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PRINCIPAL INVESTIGATOR: Kimberly Sullivan, Ph.D.

CONTRACTING ORGANIZATION: Boston University School of Public Health

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Kimberly Sullivan, Ph.D.

E-Mail: tty@bu.edu

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Boston University School of Public Health
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None

14. ABSTRACT

This study aims to conduct follow-up longitudinal cognitive evaluations on a sub-sample of 100 Time 3 Ft. Devens Cohort veterans, most of whom were last evaluated in-person for cognitive functioning and with neuroimaging in the mid-1990s, to compare objective measurement of reported decline; and to determine cross-sectional blood and neuroimaging biomarkers (blood and structural volumetrics will also include longitudinal analyses) at 25+ years post deployment to the Gulf region, that may be consistent with cognitive outcomes and presumed pathobiological mechanisms (oxidative stress, ROS) of GWI. These data will evaluate the utility of previously unavailable blood and neuroimaging markers of oxidative stress, to devise a new diagnostic test for GWI in subgroups of GW veterans (TBI and OP exposed), and to provide a potential objective biomarker of treatment efficacy in clinical trials.

15. SUBJECT TERMS Gulf War Illness, central nervous system, biomarkers, glutathione, MR Spectroscopy, Cognition, oxidative stress markers

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1. Introduction:

Background and Purpose: One of the earliest and longest running studies of Gulf War veteran's (GWV) health was conducted with the Ft. Devens, MA army cohort (FDC) from the VA Boston Healthcare System (VABHS). The cohort was first surveyed within 5 days of their return and multiple cross sectional survey and in-person data provided some of the earliest cognitive, neuroimaging and environmental exposure outcomes since the 1990s. These findings included early documentation of the most common health symptoms, cognitive decrements in environmentally exposed GWV, and differences in structural neuroimaging, including lower white matter volumes (Proctor et al., 1998; White et al., 2001; Heaton et al., 2007). The FDC has been followed with longitudinal health surveys, and we have now completed a resurvey and biomarker study in which 50% of prior surveyed individuals have responded. This most recent resurvey is providing valuable information pertaining to longitudinal health symptoms and the trajectory of health symptoms over time. Additionally, we are able to use this longitudinal self-report data to monitor CDC and Kansas GWI criteria over time. However, these data are self-report and only a small portion of individuals are being seen for cross-sectional analyses of proteins in the blood (GW100046). Since subsamples of the FDC took part in cognitive and neuroimaging studies between 1994 and 1996, we are now extending these studies by reassessing neurocognitive and neuroimaging status to more fully capitalize on the longitudinal nature of this cohort and the recent findings of oxidative stress markers in GWI.

Scope: The overarching objective of this work study is to build on previous longitudinal studies to gain a better understanding of Gulf War Illness and to devise targeted treatment strategies. This study aims to conduct follow-up longitudinal cognitive evaluations on a sub-sample of 100 Time 3 FDC veterans, most of whom were last evaluated in-person for cognitive functioning and with neuroimaging in the mid-1990s, to compare objective measurement of reported decline; and to determine cross-sectional blood and neuroimaging biomarkers (blood and structural volumetrics will also include longitudinal analyses) at 25+ years post deployment to the Gulf region, that may be consistent with cognitive outcomes and presumed pathobiological mechanisms (oxidative stress, ROS) of GWI. These data will evaluate the utility of previously unavailable blood and neuroimaging markers of oxidative stress, to devise a new diagnostic test for GWI in subgroups of GWV (TBI and OP exposed), and to provide a potential objective biomarker of treatment efficacy in clinical trials.

2. Key Words: Gulf War Illness, central nervous system, biomarkers, glutathione, MR Spectroscopy, Cognition, oxidative stress

3. Accomplishments:

- What were the major goals of the project?
    - The major goals of the project as stated in the approved SOW for year 3 is listed in the table below. Specifically, during year 3, the primary goals were to screen, recruit, and complete longitudinal assessments of FDC veterans, as well as data cleaning and MRI/MRS post-image processing. Milestones/target dates for important activities or phases of these dates are listed in the table and actual completion dates are listed below.

Tasks	Timeline
Task 1. Obtain necessary authorization prior to initiation of human subjects	**Months**
1a. Obtain Institutional Review Board (IRB) approval for research sites at VA Boston (VABHS), Boston University Medical Campus (BUMC), and Nova University (NSU) for protocols	1-4
1b. Obtain DOD Human subjects Research Protections Office (HRPO) approvals	5-7
1c. Complete hiring of necessary staff and ensure all mandatory IRB research related trainings are completed by all staff members	1-8
Task 2. Preparation and Training for Clinical Study Procedures	**Months**
2a. Obtain Time 3 cognitive and MRI neuroimaging data for longitudinal analyses and participant contact information from the Ft. Devens cohort (FDC) study through the share drive at VABHS.	1-2
2b. Develop manuals for neuropsychological testing protocol, structural MRI and Magnetic Resonance Spectroscopy (MRS) of glutathione oxidative stress marker (GSH) protocols and blood specimen collection protocols for several oxidative stress markers.	1-6
2c. Train researchers and staff on cognitive, neuroimaging and phlebotomy protocols and quality control measures.	6-9
Task 3. Screening, recruitment and longitudinal assessment of FDC Gulf War veterans	**Months**
3a. Obtain informed consent from potentially eligible GW veterans	9-36
3b. Assess 150 FDC veterans and obtain demographics, medical history, self-report questionnaires and neuropsychological testing for planned longitudinal analyses.	9-36
3c. Perform brain GSH MR Spectroscopy and structural MRI imaging and blood draw for oxidative stress markers from 100 Gulf War veterans for cross-sectional study.	9-36
Task. 4. Data Cleaning and MRI/MRS image Post-processing	**Months**
4a. Post-process MRI/MRS neuroimaging data for data analysis.	18-40
4b. Score neuropsychological test data and upload summary data to VA Share drive for entry, cleaning and analyses.	18-38
4c. Ship blood samples to Nova University for analysis of GSH oxidative stress markers including (HNE, 8-iso-PGF2α).	18-36
4d. Perform analyses of plasma oxidative stress markers.	18-40
Task. 5. Merge Data and Perform Interim Data analyses	**Months**
5a. Data entry of all questionnaires, cognitive evaluations and quality control measures will be ongoing.	18-42

5b. Interim Statistical analyses of data obtained from cognitive evaluations, blood markers, neuroimaging and questionnaire data will be performed periodically.	18-42
5c. Annual reports of progress will be written.	18-36
Task 6. Perform Final Data Analysis and Prepare Manuscripts for Publication (months 42-48)	**Months**
6a. Perform cross-sectional analyses comparing central and peripheral markers of oxidative stress in brain MRS (GSH) and plasma (HNE, 8-iso-PGF2α) compared with cognitive functioning and health symptom report in FDC veterans.	42-45
6b. Perform longitudinal analyses of structural MRI imaging, cognitive, and health symptom outcomes from Time 3 and Time 6 in FDC veterans.	42-46
6c. Write final study report	47-48
6d. Present findings at scientific meetings	42-48
6e. Prepare manuscripts for submission for cross-sectional and longitudinal studies.	42-48

- What was accomplished under these goals?
- Task 1:
  - We obtained necessary authorization prior to initiation of study (IRB approvals, DoD HRPO approvals).
  - We completed the hiring of staff and ensured that all mandatory trainings are now completed.
- Task 2:
  - We have obtained Time 3 cognitive and MRI neuroimaging data for the longitudinal analysis and participant contact information form the Ft. Devens cohort (FDC) study and Treatment Seeking Cohort (TSC) through the share drive at VA Boston Health Care System (VABHS).
  - We developed manuals for the neuropsychological testing protocol as well as structural MRI and MRS of glutathione oxidative stress marker (GSH) protocols and blood specimen collection protocols for oxidative stress markers.
  - We have trained the researchers and staff on cognitive neuroimaging and phlebotomy protocols and questionnaires.

Task 3:

- We have obtained informed consent and longitudinal assessment of 63 FDC veterans.

Task 4:

- The neuropsychological test data was scored and uploaded to the VA share drive for the 63 subjects who have complete assessments.
- Structural MRI and MRS of GSH data has been collected for 14 subjects who have completed neuropsychological assessments
- Four shipments of blood samples has been sent to the NOVA university for analysis, for the 62 subjects who have complete assessments.

