largely facilitated through data sharing by the Boston Biorepository and Integrative Network (BBRAIN) for the GWI repository. All biological and clinical data utilized within this study were from the following case-control veteran cohorts: (1) the Roskamp Institute Neurology Clinic (RINC) cohort ($n=69$), (2) the Boston Gulf War Illness Consortium (GWIC) ($n=38$), (3) the Fort Devens cohort ($n=57$) [22], (4) the San Francisco Veterans Affairs Health Care System (SFVAHCS) cohort ($n=142$) that consisted of consecutive Gulf War veteran recruitment from 2014 to 2018 [25, 26], and (5) the Georgetown University cohort ($n=45$) [27]. Among all cohorts, either the Centers for Disease Control (CDC) chronic multi-symptom illness definition or the Kansas GWI criteria determined GWI status [28, 29]. More specifically, the Kansas GWI criteria requires that GW veterans must show symptoms in at least three of six symptom domains (fatigue/sleep problems, somatic pain, neurological/cognitive/mood symptoms, gastrointestinal symptoms, respiratory symptoms, and skin abnormalities) to ascertain a GWI diagnosis, whereas the CDC case criteria requires that a veteran must exhibit at least two chronic symptoms that present longer than six months from the following categories: pain, fatigue, and mood/cognition. Controls were either veterans deployed to the 1990–91 GW or healthy sedentary civilian controls that did not meet the Kansas GWI criteria or any of the exclusionary criteria. Primarily, participants were excluded if they reported a confounding diagnosis in their medical history [30]. Self-report of pesticide exposure was based on field use of pesticides, including sprays, fogs, pest-strips, fly baits, and the personal use of flea collars and pesticide-sprayed uniforms. These variables were combined into a binary pesticide exposure

questionnaire where reporting yes for any of the pesticide categories established pesticide exposure. Furthermore, self-reported exposure to chemical alarms, oil well fires, SCUD missiles, CARC paint, and the consumption of PB pills were also each coded as separate binary variables. Data on the sex of the participants were gathered via self-report as well, with the options being male and female. As per the National Institute of Health, sex refers to the biological differences between males and females, as previously described [30]. Similarly, self-reported information of symptoms pertaining to fatigue, cognitive problems, depression/depressed mood, and sleep disturbances was also collected and analyzed as a dichotomous binary variable (yes/no). Participant recruitment, blood collection, and blood storage procedures have been described previously [30]. All samples were collected using similarly written standard operating procedures for performing phlebotomy for blood collection.

APOE genotyping

The Ft. Devens, GWIC, and RINC samples: Within the Ft. Devens and RINC locations, blood samples were drawn into EDTA tubes to prepare plasma and preserve the blood cells for DNA extraction. With the GWIC and Georgetown University samples, DNA from white blood cells was extracted using the Gentra Puregene Blood Kit (Qiagen) as per the manufacturer's instructions (Qiagen). Genotyping of APOE using extracted DNA was performed using the EzWay direct ApoE genotyping kit (Cosmo Bio) as described by the providers. Amplified DNA fragments corresponding to different genotypes were separated by electrophoresis in Ethidium Bromide (EtBr) stained 2% agarose and 1% MetaPhor agarose gels. The presence of the $\epsilon 4$ allele identified with the EzWay direct ApoE genotyping kit was confirmed using polymerase chain reaction-restriction fragment-length polymorphism as follows: a section of the ApoE gene (218 bp) was amplified by PCR using the following primers: 5'-TCCAAGGAG-GTGCAGGCGGCGCA-3' (upstream) and 5'-GCCCCGGCCTGGTACACTGCCA-3' (downstream), ~250ng of DNA, 5 μ L of Expand High Fidelity Buffer, with 15 mM MgCl₂ 10x concentrated (Sigma), 2.5 μ L of each primer (10 μ M), 1 μ L of dNTP (10 mM), 5% dimethyl sulfoxide, 4 μ L of MgCl₂ (25 mM) and 0.75 μ L of Expand High Fidelity Enzyme mix (Sigma) in a final volume of 50 μ L. The DNA was denatured at 95 °C for five minutes, followed by 35 cycles of denaturation (95 °C, 1 min), annealing (65 °C, 1 min), and extension (72 °C, 1 min), with a final extension at 72 °C for four minutes. 15 μ L of PCR product was used to confirm the presence of the expected band (218 bp) by electrophoresis in EtBr-stained 2% agarose gels, while the remaining 35 μ L of PCR product was precipitated by adding 3.5 μ L of Sodium Acetate (3 M, pH 5.2) and 100 μ L of

100% ethanol to 35 μ L of amplified DNA. After incubating for 30 min at -80 °C, samples were centrifuged at 14,000 rpm at 4 °C for 10 min, and DNA-containing pellets were resuspended in nuclease-free water. Enzymatic digestion using 0.5 μ L of Cfo-I restriction enzyme (10 μ g/ μ L), 1.5 μ L of restriction enzyme 10X buffer, 0.2 μ L of acetylated BSA (10 μ g/ μ L), and 13 μ L of precipitated DNA was performed for 1.5 h at 37 °C to identify digestion profiles reflecting the presence of the $\epsilon 4$ allele. The restriction fragments corresponding to one or two copies of the $\epsilon 4$ allele were then separated by electrophoresis in 2% EtBr-stained and 1% MetaPhor agarose gels. For the SFVAHCS, DNA was isolated from saliva samples using Oragene kits (DNA Genotek, Ottawa, ON, Canada) in the laboratory of Dr. Joachim Hallmayer at Stanford University, as previously described [31]. For the Georgetown University cohort, APOE genotyping was performed using the TaqMan assay as previously described [32].

Statistical analyses The Pearson χ^2 statistics were used to compare demographic differences in race/ethnicity, exposure status, and sex and determine significant differences in the Hardy-Weinberg equilibrium (HWE) for APOE genotypes. The age of the controls and veterans with GWI was examined using a t-test for independent samples. Binary logistic regression modeling was used to calculate the odds ratios (OR) and associated 95% confidence intervals (CI). To evaluate possible interactions between GW exposures and the $\epsilon 4$ allele status, stepwise forward likelihood ratio selection in logistic regression was used where the final step was retained in the model. Terms in this model included independent GW exposures and their interactions with PB and the presence of the $\epsilon 4$ allele while still adjusting for age, sex and race/ethnicity. Given the exploratory nature of the study, no a priori power calculations were performed and all available data were utilized. A post-hoc power analysis of the data was conducted with the G-Power software. Using the OR for the differences between the diagnostic category of GWI and controls, the observed power of 72% was detected as alpha at 0.05, and for the observed interactions, a power of 97% at alpha 0.05 based on a sample of 220 GWI and 131 control individuals SPSS version 26 was used to analyze these data.