- How were the results disseminated to communities of interest?
  - Seven Ft. Devens Cohort longitudinal manuscripts have been published to date.
    - Zundel CG, Price K, Grasso CM, Spiro A 3rd, Heeren T, Sullivan K, Krengel MH. The impact of neurotoxicant exposures on posttraumatic stress disorder trajectories: The Ft. Devens Gulf War Veterans Cohort. J Trauma Stress. 2022 Jun;35(3):955-966. doi: 10.1002/jts.22802. Epub 2022 Feb 12. PMID: 35150175; PMCID: PMC9541763.
    - Krengel MH, Zundel CG, Heeren T, Yee M, Spiro A, Proctor SP, Grasso CM, Sullivan K. Health symptom trajectories and neurotoxicant exposures in Gulf War veterans: the Ft. Devens cohort. Environ Health. 2022 Jan 8;21(1):7. doi: 10.1186/s12940-021-00812-0. PMID: 34998396; PMCID: PMC8742929
    - Zundel, C. G., Krengel, M. H., Heeren, T., Yee, M. K., Grasso, C. M., Janulewicz Lloyd, P. A., Coughlin, S. S., & Sullivan, K. (2019). Rates of Chronic Medical Conditions in 1991 Gulf War Veterans Compared to the General Population. International journal of environmental research and public health, 16(6), 949.
    - Zundel, C. G., Heeren, T., Grasso, C. M., Spiro, A., 3rd, Proctor, S. P., Sullivan, K., & Krengel, M. (2020). Changes in Health Status in the Ft. Devens Gulf War Veterans Cohort: 1997-2017. Neuroscience insights, 15, 2633105520952675. https://doi.org/10.1177/2633105520952675
    - Yee, M. K., Zundel, C. G., Maule, A. L., Heeren, T., Proctor, S. P., Sullivan, K. A., & Krengel, M. H. (2020). Longitudinal Assessment of Health Symptoms in Relation to Neurotoxicant Exposures in 1991 Gulf War Veterans: The Ft. Devens Cohort. Journal of occupational and environmental medicine, 62(9), 663–668. https://doi.org/10.1097/JOM.0000000000001910
    - Clara G. Zundel, B.S., Kathryn Price, B.A., Claudia M. Grasso, B.A., Avron Spiro III PhD, Susan P. Proctor, DSc, Kimberly Sullivan, PhD, and Maxine H. Krengel, PhD. The Impact of Neurotoxicant Exposures on Posttraumatic Stress Disorder Trajectories: The Ft. Devens Gulf War Veterans Cohort. J Trauma Stress. 2022 Jun;35(3):955-966. doi: 10.1002/jts.22802. Epub 2022 Feb 12.
    - Maxine H. Krengel, Clara G. Zundel, Timothy Heeren, Megan Yee, Avron Spiro, Susan P. Proctor, Claudia M. Grasso, Kimberly A. Sullivan. Health Symptom Trajectories and Neurotoxicant Exposures in Gulf War Veterans: The Ft. Devens Cohort. Environ Health. 2022 Jan 8;21(1):7. doi: 10.1186/s12940-021-00812-0.
- What do you plan to do during the next reporting period to accomplish the goals?
  - We will continue to adjust the study protocol to accommodate possible COVID-19 requirements of Boston University to continue to facilitate enrollment in this study.
  - We plan to continue enrolling individuals that have responded to our successful recruitment efforts and have agreed to participate in this study in the past 12 months.
  - We will continue to recruit until we have reached our anticipated goal of 100 participants by the next reporting period and present the preliminary results at appropriate National and International meetings.
  - We plan to recruit 50 more study participants by the next reporting period and present the preliminary results at appropriate National and International meetings.
  - We plan to complete publication of other manuscripts of preliminary results from the GWI case- control medical conditions, and trajectory of symptom analyses and the cognitive, MR Spectroscopy glutathione brain imaging and blood oxidative

stress markers during the next reporting period.
- We have received IRB approval for a no cost extension, to continue our efforts of recruiting participants for additional MRI with MRS data and blood specimens.

4. Impact:

- What was the impact on the development of the principal discipline(s) of the project?
  - Gulf War Illness (GWI) can have a dramatic impact on the lives and well-being of GW veterans who experience chronic and often debilitating symptoms. The results of this study will help address a critical knowledge gap regarding the nature of continued cognitive symptoms and other chronic health effects of GWI.
  - This project will distinguish itself by examining the nature and trajectory of symptoms by adding objective markers of longitudinal neurocognitive decline, traditional structural MRI imaging and cutting-edge MRS brain imaging techniques of oxidative stress markers (glutathione) compared with plasma oxidative stress markers. When combined with the prior rich 20+ year longitudinal data from the Ft. Devens and Treatment-seeking cohorts, this provides an unprecedented opportunity to further characterize objective biomarkers of illness in a well-characterized cohort of GW veterans.
  - Defects in modulation of oxidative stress may well predispose individuals to damage from reactive oxygen species (ROS) from environmental exposures, TBI or other sources that could potentially be used as a diagnostic marker of illness.
  - This analysis also offers an opportunity to determine whether a given therapeutic strategy such as antioxidants including co-enzyme Q-10 or n-acetyl cysteine supplementation in subgroups with low brain glutathione levels may be chosen as a treatment option to improve a susceptible individual's ability to modulate oxidative stress, reduce accelerated aging and improve the symptoms of GWI utilizing a personalized medicine approach.

- What was the impact on other disciplines?
  - A major advantage of work with the Ft. Devens cohort showing mTBI to be related to rates of GWI in our two recent papers suggests that the results of this study with oxidative stress glutathione markers may be relevant not only to GWI but also to other veteran and civilian groups with mTBI and neurotoxicant exposures as part of a multiple-hit hypothesis.
  - Blood and neuroimaging-based biomarkers of GWI provide an effective way to enhance its management:
    - It can be used as a diagnostic and prognostic tool with the ability to provide information about rate of disease progression.
    - It would help in identification of novel and effective treatments for multiple disorders and environmental exposure groups (i.e. pesticides, nerve agents).
    - It could be used for monitoring therapeutic efficacy for multiple disorders
    - It could provide a cost-effective option for recruitment into clinical trials

- What was the impact on technology transfer?
  - The biomarker that we hope to develop will be cost effective, available, and do not need expensive technicians if we can identify an oxidative stress biomarker in blood that be correlated with MR spectroscopy brain imaging markers that we will also collect and analyze.

- What was the impact on society beyond science and technology?

- Our blood and brain imaging biomarkers should improve the quality of life for the veterans of the GW who have GW illness because:
  - Our biomarkers can provide objective evidence thereby validating the chronic health symptoms of ill GW veterans.
  - Our biomarkers should lead to studies to develop treatment of brain injury that may lead to improvement of their clinical condition.

5. Changes for approach and reasons for change:

- Changes: Tele-health neuropsychological measures per COVID-19 restrictions

We were granted an IRB amendment to recruit participants that have already completed the neuropsychological assessments without having prior imaging done, to aid in increasing participant numbers for the structural MRI and MRS of glutathione oxidative stress marker (GSH) protocols and blood specimen collection protocols for oxidative stress markers.

Changes that had significant impact on expenditures

- We were granted a no cost extension year to complete subject recruitment efforts.

- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:
  - Significant changes in use or care of human subjects: None
  - Significant changes in use or care of vertebrate animals: None
  - Significant changes in use of biohazards, and/or select agents: None

6. Products:

- **Publications, conference papers, and presentations**
  - **Journal Publications**

- Zundel, C.G., Krengel, M. H., Yee, M.K., Grasso, C.M., Januelwicz Lloyd, P., Sullivan, K.A. Rates of Chronic Medical Conditions in 1991 Gulf War Veterans Compared to the General Population. International Journal of Environmental Research and Public Health
- Yee MK, Janulewicz PA, Seichepine DR, Sullivan KA, Proctor SP, Krengel MH. Multiple Mild Traumatic Brain Injuries Are Associated with Increased Rates of Health Symptoms and Gulf War Illness in a Cohort of 1990-1991 Gulf War Veterans. Brain Sci. 2017 Jul 9;7(7). pii: E79. doi: 10.3390/brainsci7070079.
- Yee, M., Seichepine, D., Januelwicz Lloyd P., Sullivan, K., Proctor, SP & Krengel, M. Traumatic brain injury, health and rate of chronic multisymptom illness in veterans from the 1990-1991 Gulf War. Journal of Head Trauma Rehabilitation. 2016 Sep-Oct;31(5):320-8. doi: 10.1097/HTR.0000000000000173.
- Yee, M. K., Zundel, C. G., Maule, A. L., Heeren, T., Proctor, S. P., Sullivan, K. A., & Krengel, M. H. (2020). Longitudinal Assessment of Health Symptoms in Relation to Neurotoxicant Exposures in 1991 Gulf War Veterans: The Ft. Devens Cohort. Journal of occupational and environmental medicine, 62(9), 663–668. https://doi.org/10.1097/JOM.0000000000001910
- Zundel, C. G., Heeren, T., Grasso, C. M., Spiro, A., 3rd, Proctor, S. P., Sullivan, K., & Krengel, M. (2020). Changes in Health Status in the Ft. Devens Gulf War Veterans Cohort: 1997-2017. Neuroscience insights, 15, 2633105520952675. https://doi.org/10.1177/2633105520952675

- Zundel CG, Price K, Grasso CM, Spiro A 3rd, Heeren T, Sullivan K, Krengel MH. The impact of neurotoxicant exposures on posttraumatic stress disorder trajectories: The Ft. Devens Gulf War Veterans Cohort. J Trauma Stress. 2022 Jun;35(3):955-966. doi: 10.1002/jts.22802. Epub 2022 Feb 12. PMID: 35150175; PMCID: PMC9541763.
- Maxine H. Krengel, Clara G. Zundel, Timothy Heeren, Megan Yee, Avron Spiro, Susan P. Proctor, Claudia M. Grasso, Kimberly A. Sullivan. Health Symptom Trajectories and Neurotoxicant Exposures in Gulf War Veterans: The Ft. Devens Cohort. Environ Health. 2022 Jan 8;21(1):7. doi: 10.1186/s12940-021-00812-0.

  - **Books or other non-periodicals, one-time publications**
    - None
  - **Other publications, conference papers, and presentations**
    - Price, K., Orlinsky L., Zundel, CG., Rivoira, P., Sullivan, K., Krengel, MH. (2022) Longitudinal Data Collection of Neurotoxicant Exposures and Health Symptoms in Gulf War Veterans. Poster Presentation- Military Health System Research Symposium, Kissimmee, Florida
    - Price, K., Orlinsky L., Zundel, CG., Rivoira, P., Sullivan, K., Krengel, MH. (2022) Longitudinal Data Collection of Neurotoxicant Exposures and Health Symptoms in Gulf War Veterans. International Neuropsychological Society - Poster Presentation, Barcelona, Spain
    - Krengel, MK. Mild Traumatic Brain Injury and Cognitive, Health and Emotional Symptoms (2022)- Traumatic Brain Injury Symposium Presentation- International Neuropsychological Society -Barcelona, Spain
    - Price, K., Orlinsky, L., Krengel, MH., Sullivan, K., Zundel, CG. Neuropsychological Functioning in Gulf War Illness: A Longitudinal Case Study. International Neuropsychological Society -Poster Presentation, New Orleans, Louisiana
    - Price, K., Zundel, C.G., Grasso, C.M., Spiro, A., Heeren, T., Sullivan, K.A., & Krengel, M.H. (*In Review*) Impact of Neurotoxicant Exposures on Post Traumatic Stress Disorder Trajectories; The Ft. Devens Gulf War Veterans Cohort. American Psychological Association 2021 Conference.
    - Price, K., Zundel, C.G., Krengel, M.K. (2020). Longitudinal Change in PTSD Symptomatology and Associations with Neurotoxicant Exposures in Gulf War Veterans. International Neuropsychology Society 2020 Conference, Vienna, Austria
    - Price, K., Zundel, C.G., Krengel, M.K. (2020). Longitudinal Change in PTSD Symptomatology and Associations with Neurotoxicant Exposures in Gulf War Veterans. University of Massachusetts Boston – Psychology Honors Student Presentation Day.
    - Zundel, C.G., Yee, M.K., Sullivan, K., Krengel, M.K. (2020). A Longitudinal Assessment of Health Symptoms and the Associations with Neurotoxicant Exposures in 1991 Gulf War Veterans: The Ft. Devens Cohort. International Neuropsychology al Society 2020 Conference, Denver, CO.
    - Grasso, C.M., Zundel, C.G., Krengel, M.H. (2020). Exposures, Healht, and Neuropsychological Outcomes in Gulf War Veterans 25+ Years Post War. International Neuropsychological Society 2020 Conference, Denver, CO, Massachusetts Neuropsychological Society Science Symposium, and VA Research Week.