Results

Characteristics of the study population

There were no significant differences between controls (n=131) and veterans with GWI (n=220) for age, sex, and racial/ethnic differences (Table 1). The mean age of controls was 53 years (± 1.0 SE), whereas veterans with GWI had an average age of 52 (± 0.5 SE) at the time of the blood collection. Female sex made up 26% of the controls and 21% of the GWI cases. Self-reported exposure

Table 1 General demographics and GW exposures

	GW (n = 220)	Control (n = 131)
Demographics		
$\epsilon 4$ + carrier (%)	64 (29.1%)*	15 (19.1%)
Female (%)	47 (21.4%)	34 (26.0%)
Race (%)		
Caucasian	171 (79.9%)	106 (80.9%)
Latino	8 (3.7%)	6 (4.6%)
African American	21 (9.8%)	13 (9.9%)
Other	14 (6.5%)	6 (4.6%)
Mean age ($\pm$ SEM)	52.1 ($\pm$ 0.5)	53 ($\pm$ 1.0)
	GW (n = 161)	Controls (n = 85)
Exposures (Yes%)		
Oil well fire smoke	141 (87.6%)**	63 (74.1%)
Chemical alarm	139 (86.9%)**	47 (56.0%)
Scud missiles	79 (50.3%)**	28 (33.3%)
Pesticides	132 (82.0%)**	42 (49.4%)
PB	142(87.7%)**	47 (55.3%)
CARC paint	59 (37.8%)**	12 (14.5%)

Note: ** $p < 0.01$, * $p < 0.05$; race information unavailable for 6 GWI participants.

Table 2 APOE genotype and allele frequencies

	GW (n = 220)	Control (n = 131)
APOE genotype frequencies		
$\epsilon 2/\epsilon 2$	0 (0%)	1 (0.8%)
$\epsilon 2/\epsilon 3$	27 (12.3%)	17 (13.0%)
$\epsilon 2/\epsilon 4$	9 (4.1%)	3 (2.3%)
$\epsilon 3/\epsilon 3$	129 (58.6%)	88 (67.2%)
$\epsilon 3/\epsilon 4$	43 (19.5%)	22 (16.8%)
$\epsilon 4/\epsilon 4$	12 (5.5%)	0 (0%)
APOE allele frequencies		
$\epsilon 2$	36 (8.2%)	22 (8.4%)
$\epsilon 3$	328 (74.6%)	215 (82.1%)
$\epsilon 4$	76 (17.3%)	25 (9.5%)

Note: While APOE genotypes in controls were in Hardy-Weinberg equilibrium (HWE), APOE genotypes were not in HWE in GWI cases ($p = 0.012$).

Table 3 Association between APOE $\epsilon 4$ presence and GWI diagnosis

	Model I OR [95% CI]	Model II OR [95% CI]
$\epsilon 4$ +	1.84 [1.07–3.15]*	1.99 [1.23–3.21]**
Female	0.75 [0.44–1.26]	0.73 [0.44–1.25]
Ethnicity		
Latino	0.75 [0.25–2.25]	0.72 [0.24–2.19]
African American	0.92 [0.43–1.96]	0.88 [0.41–1.88]
Other	1.55 [0.57–4.20]	1.58 [0.58–4.30]
Age	0.99 [0.96–1.01]	0.99 [0.96–1.01]

Note: * $p \leq 0.05$, ** $p \leq 0.01$; Model I is $\epsilon 4$ carrier status, and model II is $\epsilon 4$ dose ($\epsilon 4$ non-carriers, homozygous or heterozygous). Both models are adjusted for age, sex, and race/ethnicity.

Table 4 Association between the APOE $\epsilon 4$ allele and GWI diagnosis in the presence of known GW chemicals

	OR [95% CI]
Age	0.95 [0.91–0.99]*
PB x Pesticides	4.10 [2.12–7.91]**
PB x chemical alarm	3.30 [1.56–6.97]**
Oil well x $\epsilon 4$ + carriers	2.46 [1.10–5.66]*

Note: * $p \leq 0.05$, ** $p \leq 0.01$; This is a stepwise forward LR model (displaying last final step selected). In addition to the variables in Table 2, GW exposure variables included reporting of exposure to oil well fires, hearing chemical alarms, scud missiles explosions, CARC paint, pesticide, PB intake, interaction between PB and pesticide exposure, and interaction between GW exposures and $\epsilon 4$. Also, since we had exposure data available for the limited dataset, analyses were restricted to 85 controls and 161 GWI subjects.

to oil well fires, chemical alarms, CARC paint, SCUD missiles, pesticides, and the consumption of PB pills was higher among veterans with GWI compared to controls (Table 1, $p < 0.05$). Table 2 shows that the presence of the $\epsilon 4$ allele was significantly higher among veterans with GWI (29%) compared to controls (19%). The frequency of the $\epsilon 2$ allele was 8.4% in controls and 8.2% in GWI, while the $\epsilon 3$ allele was 82.1% in controls and 74.6% in GWI, and the $\epsilon 4$ allele was 9.5% in controls and 17.3% in GWI (Table 2). While the APOE allele frequencies were in HWE for controls, there was a distortion of the $\epsilon 3$ and $\epsilon 4$ alleles. Consequently, there was a lack of HWE among GWI cases ($p < 0.05$). No independent association existed between the presence of the $\epsilon 4$ allele with sex, race and GW exposures.

Association of the $\epsilon 4$ allele with GWI diagnosis and its interaction with GW theatre exposures

We examined whether the presence of the $\epsilon 4$ allele was associated with the GWI diagnosis. Model I, in Table 3, shows that $\epsilon 4$ carrier status, as either homozygous or heterozygous, is significantly higher among those with a GWI diagnosis compared to healthy GW veterans (OR=1.84, 95% CI [1.07–3.15], $p \leq 0.05$), even when adjusted for age, sex, and race/ethnicity. The association between the presence of the $\epsilon 4$ allele and GWI diagnosis strengthened further when the $\epsilon 4$ allele dose, accounting for heterozygous versus homozygous status, was incorporated in Model II (OR=1.99, 95% CI [1.23–3.21], $p \leq 0.01$), even after adjusting for age, sex, and race/ethnicity (Table 3). The $\epsilon 4$ allele dose remained independently associated with the GWI diagnosis, when other GW exposures were included in the model (OR=2.06, 95% CI [1.05 to 4.03], $p \leq 0.05$). We then examined whether there was an interaction between GW exposures and the $\epsilon 4$ allele. Table 4 shows that while a higher age was associated with lower odds of GWI diagnosis

(OR=0.95, 95% CI [0.91–0.99], $p \leq 0.05$), chemical alarm recollection (OR=3.06, 95% CI [1.49–6.32], $p \leq 0.01$), PB pill and pesticide use (OR=4.10, 95% CI [2.12–7.91], $p \leq 0.01$) and exposure to oil well fires with the presence of the $\epsilon 4$ allele were all associated with an increased odds of having a GWI diagnosis (OR=2.46, 95% CI [1.07–5.62], $p \leq 0.01$). While a diagnosis of GWI was significantly associated with the self-reporting of cognitive problems (OR=6.85, 95% CI [2.07–22.75], $p \leq 0.01$), there was a non-significant interaction between the presence of the $\epsilon 4$ allele and GWI diagnosis that correlated with higher odds for reporting cognitive problems (OR=4.0, 95% CI [0.21 to 22.78], $p > 0.05$).

Discussion

The role of the APOE $\epsilon 4$ allele in modifying the biological responses to environmental exposures is well documented, where some recent studies have indicated that $\epsilon 4$ carriers display poor cognitive health even at a young age in urban areas with high air pollution [33–35]. While the exact mechanisms remain unclear, these studies suggest that the $\epsilon 4$ allele carriers may be more susceptible to developing CNS conditions that are associated with environmental exposures. In that regard, environmental exposure to a mixture of toxic chemicals, organophosphates pesticides, anti-nerve agents, and airborne hazards associated with oil well fires are widely implicated in GWI pathogenesis [1]. Hence, the observed association between the presence of the $\epsilon 4$ allele and GWI diagnosis is entirely consistent with the role of the $\epsilon 4$ allele in altering the response to environmental exposures and subsequent risk of neurological conditions, possibly due to a lower cognitive reserve in $\epsilon 4$ carriers that becomes exacerbated after environmental exposures [36].

Our current study shows for the first time that the presence of the $\epsilon 4$ allele is independently associated with a diagnosis of GWI, even after adjusting for demographic factors and correcting for independent influences of other self-reported GW exposures. Additionally, the observed $\epsilon 4$ allele frequencies in controls were similar to the general population [37]. In addition, the $\epsilon 4$ allele frequency corresponded with the self-reported exposure to oil well fires by increasing the odds of having received a GWI diagnosis in a GXE interaction. We did not observe any relationship between the presence of the $\epsilon 4$ allele and race/ethnicity, which did not differ between the case and control groups, making it unlikely that race/ethnicity could be a confounding factor for the association between APOE and GWI. While the self-reporting of exposure to GW chemicals and oil-well fires remains a key limitation of the study, the interaction of self-reported exposure to oil-well fires with the $\epsilon 4$ allele suggests the potential validity of this self-report measure by those truly exposed in the GW. Cognitive problems

were significantly associated with a GWI diagnosis, but the interaction between the $\epsilon 4$ allele and GWI status with cognitive impairment did not reach statistical significance. This is possibly due to the sample size limitation and the confounding influence of self-reported symptoms. Nevertheless, these studies contribute value to the current literature on gene-exposure outcomes in GWI research [38, 39] and show that genetic factors, such as the $\epsilon 4$ allele, may have interacted with GW exposures and precipitated this illness among soldiers who were deployed to the 1990–1991 GW.

There have been several studies investigating a genetic component in the risk of developing GWI. Since self-reported PB pill intake among GW veterans is associated with a diagnosis of GWI, Steele and colleagues investigated different variants of the butyrylcholinesterase (BCHE) gene [38]. Veterans with GWI who reported experiencing illness after PB consumption were enriched with less common BCHE variants which were also associated with lower BuChE enzymatic activity in degrading acetylcholine, suggesting a pre-existing genetic vulnerability as a consequence of PB exposure among veterans with GWI in a GXE relationship [38]. Haley and colleagues reported an association between the Q192R polymorphism of paraoxonase (PON1) with GWI diagnosis, as well as a significant GXE interaction for the hearing of chemical alarms and the presence of this polymorphism within GWI diagnosis [39]. Hence, these studies support a significant GXE component in GWI.

To date, the current study remains the first to indicate an influence of the $\epsilon 4$ allele and exposure to oil well fires on the development of GWI. A 13-year follow-up study of GW veterans suggested an increased risk of brain cancer among those with self-reports of exposure to oil well fires [40]. An association with asthma among GW veterans exposed to oil well fires is also reported [41]. To date, there are no reports of an association between exposure to oil well fires and neurological illnesses among GW veterans. While we were unable to examine the associations between self-report of oil well fires and neurological symptoms given the small sample size, recent studies highlight an association of the $\epsilon 4$ allele and air pollution with increasing the risk of cognitive impairment. In the Mexico City metropolitan area, where air pollution is thought to be the worst in the world, studies conducted with children showed that those who had the $\epsilon 4$ allele displayed poor cognition, particularly with respect to attention, short-term memory, and verbal fluency. Other studies have shown that these effects are more pronounced in female $\epsilon 4$ carriers who also display features of metabolic disorders in addition to a low intellectual quotient [35]. Similarly, prolonged exposure to debris piles of the World Trade Center for nearly 15 weeks seems to have increased the prevalence of cognitive impairment

among first responders with an $\epsilon 4$ allele [42]. Collectively, these studies suggest that the $\epsilon 4$ allele may interact with airborne hazards, in both urban and military settings, and modify the risk of developing neurological conditions.

We did not see a positive interaction between the $\epsilon 4$ allele, pesticide exposure, and the risk of having a GWI diagnosis. Pesticide-mediated pathobiology appears to associate uniquely with cognitive decline in the context of aging [43]. Since our study was restricted to the diagnosis of GWI, studies are required to determine whether pesticides and the $\epsilon 4$ allele may modify the risk of cognitive decline as veterans with GWI age.

The mechanistic underpinning of GXE in GWI may be helpful for developing therapeutic approaches, particularly in the context of aging and chronic age-related health conditions, which are suggested to represent a high burden of morbidity among GW veterans with GWI. Interestingly, the apolipoprotein E (apoE) protein is involved in lipid transport, and the $\epsilon 4$ allele lowers apoE availability within high-density lipoprotein (HDL) particles. Similarly, the PON1 enzyme is found in circulating high-density lipoprotein (HDL) particles [44]. The Q192R PON1 polymorphism and the $\epsilon 4$ allele are both implicated in modifying the risk of cardiovascular disease, secondary to their role in lipid metabolism [45–47]. Therefore, it is possible that environmental toxicants, by interacting with these genes, may have modified lipid metabolism pathways that contribute to the pathogenesis of GWI. In that regard, our prior studies have shown increased triglycerides in the blood of veterans diagnosed with GWI [30]. These studies collectively implicate that the underlying genetic component of cardiovascular risk as a key driver of the persistent pathophysiology of GWI and this warrants further investigation. A number of studies point to the proper maintenance of cardiovascular and metabolic risk factors; proper physical activity, dietary, and lifestyle habits may impart some benefits to reducing the burden of cognitive decline and neurodegeneration with age [48, 49]. Such efforts must be investigated among this high-risk and vulnerable population of GW veterans given their elevated genetic burden of AD risk factors, as seen in the current study.