- Zundel, C.G., Yee, M.K., Maule, A., Grasso, C.M., Sullivan, K., Krengel, M. (2019). Health Symptoms Associated with Gulf War-Specific Exposures in Male and Female Veterans: A Longitudinal Assessment. International Neuropsychological Society 2019 Conference, New York, NY.
- Cohen, A.B., Zundel, C.G., Krengel, M.K., Sullivan, K.A. (2018). Preliminary Findings of Gender Differences and Symptomatology within Gulf War Veterans. Massachusetts Neuropsychology Society Science Symposium 2018
- Zundel, C.G., Lad, S.S., Yee, M.K., Grasso, C.M., Janulewicz Lloyd, P.A., Sullivan, K.A., Krengel, M.H. Rates of medical conditions: Do Gulf War veterans differ from the general population? Presented at international Neuropsychological Society 2018 Conference, Washington D.C.
- Krengel MH, Yee M, Nolan T, Janulewicz Lloyd PA, Sullivan K & Seichepine DR. Multiple Self-Reported Exposures to Mild Traumatic Brain Injury and Neurotoxicants Predict Current Total Health Symptoms in a Cohort of 1990-1991 Gulf War Veterans. Journal of International Neuropsychological Society. Supplement 1, March 2016.
- Yee, M., Seichepine DR, Nolan T, Janulewicz Lloyd PA, Sullivan K & Krengel MH. Multiple Self-Reported Brain Injuries are Associated with Increased Health Symptoms in a Cohort of 1990-1991 Gulf War Veterans. Journal of International Neuropsychological Society. Supplement 1, March 2016.

- **Other products**

Website(s) or other Internet site(s)

None

Technologies or techniques

None

7. Participants and other Participating Organizations

Site 1: VA Boston Health Care System (VABHS) (BUMC) 150 S. Huntington Avenue
Boston, MA 02130
Initiating PI: Maxine Krengel, PhD

Site 2: Boston University Medical Campus
715 Albany Street
Boston, MA 02118
Partnering PI: Kimberly Sullivan, PhD Co-Investigator: Carole Palumbo, PhD
Co-Investigator: Ronald Killiany, PhD

Site 3: Nova Southeastern University (NSU)
3200 South University Drive
Fort Lauderdale, Florida 33328-2018
Co-Investigator: Richard C. Deth, PhD Collaborator: Nancy Klimas, MD

Study Sites Responsibilities

Site 1: Dr. Krengel and her VABHS team will be responsible for recruiting FDC study participants and conducting cognitive evaluations and phlebotomy to send to NOVA investigators. Specifically, she will oversee the recruitment and blood draws/cognitive evaluations of FDC study participants and the processing of plasma samples that will be shared for the proposed study. Dr. Krengel will also oversee the experimental design, data analysis and interpretation and presentation of study results in collaboration with Dr. Sullivan and the other study investigators. **Tasks 1-6**

Site 2: Dr. Sullivan and her BUMC team will be responsible for performing the MRS/MRI imaging protocols and post-processing the imaging data for cross-sectional (MRS) and longitudinal analyses (structural MRI). Specifically, she will oversee the imaging acquisition and post-processing protocols in collaboration with Drs. Killiany and Palumbo. Dr. Sullivan will also assist with the experimental design, data analysis and interpretation and presentation of study results in collaboration with Dr. Krengel and the other study investigators. **Tasks 1-6**

Site 3: Dr. Deth and Klimas will be responsible for receiving the plasma samples from the Boston site and performing oxidative stress marker analyses for GSH, HNE and 8-iso-PGF2α for
100 blood samples (50 GWI, 50 controls). Dr. Deth will also assist with the experimental design, interpretation of data, report and manuscript writing and presentation of results at scientific meetings. **Tasks 1, 2, 4, 5, 6**

8. Special Reporting requirements:
None

9. Appendixes.

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Environmental Health

RESEARCH Open Access

Health symptom trajectories and neurotoxicant exposures in Gulf War veterans: the Ft. Devens cohort

Maxine H. Krengel[1*†], Clara G. Zundel[1,2†], Timothy Heeren[3], Megan Yee[1,4], Avron Spiro[5,6,7], Susan P. Proctor[1,8], Claudia M. Grasso[1] and Kimberly Sullivan[4]

Abstract

Background: Thirty years ago, Gulf War (GW) veterans returned home with numerous health symptoms that have been associated with neurotoxicant exposures experienced during deployment. The health effects from these exposures have been termed toxic wounds. Most GW exposure-outcome studies utilize group analyses and thus individual fluctuations in symptoms may have been masked. This study investigates health symptom trajectories in the same veterans over 25 years.

Methods: Veterans were categorized into 5 a priori trajectory groups for each health symptom and Chronic Multisymptom Illness (CMI) clinical case status. Multinomial logistic regression models were used to investigate associations between these trajectories and neurotoxicant exposures.

Results: Results indicate that more than 21 Pyridostigmine Bromide (PB) pill exposure was associated with consistent reporting of fatigue, pain, and cognitive/mood symptoms as well as the development of six additional symptoms over time. Chemical weapons exposure was associated with both consistent reporting and development of neurological symptoms over time. Reported exposure to tent heater exhaust was associated with later development of gastrointestinal and pulmonary symptoms. Veterans reporting exposure to more than 21 PB pills were more than 8 times as likely to consistently meet the criteria for CMI over time.

Conclusion: This study highlights the importance of the continued documentation of the health impacts experienced by GW veterans', their resulting chronic health symptoms, and the importance of exposure-outcome relationships in these veterans now 30 years post-deployment.

Keywords: Gulf War, Veterans, Toxic wounds, Neurotoxicant exposure, Longitudinal Design, Health symptoms

Background

Thirty years ago, Gulf War (GW) veterans returned home from deployment with a constellation of health symptoms: some have been chronic, some have emerged over time, and others have remitted [1, 2]. Collectively, these symptoms are termed Gulf War Illness (GWI) and have been associated with central nervous system (CNS) dysfunction as a result of neurotoxicant exposures during the war and resultant toxic wounds [3, 4]. These exposures include chemical warfare agents (sarin/cyclosarin), pesticide sprays and creams, pyridostigmine bromide (PB) prophylactic anti-nerve gas pills, smoke from oil well fires, tent heater exhausts, and others [3–6].

In the early years, research focused on determining the symptoms that characterized GWI, and identifying

*Correspondence: maxine.krengel@va.gov
†Maxine H. Krengel and Clara G. Zundel contributed equally to this work.
[1] Research Service, VA Boston Healthcare System, 150 South Huntington Ave. 8A-90, Jamaica Plain, Boston, MA 02130, USA
Full list of author information is available at the end of the article

potential neurotoxicant exposures that could be etiologically related to these symptoms. However, studies which compared GWI cohorts to controls did not allow for the comparison of the separate GWI health symptom trajectories of an individual veteran. The majority of the studies employ the two most widely used case criteria for GWI, the Center for Disease Control (CDC)'s Chronic Multisymptom Illness (CMI) criteria and the Kansas GWI criteria [7, 8]. To date, these two criteria are recommended by the Institute of Medicine (IOM) and Department of Defense (DoD) for use in GWI research studies. However, both criteria were derived over 20 years ago and reflect what the illness looked like initially post-deployment. In addition, these criteria ask veterans whether they have experienced these symptoms over the past 6 months. Therefore, the case criteria only capture current symptoms and do not account for potential changes in symptoms over time [9]. This has made obtaining service-related benefits difficult for veterans with toxic wounds.

Specifically, the CDC criteria include the following symptom domains: fatigue, mood and cognition, and pain, whereas the Kansas criteria also include gastrointestinal, respiratory, and skin domains [7, 8]. Additionally, the Kansas criteria exclude concomitant illnesses that could account for their chronic health symptoms, including neurologic disorders such as Parkinson's disease, dementia, and stroke [8]. Relying on these case definitions 30 years post-deployment, researchers and clinicians may not be adequately addressing the sensitivity and specificity of the illness as a whole, as some symptoms may have decreased and new symptoms may have emerged over time [1, 10].

Importantly, these two criteria vary greatly in the presumptive rates of illness. The CDC criteria is likely to have increased rates of cases among various cohorts of GW veterans relative to the Kansas criteria (i.e. higher sensitivity but lower specificity) because the Kansas criteria encompasses more bodily systems and excludes those with other neurologic or other chronic medical conditions [7, 8]. For example, the Kansas criteria excludes diabetes and stroke, when studies have shown that these conditions are increasing in GW veterans over time and are related to specific neurotoxicant exposures (PB and sarin exposure) [2, 10]. These exposures may also be related to delayed or latent health symptoms that develop over time and can result in additional toxic wounds [1]. These strict Kansas criteria exclusions may have helped to characterize the GW veteran population initially, but now, 30 years later, may not be as applicable and may be excluding the very veterans who are the sickest and most affected by their deployment. For instance, in our recent study utilizing the population-based Ft. Devens Cohort (FDC) of GW veterans, we found that rates of both the CDC and Kansas case criteria have increased substantially (by 20%), over the course of 20 years in these largely non-treatment seeking veterans [2]. However, rates of Kansas GWI criteria continue to be lower than the CDC's CMI criteria when those with chronic medical conditions are excluded (rates of cases at 66 and 79% respectively) [2].

An additional criticism of these criteria is that some of the symptoms may be sensitive to changes that are associated with normal aging (i.e., joint pain and sleep dysfunction), and thus may not reflect the actual deployment-related illness [11]. In a recent paper using the Department of Veteran Affairs' Millennium Cohort, rates of CMI were compared between GW-era and non-era veterans over five separate time periods [12]. It was found that rates of CMI increased in all veteran groups over time, especially in those deployed to the Gulf region. The increased rates of CMI over time in other potentially non-exposed cohorts may suggest that the symptoms used in this criterion may be susceptible to non-deployment related factors, including normal aging. Further, as the GW veteran population ages and the initial rule-out diagnoses of the Kansas criteria are increasing in prevalence, it is imperative that we assess and potentially revise the discrete symptoms and exclusionary criteria. Reevaluating the diagnostic criteria will lessen the risk of excluding those individuals whose symptoms and medical conditions are related to GW deployment and who also have neurotoxicant-induced accelerated age-related disorders. Additionally, a revised criteria should exclude those who might erroneously meet criteria due to mild symptoms that are expected for their current age-group (i.e., joint paint and sleep dysfunction that increased with age) [10].

A recent re-survey (2014–2016) of the Gulf War Era Cohort and Biorepository (GWECB) was conducted to assess the current rates of GWI using both the CDC and the Kansas criteria, and to evaluate the utility of these criteria now to distinguish health problems reported by GW deployed veterans and GW era veterans [13]. The results of this study replicate what was found in Porter, et al [12], in that 80% of non-deployed era veterans reported symptoms consistent with the CDC GWI criteria, suggesting that this criteria has reduced specificity [13]. For the Kansas criteria, deployment status was associated with increased odds of reporting 27 of the 29 symptoms, suggesting its continued utility [13]. However, roughly 40% of the GWECB cohort had at least one medical or psychiatric condition that is considered an exclusion condition in the Kansas criteria. This is a more than 30% increase from the original Kansas cohort, further emphasizing

the fact that these exclusion criteria need to be re-evaluated as GW veterans continue to age [13].

One possible explanation for variability of case status and corresponding health symptoms across samples reported in the literature is that different exposures may result in different types, rates, and longevity and trajectory of health symptoms [9, 14, 15]. Therefore, it is vitally important to continue to characterize the illness by toxicant exposures, as some toxicant exposures are known to exert latent or delayed health effects, for instance, chronic respiratory problems and cancers including brain tumors [16–19]. Although exposure outcome studies have been conducted with both preclinical and clinical models, most are conducted with exposure mixtures or by case status (GWI versus controls), so it is often difficult to conclude which symptoms result from specific exposures. Additionally, exposure-outcome relationship studies are often flawed because of the small sample sizes, limited exposure data, reliance on self-report or retrospective recall of exposure, cross-sectional analyses, or the use of treatment seeking populations with numerous health complaints [3, 4].

It is especially important to conduct exposure-outcome studies longitudinally in population-based non-treatment seeking cohorts. To date, several longitudinal studies of health symptoms and rates of case criteria have been conducted in GW veterans from multiple countries including the US, UK and Australia [1, 2, 9, 12, 20–22]. However, few studies have analyzed exposure-based outcomes longitudinally [1, 12]. We recently reported on data from the longest-running population-based cohort of GW veterans who returned from the war through Ft. Devens, MA (FDC) where we examined exposures related to health symptom outcomes [1]. We found increased rates of mood and cognitive outcomes in those reporting exposures to tent heaters, PB pills, and chemical warfare agents, and that the self-reported rates of these symptoms increased over time [1]. However, the analyses for this paper examined changes at the group rather than the individual level. Thus, it was not clear if specific symptoms fluctuated over time within individual veterans. For example, while the study showed that many symptoms increased over time, whether individual GW veteran symptom reporting increased, decreased, or fluctuated over time was not examined.

Assessing individual veteran health symptom trajectories is also important when analyzing trends in rates of a chronic disorder such as GWI. For example, there are many ways in which an individual could meet criteria for the illness. In analyses investigating rates of illness longitudinally, it is often unclear if the individuals are arriving at clinical status via the same symptoms, or potentially if some of their symptoms have recovered or if new ones have emerged, thus having the veteran continue to meet GWI criteria but through different combinations of symptoms. For example, there are 27 different ways in which an individual can meet the CDC's CMI criteria [23]. Through group analyses, this may indicate that individuals have consistently met the criteria over time, when in fact, various symptoms may have fluctuated or appeared over time, but the individual's case status remained unchanged (i.e. they still met criteria but by a different combination of symptoms).

Therefore, the most accurate way of assessing changing symptoms over time is to evaluate health symptom reporting longitudinally, in a way that assesses individual fluctuations in reporting, that may be masked in group analyses. To do this, each individual can be assigned into a trajectory group for every health symptom (i.e., no symptom, develops symptom, mixed reporting of the symptom, remission of symptom, and consistent report of symptom). The FDC, a cohort of deployed GW veterans who have been followed since post-deployment in 1991 and at three time points since (1992–1993, 1997–1998, and 2013–2017), is one of the few cohorts which allows for this type of analysis. In this paper we evaluated the same 15 health symptoms as well as clinical case status (GWI) in three separate time periods over 25 years, comparing individual veterans' fluctuation in symptoms, or 'symptom trajectories', over time. In addition, given the exposure data compiled from this cohort over the years (beginning almost immediately post-deployment before some exposures were identified as potentially causative), we analyzed different trajectories of symptoms and CMI clinical case status in association with self-reported neurotoxicant exposures in the GW theatre.

Methods

Participants and surveys

The FDC has been described previously [1, 2]. Briefly, this was a cohort of Active Duty, Reserve, and National Guard Army veterans who were deployed to the GW and returned home through Ft. Devens, MA. The initial baseline survey in 1991 was designed to assess psychological health and combat exposure. Subsequent follow-up questionnaires (1992, 1997–1998, and 2013–2017) assessed long term health, psychological and functional well-being, including Post-traumatic stress disorder as measured by the Mississippi Scale for Combat Related PTSD ([24] as well as self-report of GW specific neurotoxicant exposures. The current study uses a subset of the FDC participants ($N=293$) who completed the Health Symptom Checklist at all three follow-up time points (1992, 1997–1998, and 2013–2017). All participants gave their informed consent for inclusion at each timepoint, before they participated in the surveys. Institutional review

board approvals were obtained from VA Boston Healthcare System and Boston University prior to initiating the surveys. The timeline of the FDC is displayed in Fig. 1.

The health symptom checklist

The minor differences in Health Symptom Checklist (HSC) versions across the three follow-up surveys have been explained in a previous study [1]. In brief, the HSC is a list of health symptoms originally adapted from Bartone, Ursano [25]. Follow-up 1 contained a 20-item HSC, in which veterans were asked to indicate the frequency of symptoms over the past 4 weeks using a Likert-scale rating (none, a little, often, very often) [26], Follow-up 2 contained a 52-item HSC, in which veterans were asked to indicate whether they experienced each health symptom over the past 4 weeks (yes or no) [27, 28], and Follow-up 3 contained a 34-item HSC, in which veterans were asked to endorse whether they experienced a symptom (yes or no) over the past 30 days [1]. A total of 15 symptoms were consistently assessed at all three timepoints. All responses were dichotomized as present or absent. If a veteran indicated not experiencing a symptom, and also endorsed a frequency, the response was recoded as being present.

CMI criteria

Chronic Multisymptom Illness (CMI) by the Center for Disease Control (CDC) criteria were determined as the presence of persistent symptoms over six months in two out of three domains: fatigue, cognitive/mood, and musculoskeletal [7]. Because a limited number of symptoms [15] were consistently assessed at all three timepoints, our measure of the CMI criterion included: 1 symptom in the fatigue domain (overly tired/lack of energy), 4 in the cognitive/mood domain (depressed mood, difficulty concentrating, nervous or tense, trouble sleeping), and 1 in the musculoskeletal domain (joint pain).

Gulf War exposure characterization

The full explanation of how GW exposures were characterized in the FDC has been reported previously [1]. In brief, to minimize the length of time between deployment and recall, exposure data were taken from the Follow-up 2 survey, 6 years after deployment. In this paper we report the associations between health symptom trajectories and 4 exposures, including PB pills, chemical weapon alert, Khamisiyah weapons depot notification, and tent heater exposure.

Participants were asked to indicate how many anti-nerve gas (PB) pills they took in the GW, on a scale of 0, 1–2, 3–10, 11–21, and > 21. Responses were coded into whether they took more than 21 PB pills, dichotomized into yes (> 21) and no (≤ 21) responses, as the blister pack given troops included 21 pills and 21 pills has previously been associated with higher rates of GWI symptoms [29]. Similarly, participants were asked to indicate how many times they were on "formal alert" for a chemical attack (e.g., had to put on full MOPP gear) on a scale of 0, 1–2, 3–10, 11–20, and 20 or more times. Responses were coded into whether they were on "formal alert" for more than 20 times, dichotomized into yes (> 20) and no (≤ 20) responses, as self-reported frequency of chemical alarms has been associated with adverse effects on brain structure, with the strongest effect observed for both 7–30 days and 30+ days of hearing chemical alarms [30].