Conclusion

For over 30 years, veterans with GWI have been experiencing adverse CNS health effects caused by exposure to a mixture of toxic chemicals and airborne hazards present during the 1990–1991 GW. In this study, we show that veterans with GWI also have higher frequencies of the $\epsilon 4$ allele, which may have played a GxE role in the risk of developing GWI. Since the $\epsilon 4$ allele also increases the risk of cardiovascular and neurodegenerative disorders with age, the current study highlights the need to focus

on the aging aspect since veterans with GWI are middle age and may be prematurely vulnerable to these disorders because of their potential gene-environment interaction susceptibility.

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Authors' contributions

LA, AN, LC, FC, MK and KS designed research and was assisted by MM, MH, AK, and JB, who provided critical comments; AN, DN, and LA assisted with designing the study; GB, AN, DN, and GA collected data; LA, AN, NK, GB, LC, JB, MH and KS coordinated participant recruitment, medical histories, and clinical data; AN and LA analyzed data; LA, AN, GA, DN and KS wrote the paper that was reviewed by all the authors. All authors read and approved the final manuscript.

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Data Availability

These are available through the BBRAIN program upon a written request.

Declarations

Competing interests

The authors declare no competing interests.

Disclaimers

The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the Department of Defense, the Department of Veterans Affairs or the United States Government.

Conflicts

Nothing to disclose.

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References

1. White RF, Steele L, O'Callaghan JP, et al. Recent research on Gulf War illness and other health problems in veterans of the 1991 Gulf War: Effects of toxic exposures during deployment. *Cortex*. 2016;74:449–75.
2. Proctor SP, Heaton KJ, Heeren T, White RF. Effects of sarin and cyclosarin exposure during the 1991 Gulf War on neurobehavioral functioning in US army veterans. *Neurotoxicology*. 2006;27:931–9.
3. Zundel CG, Heeren T, Grasso CM, et al. Changes in Health Status in the Ft. Devens Gulf War Veterans Cohort: 1997–2017. *Neurosci Insights*. 2020;15:2633105520952675.
4. Weintraub S, Teylan M, Rader B, et al. APOE is a correlate of phenotypic heterogeneity in Alzheimer disease in a national cohort. *Neurology*. 2020;94:e607–e12.
5. Angelopoulou E, Paudel YN, Papageorgiou SG, Piperi C. APOE genotype and Alzheimer's Disease: the influence of Lifestyle and Environmental factors. *ACS Chem Neurosci*. 2021;12:2749–64.

6. Reinvang I, Winjevoll IL, Rootwelt H, Espeseth T. Working memory deficits in healthy APOE epsilon 4 carriers. *Neuropsychologia*. 2010;48:566–73.
7. Sinclair LJ, Button KS, Munafò MR, Day IN, Lewis G. Possible association of APOE genotype with Working Memory in Young adults. *PLoS ONE*. 2015;10:e0135894.
8. Palmer Alfred ND, Raffield LM, Hardy JC, et al. APOE genotypes associate with cognitive performance but not cerebral structure: diabetes heart study MIND. *Diabetes Care*. 2016;39:2225–31.
9. Eramudugolla R, Bielak AA, Bunce D, Eastale S, Cherbuin N, Anstey KJ. Long-term cognitive correlates of traumatic brain injury across adulthood and interactions with APOE genotype, sex, and age cohorts. *J Int Neuropsychol Soc*. 2014;20:444–54.
10. Li L, Bao Y, He S, et al. The Association between Apolipoprotein E and Functional Outcome after Traumatic Brain Injury: a Meta-analysis. *Med (Baltim)*. 2015;94:e2028.
11. Yue JK, Robinson CK, Burke JF, et al. Apolipoprotein E epsilon 4 (APOE-epsilon4) genotype is associated with decreased 6-month verbal memory performance after mild traumatic brain injury. *Brain Behav*. 2017;7:e00791.
12. Clouston SAP, Diminich ED, Kotov R, et al. Incidence of mild cognitive impairment in World Trade Center responders: long-term consequences of re-experiencing the events on 9/11/2001. *Alzheimers Dement (Amst)*. 2019;11:628–36.
13. Wang SXY, Zhang J, Meng H, Zhang J, Li X, Zhang Z, Li H, Pan H, Lu X, Zhang Q, Niu Q. Interaction between aluminum exposure and ApoE4 gene on cognitive function of in-service workers. *Chemosphere*. 2023; 232.
14. Kulick ER, Elkind MSV, Boehme AK, et al. Long-term exposure to ambient air pollution, APOE-epsilon4 status, and cognitive decline in a cohort of older adults in northern Manhattan. *Environ Int*. 2020;136:105440.
15. O'Hara R, Sommer B, Way N, Kraemer HC, Taylor J, Murphy G. Slower speed-of-processing of cognitive tasks is associated with presence of the apolipoprotein epsilon4 allele. *J Psychiatr Res*. 2008;42:199–204.
16. Luo X, Jiaerken Y, Yu X, et al. Affect of APOE on information processing speed in non-demented elderly population: a preliminary structural MRI study. *Brain Imaging Behav*. 2017;11:977–85.
17. Rayhan RU, Stevens BW, Timbol CR, et al. Increased brain white matter axial diffusivity associated with fatigue, pain and hyperalgesia in Gulf War illness. *PLoS ONE*. 2013;8:e58493.
18. Sullivan K, Kregel M, Bradford W, et al. Neuropsychological functioning in military pesticide applicators from the Gulf War: Effects on information processing speed, attention and visual memory. *Neurotoxicol Teratol*. 2018;65:1–13.
19. Lu K, Nicholas JM, Pertzov Y, et al. Dissociable effects of APOE-epsilon4 and beta-amyloid pathology on visual working memory. *Nat Aging*. 2021;1:1002–9.
20. Janulewicz PA, Kregel MH, Maule A, et al. Neuropsychological characteristics of Gulf War illness: a meta-analysis. *PLoS ONE*. 2017;12:e0177121.
21. Jeffrey MG, Kregel M, Kibler JL, et al. Neuropsychological findings in Gulf War Illness: a review. *Front Psychol*. 2019;10:2088.
22. Yee MK, Zundel CG, Maule AL et al. Longitudinal Assessment of health symptoms in relation to Neurotoxicant Exposures in 1991 Gulf War Veterans: the Ft. Devens Cohort. *J Occup Environ Med*. 2020.
23. Gomez-Soria I, Ferreira C, Olivan Blazquez B, Magallon Botaya RM, Calatayud E. Short-term memory, attention, and temporal orientation as predictors of the cognitive impairment in older adults: a cross-sectional observational study. *PLoS ONE*. 2021;16:e0261313.
24. Ramos AA, Galiano-Castillo N, Machado L. Cognitive Functioning of Unaffected First-degree Relatives of Individuals With Late-onset Alzheimer's Disease: A Systematic Literature Review and Meta-analysis. *Neuropsychol Rev*. 2022.
25. Chao LL, Reeb R, Esparza IL, Abadjian LR. Associations between the self-reported frequency of hearing chemical alarms in theater and regional brain volume in Gulf War Veterans. *Neurotoxicology*. 2016;53:246–56.
26. Chao LL. The prevalence of mild cognitive impairment in a convenience sample of 202 Gulf War Veterans. *Int J Environ Res Public Health*. 2020; 17.
27. Rayhan RU, Stevens BW, Rakits MP, et al. Exercise challenge in Gulf War Illness reveals two subgroups with altered brain structure and function. *PLoS ONE*. 2013;8:e63903.
28. Fukuda K, Nisenbaum R, Stewart G, et al. Chronic multisymptom illness affecting Air Force veterans of the Gulf War. *JAMA*. 1998;280:981–8.
29. Steele L. Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. *Am J Epidemiol*. 2000;152:992–1002.
30. Oberlin S, Nkiliza A, Parks M, et al. Sex-specific differences in plasma lipid profiles are associated with Gulf War Illness. *J Transl Med*. 2022;20:73.
31. Laws SM, Clarnette RM, Taddei K, et al. APOE-epsilon4 and APOE – 491A polymorphisms in individuals with subjective memory loss. *Mol Psychiatry*. 2002;7:768–75.
32. DiBattista AM, Stevens BW, Rebeck GW, Green AE. Two Alzheimer's disease risk genes increase entorhinal cortex volume in young adults. *Front Hum Neurosci*. 2014;8:779.
33. Calderon-Garciduenas L, Franco-Lira M, Henriquez-Roldan C, et al. Urban air pollution: influences on olfactory function and pathology in exposed children and young adults. *Exp Toxicol Pathol*. 2010;62:91–102.
34. Calderon-Garciduenas L, Mora-Tiscareno A, Franco-Lira M, et al. Decreases in short term memory, IQ, and altered brain metabolic ratios in Urban Apolipoprotein epsilon4 children exposed to Air Pollution. *J Alzheimers Dis*. 2015;45:757–70.
35. Calderon-Garciduenas L, Jewells V, Galaz-Montoya C, et al. Interactive and additive influences of gender, BMI and apolipoprotein 4 on cognition in children chronically exposed to high concentrations of PM2.5 and ozone. APOE 4 females are at highest risk in Mexico City. *Environ Res*. 2016;150:411–22.
36. Weisskopf MG, Sullivan KA. Invited perspective: causal implications of gene by Environment Studies Applied to Gulf War Illness. *Environ Health Perspect*. 2022;130:51301.
37. Jia L, Xu H, Chen S, et al. The APOE epsilon4 exerts differential effects on familial and other subtypes of Alzheimer's disease. *Alzheimers Dement*. 2020;16:1613–23.
38. Steele L, Lockridge O, Gerkovich MM, Cook MR, Sastre A. Butyrylcholinesterase genotype and enzyme activity in relation to Gulf War illness: preliminary evidence of gene-exposure interaction from a case-control study of 1991 Gulf War veterans. *Environ Health*. 2015;14:4.
39. Haley RW, Kramer G, Xiao J, Dever JA, Teiber JF. Evaluation of a Gene-Environment Interaction of PON1 and low-level nerve Agent exposure with Gulf War Illness: a prevalence case-control study drawn from the U.S. Military Health Survey's National Population Sample. *Environ Health Perspect*. 2022;130:57001.
40. Barth SK, Kang HK, Bullman TA, Wallin MT. Neurological mortality among U.S. veterans of the Persian Gulf War: 13-year follow-up. *Am J Ind Med*. 2009;52:663–70.
41. Cowan DN, Lange JL, Heller J, Kirkpatrick J, DeBakey S. A case-control study of asthma among U.S. Army Gulf War veterans and modeled exposure to oil well fire smoke. *Mil Med*. 2002;167:777–82.
42. Daniels RD, Clouston SAP, Hall CB et al. A workshop on cognitive aging and impairment in the 9/11-Exposed Population. *Int J Environ Res Public Health*. 2021; 18.
43. Hayden KM, Norton MC, Darcey D, et al. Occupational exposure to pesticides increases the risk of incident AD: the Cache County study. *Neurology*. 2010;74:1524–30.
44. Rainwater DL, Rutherford S, Dyer TD, et al. Determinants of variation in human serum paraoxonase activity. *Heredity (Edinb)*. 2009;102:147–54.
45. Andersen HR, Wohlfahrt-Veje C, Dalgard C, et al. Paraoxonase 1 polymorphism and prenatal pesticide exposure associated with adverse cardiovascular risk profiles at school age. *PLoS ONE*. 2012;7:e36830.
46. Mahley RW, Apolipoprotein E. From cardiovascular disease to neurodegenerative disorders. *J Mol Med (Berl)*. 2016;94:739–46.
47. Darand M, Salehi-Abargouei A, Vahidi Mehrjardi MY, Feizi A, Seyedhossaini SM, Askari G. The association of the paraoxonase 1 Q192R polymorphism with coronary artery disease (CAD) and cardiometabolic risk factors in Iranian patients suspected of CAD. *Front Cardiovasc Med*. 2022;9:1037940.
48. Ngandu T, Lehtisalo J, Korkki S, et al. The effect of adherence on cognition in a multidomain lifestyle intervention (FINGER). *Alzheimers Dement*. 2022;18:1325–34.
49. Bantugan MA, Xian H, Solomon V et al. Associations of ApoE4 status and DHA supplementation on plasma and CSF lipid profiles and entorhinal cortex thickness. *J Lipid Res*. 2023: 100354.

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Is Gulf War Illness a prolonged early phase tauopathy?

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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 neurotoxins 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 (Falnkar & Baas, 2009; Hernandez et al., 2015; Morfini et al., 2009). One hypothesis is that the neurotoxins 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 neurotoxins 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., restraint 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.