Veteran's exposure to the Khamisiyah weapons demolition was determined. In March of 1991, the Army Corp of engineers detonated underground munition bunkers which unbeknownst to them housed thousands of Iraqi rockets, with chemical weapons sarin/cyclosarin in the tips of the rockets. When the explosions occurred, it was estimated that over 100,000 GW veterans were exposed to low-level sarin/cyclosarin due to their proximity to the air plumes from the explosions [31, 32]. The Department of Defense (DoD) eventually notified exposed veterans by letter and a registry was established. A list of FDC veterans who received notification letters

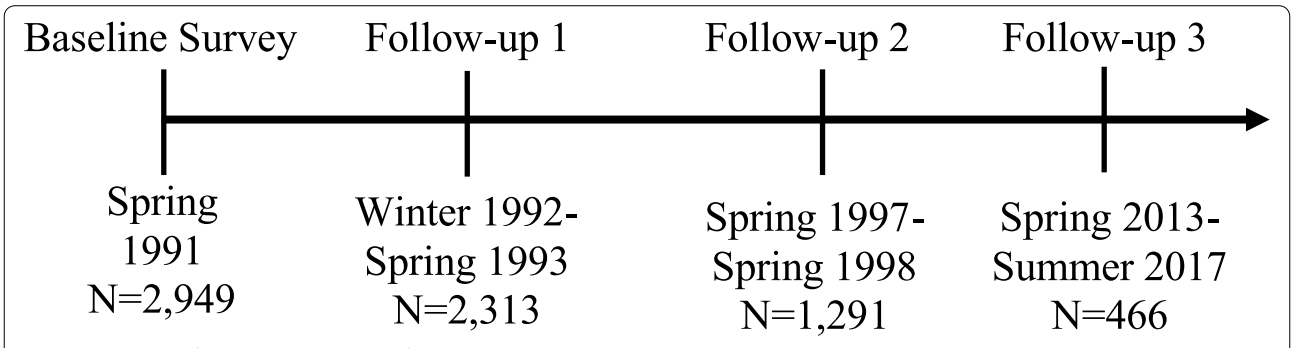

Fig. 1 Ft. Devens cohort reunion survey timeline

of potential exposure to sarin based on their proximity to the Khamisiyah weapons depot detonations and presumed to be exposed to low levels of sarin/cyclosarin based on wind plume modeling in 2000 was obtained from the DoD. These exposures were categorized as a dichotomous variable, yes (received a notification letter from the 2000 DOD exposure model) or no (never received a notification letter) for Khamisiyah exposure status as a proxy for sarin chemical weapons exposure during their deployment [32].

Lastly, participants were also asked whether they had a tent heater or stove in the area where they slept (yes vs. no).

Symptom (Sx) trajectory groups

In order to evaluate health symptom trajectories over time, five a priori symptom trajectory groups were created based on responses at the three time points (No Sx, No Sx then Develops Sx, Mixed, Remitting Sx, Consistent Sx) for each symptom assessed (see Table 1). The No Sx group contains veterans who never endorsed the symptom on all three follow-up surveys. The No Sx then Develops Sx group contains both veterans who initially did not report the symptom on the first follow-up survey, but later endorsed the symptom on Follow-up 2 and Follow-up 3 or veterans who initially did not report the symptom on the first two follow-up surveys but endorsed on Follow-up 3. The Mixed group contains veterans whose symptoms of endorsement fluctuated across the follow-ups (i.e., endorsed on follow-up 1, not endorsed on follow-up 2, and then endorsed again on follow-up 3; and not endorsed on follow-up 1 endorse on follow-up 2 and then not endorsed again on follow-up 3). The Remitting Sx group contains veterans who initially endorsed the symptom on follow-up 1 but they did not endorse the symptom on follow-up 2 and 3, as well as veterans who initially endorsed the symptom on follow-ups 1 and 2 but did not endorse on follow-up 3. Finally, the Consistent Sx group contains veterans who endorsed the symptom on all three follow-up surveys. Participants were classified into trajectory groups for each health symptom and CMI clinical case status.

Table 1 Descriptions of Symptom Trajectory Groups

Symptom Trajectory Group	Follow-up 1	Follow-up 2	Follow-up 3
No Sx	N	N	N
No Sx, Develops Sx	N	N	Y
	N	Y	Y
Mixed	N	Y	N
	Y	N	Y
Remitting Sx	Y	Y	N
	Y	N	N
Consistent Sx	Y	Y	Y

Y = Endorsed Symptom N=Did Not Endorse Symptom

Data analysis

Demographics and characteristics of the full FDC and the current study sample were presented using descriptive statistics. A multinomial logistic regression model was built with each health symptom trajectory grouping as the dependent variable, and one of the GW-specific exposures (i.e., PB pills, self-reported exposure to chemical warfare agents, notification of proximity to Khamisiyah sarin/cyclosarin air plumes, and tent heater exposure) as the independent variable. Covariates included in the model were age, sex, and post-traumatic stress disorder (PTSD) status (score of $\geq$89 on the Mississippi PTSD Scale indicative of PTSD), all derived from the baseline survey conducted in 1991. We chose to include baseline PTSD status as a covariate to control for the cognitive, mood, and somatic symptoms that may arise from trauma, and therefore not neurotoxicant exposures [33, 34] . Additionally, for each individual exposure model, we included all other neurotoxicant exposures as covariates, to control for effects on symptoms from other exposures. Odds ratios (OR) and 95% confidence intervals (CI) were calculated from the logistic regression models. *P* values <0.05 were considered significant. All analyses were performed using SAS 9.4 (SAS, Cary, NC).

Results

Demographics and baseline characteristics

A total of 2949 FDC veterans completed the initial baseline survey in 1991. A total of 295 veterans completed all follow-up surveys. Two veterans did not have complete health symptom data for all three follow-up surveys and were thus excluded from the study sample for a final sample size of 293. The veterans in the study sample were approximately 32 years old at the time of the initial 1991 baseline survey and 54 years old by the last follow-up survey (Table 2). Veterans were predominately male (87.4%) and White (92.8%). Over 16% of the veterans were on active duty at the time of the GW and 5.5% of the study sample exceeded the clinical cutoff of the Mississippi scale for PTSD. The full cohort from the initial 1991 baseline survey did not differ from the study sample with regards to PTSD score or status. The study sample differed from the full FDC, as veterans were older, more likely to be White, and less likely to be male and initially on active duty. Demographics and characteristics for both the full FDC sample and the study sample are summarized in Table 2.

Table 2 Demographics and Characteristics

Demographics/Characteristics	Full Devens Cohort (*N* = 2949)	Study Sample (*N* = 293)
Age at baseline survey, years*	30.2 + 8.3	32.3 + 8.5
Age at follow-up 3, years		54.28 + 8.56
Male, n (%)*	2702 (91.6)	256 (87.4)
White, n (%)*	2443 (82.8)	272 (92.8)
Active Duty at time of Gulf War, n (%)* (versus Reserve, National Guard)	823 (27.9)	48 (16.4)
Mississippi PTSD scale-score	61.9 + 13.4	62.0 + 14.2
Clinical cutoff on Mississippi scale-score, n (%)	116 (3.9)	16 (5.5)
GW-Specific Neurotoxicant Exposures, n (%) Time 4 (*N* = 1291)		
Took more than 21 Pyridostigmine Bromide (PB) Pills	210 (16.3)	50 (17.1)
20 or More Times on "Formal Alert" for a Chemical Attack	263 (20.4)	70 (23.9)
Received the 2000 DoD Notification for Possible Sarin Exposure from the Khamisiyah Weapons Demolition*	1024 (34.7)	121 (41.3)
Tent Heater or Stove in the Area Where you Slept*	788 (61.0)	200 (68.3)

*$p < 0.05$

Individual symptom trajectories

The prevalence of trajectory groups for each symptom are described in Fig. 2 (sample sizes of each group are presented in Table 3). Less commonly reported symptoms (greater than 50% of veterans never endorsed across all three follow-up surveys) were crying easily, hands sweating, rapid heartbeat, dizziness, and shortness of breath. Symptoms that showed an increase in prevalence over time (greater than 20% of veterans did not report initially but reported at later follow-ups) were muscle twitching, depressed mood, trouble sleeping, fatigue, nervousness, and joint pain. The only symptom that waxed and waned over time was nervous or tense (with 20% of veterans in the mixed trajectory group). Remitting symptoms included headaches and nausea (with more than 20% of veterans in these trajectory groups). More commonly reported symptoms (greater than 30% of veterans consistently reported these symptoms across all three follow-up surveys) were fatigue, trouble sleeping, and joint pain.

The prevalence of trajectory groups for CMI clinical case status are described in Fig. 3 (sample sizes of each group are presented in Table 4). Most veterans (45%)

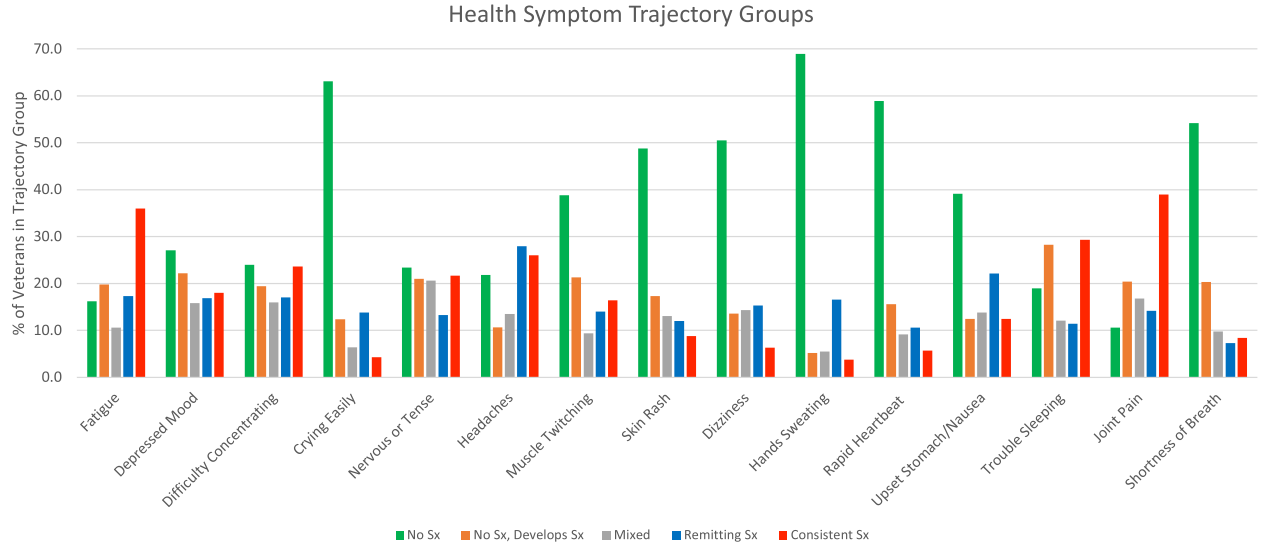

Fig. 2 Percentage of veterans by symptom trajectory group for each health symptom

Table 3 Associations between GW-Specific Neurotoxicant Exposures and Symptom Trajectories Adjusted for other exposures

Outcome	Comparison	Khamisiyah OR [95% CI]	Tent Heater OR [95% CI]	PB Pill (21 or More) OR [95% CI]	Chemical Alert (20 or More) OR [95% CI]
Difficulty Concentrating	No Sx ($n=69$)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx ($n=56$)	1.44 [0.57–3.66]	0.65 [0.26–1.61]	**3.53 [1.08–11.50]**	0.74 [0.26–2.15]
	Mixed ($n=46$)	2.50 [0.95–6.58]	0.79 [0.29–2.14]	3.70 [1.00–13.70]	0.61 [0.19–2.00]
	Remitting Sx ($n=49$)	1.87 [0.76–4.57]	0.73 [0.29–1.85]	1.56 [0.45–5.40]	1.33 [0.50–3.52]
	Consistent Sx ($n=68$)	**3.09 [1.26–57.61]**	0.69 [0.27–1.75]	2.71 [0.81–9.13]	0.95 [0.34–2.63]
Dizziness	No Sx ($n=133$)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx ($n=39$)	0.76 [0.30–1.93]	2.22 [0.83–5.91]	**4.87 [1.78–13.37]**	1.90 [0.70–5.17]
	Mixed ($n=37$)	0.82 [0.32–2.07]	1.34 [0.53–3.42]	0.35 [0.07–1.84]	2.38 [0.86–6.60]
	Remitting Sx ($n=43$)	1.09 [0.46–2.57]	1.31 [0.54–3.16]	2.33 [0.82–6.67]	1.54 [0.57–4.13]
	Consistent Sx ($n=18$)	**6.49 [1.51–27.90]**	1.11 [0.27–4.49]	**8.59 [2.07–35.59]**	0.85 [0.19–3.85]
Fatigue	No Sx (n=46)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx (n=56)	0.78 [0.28–2.16]	1.16 [0.44–3.08]	3.93 [0.98–15.78]	1.31 [0.44–3.94]
	Mixed ($n=30$)	1.42 [0.46–4.39]	1.35 [0.44–4.17]	4.06 [0.85–19.52]	0.67 [0.16–2.70]
	Remitting Sx (n=49)	1.28 [0.48–3.42]	**3.02 [1.05–8.68]**	1.80 [0.39–8.33]	0.69 [0.21–2.31]
	Consistent Sx ($n=101$)	1.30 [0.53–3.19]	1.47 [0.60–3.61]	**3.76 [1.02–13.88]**	1.03 [0.37–2.88]
Crying Easily	No Sx ($n=178$)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx ($n=35$)	1.02 [0.38–2.70]	2.23 [0.76–6.57]	1.73 [0.56–5.36]	1.15 [0.37–3.57]
	Mixed (n=18)	0.85 [0.21–3.43]	3.43 [0.62–19.01]	1.67 [0.32–8.68]	1.29 [0.27–6.13]
	Remitting Sx (n=39)	0.79 [0.29–2.16]	**4.12 [1.18–14.39]**	0.88 [0.26–3.04]	1.90 [0.66–5.47]
	Consistent Sx (n=12)	0.81 [0.15–4.28]	Sample Size Issue	2.66 [0.43–15.52]	2.02 [0.32–12.65]
Nervous or Tense	No Sx ($n=67$)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx ($n=60$)	1.12 [0.47–2.65]	0.99 [0.43–2.29]	**3.80 [1.03–14.05]**	1.21 [0.44–3.38]
	Mixed ($n=59$)	1.42 [0.58–3.45]	1.91 [0.75–4.82]	**3.99 [1.05–14.99]**	1.06 [0.36–3.13]
	Remitting Sx ($n=38$)	0.88 [0.33–2.32]	2.16 [0.80–5.88]	2.87 [0.68–12.03]	1.48 [0.49–4.45]
	Consistent Sx ($n=62$)	1.14 [0.44–2.99]	1.78 [0.65–4.84]	**7.42 [1.89–29.09]**	0.97 [0.30–3.08]
Upset Stomach/Nausea	No Sx ($n=113$)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx ($n=36$)	0.35 [0.12–1.01]	**5.42 [1.45–20.29]**	0.64 [0.15–2.69]	0.57 [0.14–2.26]
	Mixed ($n=40$)	**0.33 [0.11–0.93]**	0.96 [0.37–2.49]	0.60 [0.15–2.44]	1.74 [0.62–4.92]
	Remitting Sx ($n=64$)	**0.37 [0.16–0.82]**	1.31 [0.60–2.90]	1.40 [0.55–3.53]	0.88 [0.36–2.13]
	Consistent Sx (n=36)	0.85 [0.30–2.38]	2.68 [0.79–9.13]	**3.27 [1.03–10.31]**	1.02 [0.32–3.28]
Shortness of Breath	No Sx ($n=155$)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx ($n=58$)	0.76 [0.34–1.72]	**3.46 [1.34–8.95]**	**2.68 [1.07–6.70]**	1.61 [0.66–3.92]
	Mixed ($n=28$)	1.00 [0.29–3.38]	2.02 [0.51–8.02]	2.39 [0.60–9.54]	1.39 [0.36–5.42]
	Remitting Sx ($n=21$)	1.27 [0.43–3.72]	1.74 [0.51–5.94]	2.07 [0.55–7.78]	0.57 [0.14–2.44]
	Consistent Sx ($n=24$)	2.61 [0.74–9.24]	**0.22 [0.05–0.88]**	2.15 [0.43–10.70]	1.51 [0.38–6.02]
Depressed Mood	No Sx ($n=77$)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx ($n=63$)	2.36 [0.99–5.63]	0.93 [0.40–2.19]	**4.46 [1.34–14.80]**	0.91 [0.33–2.47]
	Mixed ($n=45$)	1.51 [0.56–4.09]	0.83 [0.32–2.15]	**3.90 [1.09–13.98]**	1.18 [0.41–3.46]
	Remitting Sx ($n=48$)	**3.19 [1.32–7.73]**	1.23 [0.49–3.08]	3.10 [0.88–10.94]	1.06 [0.39–2.93]
	Consistent Sx ($n=51$)	**2.99 [1.08–8.31]**	1.12 [0.39–3.23]	**7.01 [1.82–26.99]**	1.00 [0.31–3.24]

Table 3 (continued)

Outcome	Comparison	Khamisiyah OR [95% CI]	Tent Heater OR [95% CI]	PB Pill (21 or More) OR [95% CI]	Chemical Alert (20 or More) OR [95% CI]
Headaches	No Sx (n = 63)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx (*n* = 31)	0.34 [0.11–1.04]	0.87 [0.30–2.50]	0.69 [0.15–3.28]	1.15 [0.30–4.48]
	Mixed (n = 39)	1.00 [0.36–2.78]	0.94 [0.32–2.72]	0.65 [0.13–3.22]	**4.10 [1.29–13.04]**
	Remitting Sx (*n* = 81)	**0.34 [0.15–0.78]**	1.54 [0.65–3.62]	2.10 [0.72–6.14]	1.00 [0.35–2.91]
	Consistent Sx (*n* = 75)	0.44 [0.18–1.07]	0.76 [0.31–1.86]	2.82 [0.91–8.75]	2.09 [0.73–6.00]
Muscle Twitching	No Sx (*n* = 111)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx (*n* = 61)	1.03 [0.47–2.24]	1.26 [0.58–2.74]	1.51 [0.57–4.00]	1.26 [0.50–3.17]
	Mixed (*n* = 27)	0.87 [0.30–2.50]	0.80 [0.29–2.23]	1.49 [0.41–5.50]	0.83 [0.23–3.02]
	Remitting Sx (n = 40)	0.94 [0.38–2.27]	2.39 [0.90–6.36]	0.96 [0.30–3.08]	1.39 [0.50–3.92]
	Consistent Sx (*n* = 47)	0.98 [0.39–2.48]	**3.11 [1.07–9.06]**	2.18 [0.74–6.43]	2.23 [0.81–6.15]
	No Sx, (*n* = 138)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx (n = 49)	0.55 [0.22–1.39]	**0.40 [0.17–0.94]**	**4.72 [1.63–13.71]**	**0.17 [0.04–0.67]**
	Mixed (n = 37)	1.60 [0.61–4.23]	1.22 [0.40–3.70]	1.83 [0.58–5.82]	2.31 [0.83–6.45]
	Remitting Sx (*n* = 34)	0.77 [0.30–2.01]	1.23 [0.43–3.51]	1.64 [0.46–5.75]	0.73 [0.23–2.29]
	Consistent Sx (*n* = 25)	1.27 [0.41–3.91]	0.62 [0.19–2.00]	1.78 [0.43–7.37]	2.00 [0.60–6.62]
Hands Sweating	No Sx (*n* = 199)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx (n = 15)	0.53 [0.10–2.68]	2.53 [0.50–12.75]	3.16 [0.73–13.66]	1.43 [0.32–6.37]
	Mixed (*n* = 16)	**4.09 [1.03–16.22]**	2.43 [0.47–12.56]	**4.47 [1.06–18.81]**	1.53 [0.36–6.52]
	Remitting Sx (n = 48)	1.43 [0.60–3.40]	1.51 [0.59–3.86]	2.32 [0.86–6.26]	1.23 [0.47–3.21]
	Consistent Sx (n = 11)	5.22 [0.44–61.56]	2.36 [0.15–38.03]	3.18 [0.29–35.23]	1.65 [0.17–15.81]
Trouble Sleeping	No Sx (*n* = 55)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx (*n* = 82)	0.72 [0.31–1.69]	1.35 [0.57–3.18]	1.43 [0.40–5.12]	1.12 [0.42–3.03]
	Mixed (n = 35)	0.79 [0.27–2.27]	0.82 [0.29–2.32]	3.30 [0.82–13.26]	1.47 [0.46–4.71]
	Remitting Sx (*n* = 33)	1.09 [0.39–3.05]	1.04 [0.37–2.97]	2.57 [0.63–10.42]	0.67 [0.18–2.41]
Rapid Heartbeat	No Sx (*n* = 166)	Ref.	Ref.	Ref.	Ref.
	No Sx, Develops Sx (*n* = 44)	0.89 [0.39–2.05]	1.19 [0.52–2.74]	1.53 (0.57–4.14]	1.33 [0.54–3.27]
	Mixed (*n* = 26)	1.36 [0.46–4.06]	1.04 [0.34–3.15]	1.90 [0.51–7.11]	0.46 [0.10–2.03]
	Remitting Sx (n = 30)	1.19 [0.42–3.38]	2.64 [0.70–9.89]	1.95 [0.57–6.69]	0.64 [0.17–2.37]
	Consistent Sx (n = 16)	1.45 [0.42–5.01]	1.45 [0.38–5.59]	2.27 [0.54–9.51]	1.15 [0.29–4.54]
Joint Pain	No Sx (n = 29)	Ref	Ref	Ref	Ref
	No Sx, Develops Sx (n = 56)	1.16 [0.39–3.46]	1.04 [0.35–3.09]	Sample Size Issue	0.67 [0.20–2.25]
	Mixed (n = 46)	0.99 [0.31–3.15]	0.91 [0.30–2.79]	Sample Size Issue	0.34 [0.09–1.38]
	Remitting Sx (n = 39)	1.81 [0.58–5.68]	1.04 [0.33–3.33]	Sample Size Issue	0.64 [0.17–2.34]
	Consistent Sx (*n* = 104)	1.44 [0.50–4.15]	1.31 [0.45–3.81]	Sample Size Issue	0.84 [0.26–2.67]