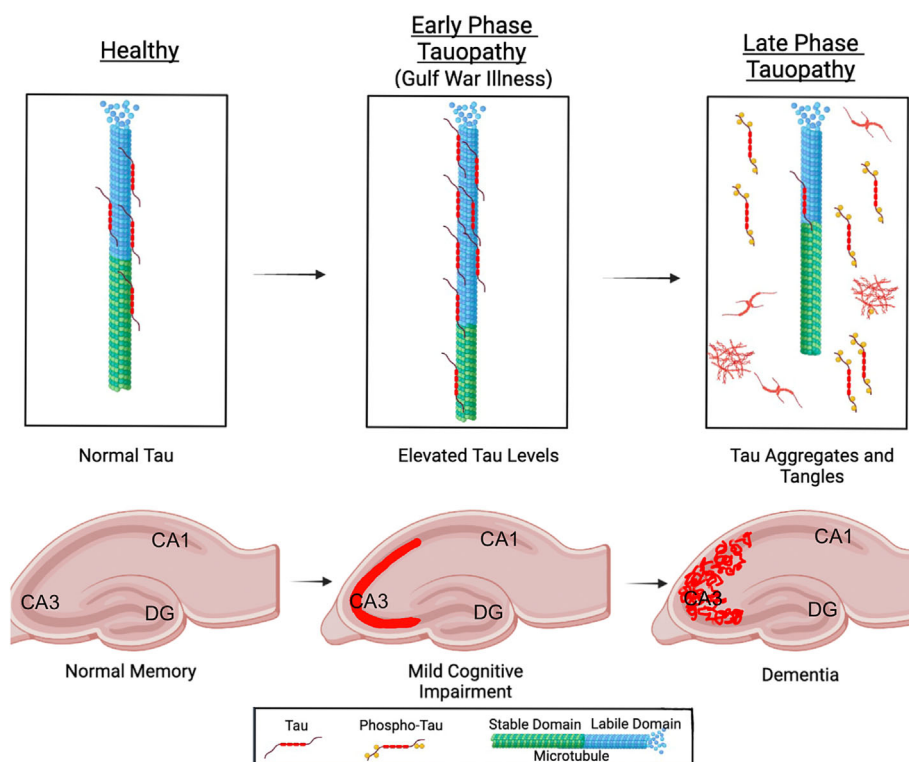
Our tau-based hypothesis for GWI is tauopathy is shown in Figure 1. As a general principle, we posit that most or all tauopathies begin as a pre-dementia early phase in which tau levels are elevated, after which the more broadly studied and recognized late phase ensues, corresponding to dementia, in 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.

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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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REFERENCES

- Abou-Donia, M. B., Conboy, L. A., Kokkotou, E., Jacobson, E., Elmasry, E. M., Elkafrawy, P., Neely, M., Bass, C. R., & Sullivan, K. (2017). Screening for novel central nervous system biomarkers in veterans with Gulf War Illness. *Neurotoxicology and Teratology*, 61, 36–46. <https://doi.org/10.1016/j.ntt.2017.03.002>
- Abou-Donia, M. B., Lapadula, E. S., Kregel, M., Quinn, E., LeClair, J., Massaro, J., Conboy, L., Kokkotou, E., Abreu, M., Klimas, N. G., Nguyen, D. D., & Sullivan, K. (2020). Using plasma autoantibodies of central nervous system proteins to distinguish veterans with Gulf War Illness from healthy and symptomatic controls. *Brain Sciences*, 10(610), 1–15. <https://doi.org/10.3390/brainsci10090610>
- Baas, P. W., & Ahmad, F. J. (2013). Beyond taxol: Microtubule-based treatment of disease and injury of the nervous system. *Brain*, 136(Pt 10), 2937–2951. <https://doi.org/10.1093/brain/awt153>
- Baas, P. W., & Qiang, L. (2019). Tau: It's not what you think. *Trends in Cell Biology*, 29(6), 452–461. <https://doi.org/10.1016/j.tcb.2019.02.007>
- Baas, P. W., Rao, A. N., Matamoros, A. J., & Leo, L. (2016). Stability properties of neuronal microtubules. *Cytoskeleton (Hoboken)*, 73(9), 442–460. <https://doi.org/10.1002/cm.21286>
- Brown, M. A., & Brix, K. A. (1998). Review of health consequences from high-, intermediate- and low-level exposure to organophosphorus nerve agents. *Journal of Applied Toxicology*, 18(6), 393–408. <https://www.ncbi.nlm.nih.gov/pubmed/9840747>
- Chao, L. L., Kriger, S., Buckley, S., Ng, P., & Mueller, S. G. (2014). Effects of low-level sarin and cyclosarin exposure on hippocampal subfields in Gulf War Veterans. *Neurotoxicology*, 44, 263–269. <https://doi.org/10.1016/j.neuro.2014.07.003>
- Chao, L. L., & Zhang, Y. (2018). Effects of low-level sarin and cyclosarin exposure on hippocampal microstructure in Gulf War Veterans. *Neurotoxicology and Teratology*, 68, 36–46. <https://doi.org/10.1016/j.ntt.2018.05.001>
- Falnikar, A., & Baas, P. W. (2009). Critical roles for microtubules in axonal development and disease. *Results and Problems in Cell Differentiation*, 48, 47–64. https://doi.org/10.1007/400_2009_2
- Gearhart, D. A., Sickles, D. W., Buccafusco, J. J., Prendergast, M. A., & Terry, A. V., Jr. (2007). Chlorpyrifos, chlorpyrifos-oxon, and diisopropylfluorophosphate inhibit kinesin-dependent microtubule motility. *Toxicology and Applied Pharmacology*, 218(1), 20–29. <https://doi.org/10.1016/j.taap.2006.10.008>
- Golomb, B. A., Allison, M., Koperski, S., Koslik, H. J., Devaraj, S., & Ritchie, J. B. (2014). Coenzyme Q10 benefits symptoms in Gulf War Veterans: Results of a randomized double-blind study. *Neural Computation*, 26(11), 2594–2651. https://doi.org/10.1162/NECO_a_00659
- Grigoryan, H., & Lockridge, O. (2009). Nanoimages show disruption of tubulin polymerization by chlorpyrifos oxon: Implications for neurotoxicity.