All analyses included age, gender, and baseline PTSD status as covariates. ***p* < 0.05**

consistently met criteria for CMI across all three follow-ups, followed by 19.5% of veterans who met criteria for CMI as time went on, 12.9% of veterans had a mixed trajectory of CMI clinical case status, and 11.1% of veterans never met criteria for CMI across all follow-ups.

Associations between individual symptom trajectories and neurotoxicant exposures

PB pill (more than 21) exposure

Veterans who reported taking more than 21 PB pills were more than twice as likely to develop difficulty

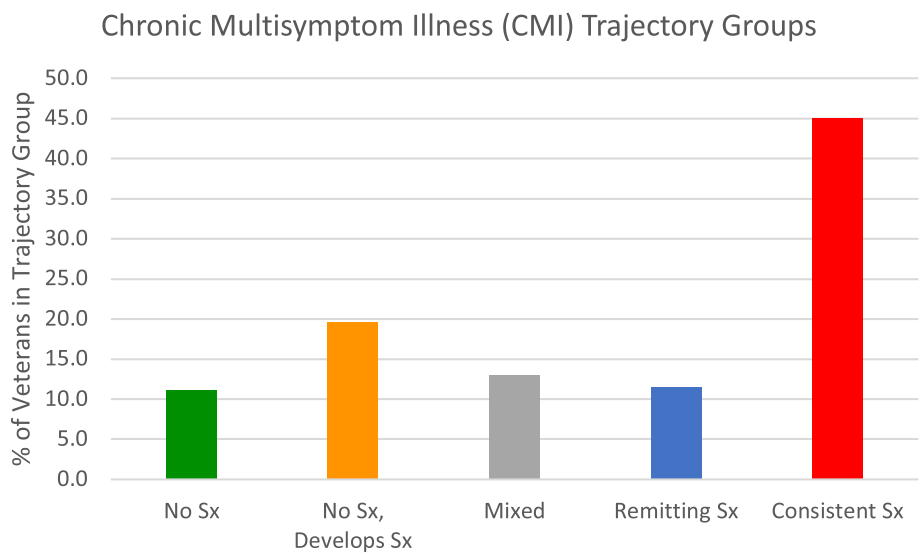

Fig. 3 Percentage of veterans by chronic multisymptom illness (CMI) trajectory group

Table 4 Associations between Exposures and CMI – GWI Trajectories

Exposure	Outcome	Comparison	OR 95% CI
Khamisiyah	CMI - GWI	No Sx (*n* = 32)	Ref
		No Sx, Develops Sx (n = 56)	1.46 [0.47, 4.49]
		Mixed (n = 37)	1.56 [0.47, 5.20]
		Remitting Sx (n = 33)	3.26 [0.97, 10.89]
		Consistent Sx (*n* = 129)	1.58 [0.56, 4.43]
Tent Heater	CMI - GWI	No Sx (n = 32)	Ref
		No Sx, Develops Sx (n = 56)	0.82 [0.28, 2.36]
		Mixed (n = 37)	1.80 [0.54, 6.06]
		Remitting Sx (n = 33)	2.02 [0.57, 7.14]
		Consistent Sx (*n* = 129)	1.10 [0.41, 2.91]
PB Pills (21 or more)	CMI - GWI	No Sx (n = 32)	Ref
		No Sx, Develops Sx (n = 56)	8.65 [0.97, 76.96]
		Mixed (n = 37)	8.51 [0.90, 80.24]
		Remitting Sx (n = 33)	3.44 [0.31, 38.45]
		Consistent Sx (n = 129)	**8.75 [1.03, 74.16]**
Chemical Alert (20 or more)	CMI - GWI	No Sx (n = 32)	Ref
		No Sx, Develops Sx (n = 56)	1.44 [0.38, 5.38]
		Mixed (n = 37)	1.11 [0.26, 4.77]
		Remitting Sx (n = 33)	1.59 [0.38, 6.69]
		Consistent Sx (n = 129)	1.35 [0.39, 4.62]

All analyses included age, gender, and baseline PTSD status as covariates. ***p* < 0.05**

concentrating, dizziness, nervous or tense, shortness of breath, depressed mood, and skin rash over time, as compared to those reporting ≤ 21 PB pill exposure (0–21 pills) (*p*'s < 0.05). This exposure was also associated with fluctuating reporting of nervous or tense, hands sweating, and depressed mood over time (*p*'s < 0.05). No associations were observed between PB pill (more than 21) exposure and symptom trajectories of headaches, muscle twitching, rapid heartbeat and crying easily (*p*'s > 0.05). Veterans who reported taking more than 21 PB pills were more than twice as likely to consistently report these symptoms over three time periods, including the following:

dizziness, fatigue, nervous or tense, trouble sleeping, upset stomach/nausea, and depressed mood as compared to those reporting ≤ 21 PB pill exposure (0–21 pills) (p's < 0.05). These results and odds ratios are reported in Table 3.

Chemical alert (20 or more) exposure

Veterans who reported experiencing 20 or more chemical alerts were more than four times as likely to fluctuate reporting of headaches over time ($p < 0.05$). Additionally, veterans who reported this exposure were *less* likely to develop skin rash over time ($p < 0.05$).

No associations were observed between chemical alert exposure (20 or more times) and symptom trajectories of hands sweating, trouble sleeping, rapid heartbeat, joint pain, difficulty concentrating, dizziness, fatigue, crying easily, nervous or tense, upset stomach/nausea, shortness of breath, depressed mood, and muscle twitching (p's > 0.05). These results and odds ratios are reported in Table 3.

Khamisiyah weapons demolition sarin air plume notification letter received –

Veterans who reported receiving a Khamisiyah notification letter from DoD were more than twice as likely to consistently report difficulty concentrating, dizziness, and depressed mood over the three time periods assessed compared to those who did not report receiving a Khamisiyah notification letter (p's < 0.05). Those reporting this exposure were also more than 3 times as likely to recover from depressed mood over time ($p < 0.05$). Those reporting this exposure were more than 4 times as likely to fluctuate on reporting hands sweating ($p < 0.05$). Those reporting this exposure were significantly less likely to fluctuate in reporting upset stomach/nausea or to recover from this symptom over time (p's < 0.05). Those reporting this exposure were significantly less likely to report headaches initially and then recover over time ($p < 0.05$). Significant associations were not observed between Khamisiyah exposure and symptom trajectories of shortness of breath, muscle twitching, skin rash, trouble sleeping, rapid heartbeat, joint pain, fatigue, crying easily, and nervous or tense (p's > 0.05). These results and odds ratios are reported in Table 3.

Tent heater exposure

Veterans who reported tent heater exposure were more than three times as likely to develop upset stomach/nausea and shortness of breath over time compared to those reporting no exposure to tent heaters (p's < 0.05). Additionally, those reporting this exposure were less likely to develop skin rash over time and less likely to consistently report shortness of breath, compared to those reporting no exposure to tent heaters ($p < 0.05$). Those who reported this exposure were more than 3 times as likely to consistently report muscle twitching over time ($p < 0.05$). Veterans who reported this exposure were also three times as likely to recover from fatigue and crying easily over time as compared to those not reporting tent heater exposure ($p < 0.05$). No associations were observed between tent heater exposure and symptom trajectories of hands sweating, trouble sleeping, rapid heartbeat, joint pain, difficulty concentrating, dizziness, nervous or tense, depressed mood, and headaches (p's > 0.05). These results and odds ratios are reported in Table 3.

Chronic multisymptom illness modified criteria (CMI)

Veterans who reported taking more than 21 PB pills were more than 8 times as likely to consistently meet the criteria for CMI over time compared to those reporting ≤ 21 PB pill exposure (0–21 pills) ($p < 0.05$). No significant associations were observed for chemical alert exposure, Khamisiyah weapons demolition exposure, or tent heater exposure for trajectories of meeting the CMI criteria (p's > 0.05). These results are summarized in Table 4 and Fig. 4.

Discussion

To our knowledge, this study is the first of its kind to evaluate individual health symptom trajectories across three time periods over more than 25 years and associations with GW-specific neurotoxicant exposures. In addition, we do not know of another study where the same individuals were compared in terms of meeting case definition of CMI and exposures across three time-periods.

Our results showed that fatigue and general aches and pain/joint pain were identified as symptoms consistently endorsed by over 30% of this cohort of GW veterans at three time periods over the span of more than 25 years. These two symptoms are currently included in both the CDC and the Kansas criteria, and consist of two of the most prominent symptom domains: Fatigue and Pain [7, 8]. Four symptoms (headache, poor concentration, poor sleep, nervousness) were consistently reported by at least 20% of the cohort who were surveyed at three time points. The symptoms of poor concentration and nervousness are in the Kansas and CMI criteria of cognition and mood. Headache, nervousness, and poor sleep are also CNS symptoms that are associated with GW deployment that should be considered in future case criteria iterations [3]. These findings of consistent health concerns over 25 years in the same individuals also add credence for these symptoms' future utility in biomarker and treatment development studies of GWI [2].