- Toxicology and Applied Pharmacology*, 240(2), 143–148. <https://doi.org/10.1016/j.taap.2009.07.015>
- Hernandez, C. M., Beck, W. D., Naughton, S. X., Poddar, I., Adam, B. L., Yanasak, N., Middleton, C., & Terry, A. V., Jr. (2015). Repeated exposure to chlorpyrifos leads to prolonged impairments of axonal transport in the living rodent brain. *Neurotoxicology*, 47, 17–26. <https://doi.org/10.1016/j.neuro.2015.01.002>
- Hernandez, F., Cuadros, R., Olla, I., Garcia, C., Ferrer, I., Perry, G., & Avila, J. (2019). Differences in structure and function between human and murine tau. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 1865(8), 2024–2030. <https://doi.org/10.1016/j.bbadis.2018.08.010>
- Hernandez, F., Merchan-Rubira, J., Valles-Saiz, L., Rodriguez-Matellan, A., & Avila, J. (2020). Differences between human and murine tau at the N-terminal end. *Frontiers in Aging Neuroscience*, 12, 11. <https://doi.org/10.3389/fnagi.2020.00011>
- Janke, C., & Montagnac, G. (2017). Causes and consequences of microtubule acetylation. *Current Biology*, 27(23), R1287–R1292. <https://doi.org/10.1016/j.cub.2017.10.044>
- Jiang, W., Duysen, E. G., Hansen, H., Shlyakhtenko, L., Schopfer, L. M., & Lockridge, O. (2010). Mice treated with chlorpyrifos or chlorpyrifos oxon have organophosphorylated tubulin in the brain and disrupted microtubule structures, suggesting a role for tubulin in neurotoxicity associated with exposure to organophosphorus agents. *Toxicological Sciences*, 115(1), 183–193. <https://doi.org/10.1093/toxsci/kfq032>
- Kneynsberg, A., Combs, B., Christensen, K., Morfini, G., & Kanaan, N. M. (2017). Axonal degeneration in tauopathies: Disease relevance and underlying mechanisms. *Frontiers in Neuroscience*, 11, 572. <https://doi.org/10.3389/fnins.2017.00572>
- Landrigan, P. J. (1997). Illness in Gulf War veterans. Causes and consequences. *The Journal of the American Medical Association*, 277(3), 259–261. <https://www.ncbi.nlm.nih.gov/pubmed/9005277>
- Lopes, S., Vaz-Silva, J., Pinto, V., Dalla, C., Kokras, N., Bedenk, B., Mack, N., Czisch, M., Almeida, O. F., Sousa, N., & Sotiropoulos, I. (2016). Tau protein is essential for stress-induced brain pathology. *Proceedings of the National Academy of Sciences of the United States of America*, 113(26), E3755–E3763. <https://doi.org/10.1073/pnas.1600953113>
- Lozupone, M., Dibello, V., Sardone, R., Castellana, F., Zupo, R., Lampignano, L., Bortone, I., Stallone, R., Altamura, M., Bellomo, A., Daniele, A., Solfrizzi, V., & Panza, F. (2023). The development of peptide- and oligonucleotide-based drugs to prevent the formation of abnormal tau in tauopathies. *Expert Opin Drug Discov*, 18(5), 515–526. <https://doi.org/10.1080/17460441.2023.2200245>
- Mackenzie Ross, S. J., Brewin, C. R., Curran, H. V., Furlong, C. E., Abraham-Smith, K. M., & Harrison, V. (2010). Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides. *Neurotoxicology and Teratology*, 32(4), 452–459. <https://doi.org/10.1016/j.ntt.2010.03.004>
- Middlemore-Risher, M. L., Adam, B. L., Lambert, N. A., & Terry, A. V., Jr. (2011). Effects of chlorpyrifos and chlorpyrifos-oxon on the dynamics and movement of mitochondria in rat cortical neurons. *The Journal of Pharmacology and Experimental Therapeutics*, 339(2), 341–349. <https://doi.org/10.1124/jpet.111.184762>
- Morfini, G. A., Burns, M., Binder, L. I., Kanaan, N. M., LaPointe, N., Bosco, D. A., Brown, R. H., Jr., Brown, H., Tiwari, A., Hayward, L., Edgar, J., Nave, K. A., Garber, J., Atagi, Y., Song, Y., Pigino, G., & Brady, S. T. (2009). Axonal transport defects in neurodegenerative diseases. *The Journal of Neuroscience*, 29(41), 12776–12786. <https://doi.org/10.1523/JNEUROSCI.3463-09.2009>
- Mummary, C. J., Borjesson-Hanson, A., Blackburn, D. J., Vijverberg, E. G. B., De Deyn, P. P., Ducharme, S., Jonsson, M., Schneider, A., Rinne, J. O., Ludolph, A. C., Bodenschatz, R., Kordasiewicz, H., Swayze, E. E., Fitzsimmons, B., Mignon, L., Moore, K. M., Yun, C., Baumann, T., Li, D., ... Lane, R. M. (2023). Tau-targeting antisense oligonucleotide MAPT(Rx) in mild Alzheimer's disease: A phase 1b, randomized, placebo-controlled trial. *Nature Medicine*, 29, 1437–1447. <https://doi.org/10.1038/s41591-023-02326-3>
- Naughton, S. X., Beck, W. D., Wei, Z., Wu, G., & Terry, A. V., Jr. (2020). Multifunctional compounds lithium chloride and methylene blue attenuate the negative effects of diisopropylfluorophosphate on axonal transport in rat cortical neurons. *Toxicology*, 431, 152379. <https://doi.org/10.1016/j.tox.2020.152379>
- O'Callaghan, J. P., Kelly, K. A., Locker, A. R., Miller, D. B., & Lasley, S. M. (2015). Corticosterone primes the neuroinflammatory response to DFP in mice: Potential animal model of Gulf War Illness. *Journal of Neurochemistry*, 133(5), 708–721. <https://doi.org/10.1111/jnc.13088>
- Okawa, Y., Ishiguro, K., & Fujita, S. C. (2003). Stress-induced hyperphosphorylation of tau in the mouse brain. *FEBS Letters*, 535(1–3), 183–189. [https://doi.org/10.1016/s0014-5793\(02\)03883-8](https://doi.org/10.1016/s0014-5793(02)03883-8)
- Parihar, V. K., Hattiangady, B., Shuai, B., & Shetty, A. K. (2013). Mood and memory deficits in a model of Gulf War Illness are linked with reduced neurogenesis, partial neuron loss, and mild inflammation in the hippocampus. *Neuropsychopharmacology*, 38(12), 2348–2362. <https://doi.org/10.1038/npp.2013.158>
- Pearson, J. N., & Patel, M. (2016). The role of oxidative stress in organophosphate and nerve agent toxicity. *Annals of the New York Academy of Sciences*, 1378(1), 17–24. <https://doi.org/10.1111/nyas.13115>
- Prendergast, M. A., Self, R. L., Smith, K. J., Ghayoumi, L., Mullins, M. M., Butler, T. R., Buccafusco, J. J., Gearhart, D. A., & Terry, A. V., Jr. (2007). Microtubule-associated targets in chlorpyrifos oxon hippocampal neurotoxicity. *Neuroscience*, 146(1), 330–339. <https://doi.org/10.1016/j.neuroscience.2007.01.023>
- Proctor, S. P., Heaton, K. J., Heeren, T., & White, R. F. (2006). Effects of sarin and cyclosarin exposure during the 1991 Gulf War on neurobehavioral functioning in US army veterans. *Neurotoxicology*, 27(6), 931–939. <https://doi.org/10.1016/j.neuro.2006.08.001>
- Qiang, L., Sun, X., Austin, T. O., Muralidharan, H., Jean, D. C., Liu, M., Yu, W., & Baas, P. W. (2018). Tau does not stabilize axonal microtubules but rather enables them to have long labile domains. *Current Biology*, 28(13), 2181–2189 e2184. <https://doi.org/10.1016/j.cub.2018.05.045>
- RAC. (2008). *Gulf War Illness and the Health of Gulf War Veterans: Scientific findings and recommendations* (Research Advisory Committee on Gulf War Veterans' Illnesses), Issue. U. S. G. P. Office.
- Rao, A. N., Patil, A., Brodnik, Z. D., Qiang, L., Espana, R. A., Sullivan, K. A., Black, M. M., & Baas, P. W. (2017). Pharmacologically increasing microtubule acetylation corrects stress-exacerbated effects of organophosphates on neurons. *Traffic*, 18(7), 433–441. <https://doi.org/10.1111/tra.12489>
- Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X., Liu, J., Barr, D. B., Slotkin, T. A., & Peterson, B. S. (2012). Brain anomalies in children exposed prenatally to a common organophosphate pesticide. *Proceedings of the National Academy of Sciences of the United States of America*, 109(20), 7871–7876. <https://doi.org/10.1073/pnas.1203396109>
- Rissman, R. A., Lee, K. F., Vale, W., & Sawchenko, P. E. (2007). Corticotropin-releasing factor receptors differentially regulate stress-induced tau phosphorylation. *The Journal of Neuroscience*, 27(24), 6552–6562. <https://doi.org/10.1523/JNEUROSCI.5173-06.2007>
- Rowe, C., Gunier, R., Bradman, A., Harley, K. G., Kogut, K., Parra, K., & Eskenazi, B. (2016). Residential proximity to organophosphate and carbamate pesticide use during pregnancy, poverty during childhood, and cognitive functioning in 10-year-old children. *Environmental Research*, 150, 128–137. <https://doi.org/10.1016/j.envres.2016.05.048>
- Saito, T., Mihira, N., Matsuba, Y., Sasaguri, H., Hashimoto, S., Narasimhan, S., Zhang, B., Murayama, S., Higuchi, M., Lee, V. M. Y., Trojanowski, J. Q., & Saido, T. C. (2019). Humanization of the entire murine Mapt gene provides a murine model of pathological human tau propagation. *The Journal of Biological Chemistry*, 294(34), 12754–12765. <https://doi.org/10.1074/jbc.RA119.009487>

- Sierra-Fonseca, J. A., & Gosselink, K. L. (2018). Tauopathy and neurodegeneration: A role for stress. *Neurobiology of Stress*, 9, 105–112. <https://doi.org/10.1016/j.ynstr.2018.08.009>
- Spillantini, M. G., & Goedert, M. (2013). Tau pathology and neurodegeneration. *Lancet Neurology*, 12(6), 609–622. [https://doi.org/10.1016/S1474-4422\(13\)70090-5](https://doi.org/10.1016/S1474-4422(13)70090-5)
- Terry, A. V., Jr., Beck, W. D., Warner, S., Vandenhuert, L., & Callahan, P. M. (2012). Chronic impairments in spatial learning and memory in rats previously exposed to chlorpyrifos or diisopropylfluorophosphate. *Neurotoxicology and Teratology*, 34(1), 1–8. <https://doi.org/10.1016/j.ntt.2011.08.015>
- Terry, A. V., Jr., Gearhart, D. A., Beck, W. D., Jr., Truan, J. N., Middlemore, M. L., Williamson, L. N., Bartlett, M. G., Prendergast, M. A., Sickles, D. W., & Buccafusco, J. J. (2007). Chronic, intermittent exposure to chlorpyrifos in rats: Protracted effects on axonal transport, neurotrophin receptors, cholinergic markers, and information processing. *The Journal of Pharmacology and Experimental Therapeutics*, 322(3), 1117–1128. <https://doi.org/10.1124/jpet.107.125625>
- Terry, A. V., Jr., Stone, J. D., Buccafusco, J. J., Sickles, D. W., Sood, A., & Prendergast, M. A. (2003). Repeated exposures to subthreshold doses of chlorpyrifos in rats: Hippocampal damage, impaired axonal transport, and deficits in spatial learning. *The Journal of Pharmacology and Experimental Therapeutics*, 305(1), 375–384. <https://doi.org/10.1124/jpet.102.041897>
- Wang, Y., & Mandelkow, E. (2016). Tau in physiology and pathology. *Nature Reviews Neuroscience*, 17(1), 5–21. <https://doi.org/10.1038/nrn.2015.1>
- Winkenwerder, W. (2003). *Environmental exposure report: Pesticides final report*. https://gulflink.health.mil/library/pest_final/index.html
- Yan, J., Sun, X. B., Wang, H. Q., Zhao, H., Zhao, X. Y., Xu, Y. X., Guo, J. C., & Zhu, C. Q. (2010). Chronic restraint stress alters the expression and distribution of phosphorylated tau and MAP2 in cortex and hippocampus of rat brain. *Brain Research*, 1347, 132–141. <https://doi.org/10.1016/j.brainres.2010.05.074>
- Yates, P. L., Case, K., Sun, X., Sullivan, K., Baas, P. W., & Qiang, L. (2022). Veteran-derived cerebral organoids display multifaceted pathological defects in studies on Gulf War Illness. *Frontiers in Cellular Neuroscience*, 16, 979652. <https://doi.org/10.3389/fncel.2022.979652>
- Yates, P. L., Patil, A., Sun, X., Niceforo, A., Gill, R., Callahan, P., Beck, W., Piermarini, E., Terry, A. V., Sullivan, K. A., Baas, P. W., & Qiang, L. (2021). A cellular approach to understanding and treating Gulf War Illness. *Cellular and Molecular Life Sciences*, 78(21–22), 6941–6961. <https://doi.org/10.1007/s00018-021-03942-3>

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