Only two symptoms, headaches (27%) and stomach aches/nausea (21%) were endorsed initially but not

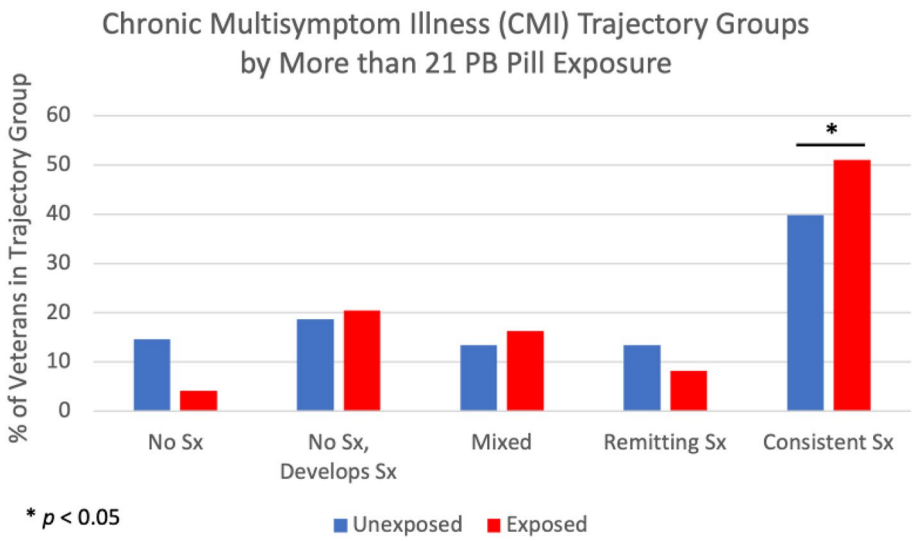

Fig. 4 Percentage of veterans in chronic multisymptom illness (CMI) trajectory group by more than 21 pyridostigmine bromide (PB) pill exposure

endorsed at later timepoints by a quarter and a fifth of the cohort respectively. We did not measure the extent to which targeted treatments were used for these symptoms, but it is possible that these individuals benefitted from such specialty clinic approaches at their local VA or private healthcare facilities.

Several symptoms were identified that were not apparent early on post-deployment but appeared later. These symptoms included fatigue, depression, nervousness, muscle aches, poor sleep, aches and pains, and shortness of breath, which were all endorsed by over 20% of the sample. Unique symptoms that were not reported above, and developed over time included mood, pain and respiratory domains, indicating their continued utility in current case criteria.

Several symptoms were never endorsed by at least 50% of our cohort. These included crying easily, hands sweating, skin rash, shortness of breath, and rapid heartbeat. These symptoms tend to represent autonomic nervous system (ANS) disturbance and although there have been reports of ANS symptoms in GW veterans, it may represent a smaller subgroup and may be consistent with particular toxicant exposure outcomes [35, 36]. Differences in symptom reporting were noted throughout the groupings, which may be related to the exposures that were experienced in theatre.

Several associations between GW-specific neurotoxicant exposures and health symptom trajectories were observed. PB exposure of more than 21 pills was associated with consistent reporting of fatigue and trouble sleeping, cognitive and mood symptoms, as well as nausea/upset stomach, and dizziness. Symptoms that developed over time in this exposed group included dizziness, difficulty concentrating, nervous/tense, shortness of breath, depressed mood, and skin rash. This exposure was not associated with any symptoms remitting over time. This exposure had associations with all symptom domains currently encompassed in the two case definitions for GWI [7, 8], suggesting a widespread effect of CNS-dysfunction affecting all major body systems, which is in line with previous studies that have found associations between this exposure and the illness (CMI) as a whole [7, 28]. They also represent many symptoms of the acetylcholinergic system which PB targets [37–39]. In fact, the current study found that veterans reporting PB exposure of more than 21 pills were more than eight times as likely to consistently meet the criteria for CMI over the span of more than 25 years, which is consistent with prior findings and expands upon the cross-sectional results of Steele, Sastre [40] and Wolfe, Proctor [28]. Over 250,000 were exposed to PB, which is a significant concern in considering a cause for the development of chronic health outcomes in these veterans [6, 38, 39]. These results suggest that an alternative to PB pills should be used in future deployments and that troops who were exposed to more than 21 PB pills should be considered under new legislative category of toxic wounds for service connection.

Self-reported exposure to 20 or more chemical alerts was associated with no consistent reporting of any of the symptoms. There were also no symptoms that developed later. However those who reported exposure to 20 or more chemical alerts were likely to show fluctuating reporting of headaches over time.

Suspected sarin exposure at the Khamisiyah weapons demolition was associated with consistent reporting of difficulty concentrating and dizziness over time, and depressed mood similar to what has been reported in previous studies and further validating the chronic CNS effects of these exposures (1, 42). However, they were also three times as likely to recover from depressed mood over time.

Tent heater exposure was associated with consistent reporting of shortness of breath and also to report the development of nausea/upset stomach and shortness of breath over time,. This latter finding is suggestive of a possible delayed or latent effect of this exposure on the gastrointestinal and respiratory systems. Veterans with this exposure were three times as likely to recover from fatigue and crying easily over time.. These findings are similar to other studies that have reported associations between tent heater exposure and neurological and pulmonary symptoms [27, 28]. However, this is the first study to our knowledge to report an association between tent heaters with gastrointestinal symptoms.

Limitations and strengths

The current analysis was limited to veterans who responded and had complete health symptom data for all three follow-up studies, which may limit the generalizability of our results, not only to the greater FDC, but to the entire GW veteran population. For example, our study sample was more likely to be male and White and less likely to have been active duty during the GW than was found in the total Devens cohort. Confirmatory studies should be conducted in more representative samples.

Second, no objective measures of GW-specific neurotoxicant exposures are available. Therefore, although we used self-report measures of these exposures, we minimized recall bias by using exposure data from Follow-up 2, which was less than 5 years after veterans returned from the GW and before many exposure-outcome reports had been published. Further, the first two follow-up surveys were conducted before the DoD notification letters of suspected sarin exposure at the Khamisiyah weapons demolition were sent. Thus, veteran's reporting of symptoms may be less biased, as they may not have known that they were exposed to sarin and would not attribute their symptoms to that exposure. In addition, exposures were reduced to binomial yes/no variables with yes denoting a specified amount based on prior research. Unfortunately, we did not have the statistical power to break these categories down. Exposure categories were not mutually exclusive. We were also limited to the exposure questions previously asked and therefore, we are not able to look at other acetylcholinesterase inhibitors and the impact on health symptoms over time.

Third, the current study was limited to using 15 health symptoms variables that were reported at all 3 follow-up surveys. It would be of interest to explore not only other health symptoms but also neuropsychological performance and neuroimaging markers longitudinally, to track disease progression, and to potentially identify new or latent effects of exposure. Next, while no data on potential treatment use were collected for this cohort, we acknowledge that treatment use could have affected not only the symptoms that were observed to resolve over time, but other symptom trajectories as well. Lastly, results should be interpreted with caution as analyses were not adjusted for multiple comparisons.

Conclusions

This study highlights the importance of the continued documentation of GW veterans' health status by GW-specific neurotoxicant exposures, and the importance of exposure-outcome relationships in these veterans now 30 years post-deployment. Importantly, several symptoms were identified that have been consistently endorsed for nearly 25 years post-deployment, suggesting chronic effects of the GW-specific neurotoxicant exposures. Therefore, the current recommended case criteria by Fukuda and Steele remain relevant based on this longitudinal analysis and could be further refined and updated with regard to exclusionary criteria and symptom inclusionary criteria based on our reported exposure-outcome relationships. Additionally, while several treatments addressing neuroinflammatory and neuroimmune mechanisms are currently being investigated through clinical trials, targeting symptom alleviation would improve veteran's quality of life until treatments for the illness as a whole become widely available.

Abbreviations

ANS: Autonomic Nervous System; CDC: Center for Disease Control; CI: Confidence Interval; CMI: Chronic Multisymptom Illness; CNS: Central Nervous System; DoD: Department of Defense; FDC: Ft. Devens Cohort; GW: Gulf War; GWI: Gulf War Illness; HSC: Health Symptom Checklist; IOM: Institute of Medicine; MA: Massachusetts; MOPP: Mission Oriented Protective Posture; NC: North Carolina; OR: Odds Ratio; PB: Pyridostigmine Bromide; PTSD: Post Traumatic Stress Disorder; SAS: Statistical Analysis System; Sx: Symptom; US: United States; UK: United Kingdom; VA: Veterans Affairs.

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Disclaimer

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Authors' contributions
MHK – Conceptualization, Methodology, Investigation, Writing – Original Draft, Writing-Review and Editing, Visualization, Supervision, Project Administration; CGZ - Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing-Original Draft, Writing-Review and Editing, Visualization, Project Administration; TH – Data Curation, Formal Analysis, Writing-Review and Editing; MY – Writing-Review and Editing, AS – Writing-Review and Editing, SPP – Writing-Review and Editing, CMG – Data Curation, Writing-Review and Editing, Visualization, Project Administration; KAS – Conceptualization, Methodology, Writing – Original Draft, Writing-Review and Editing. The author(s) read and approved the final manuscript.

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Availability of data and materials
Analyses were performed using raw data that are only available within the US Department of Veterans Affairs firewall in a secure research environment. VA privacy and data security policies and regulatory constraints provide that only aggregate summary data may be removed from the VA for publication. The authors have provided detailed results of these analyses in the paper. These restrictions are in place in order to maintain patient privacy and confidentiality. Access to these data can be granted to persons who are not an employee of the VA; however, there is an official protocol that must be followed for doing so. The authors invite those wishing to access the raw data that were used for this analysis to contact Maxine Krengel (Maxine.Krengel@va.gov) to discuss the details of the VA data access approval process.

Declarations

Ethics approval and consent to participate
All participants gave their informed consent for inclusion at each timepoint, before they participated in the surveys. Institutional review board approvals were obtained from VA Boston Healthcare System and Boston University prior to initiating the surveys.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Research Service, VA Boston Healthcare System, 150 South Huntington Ave. 8A-90, Jamaica Plain, Boston, MA 02130, USA. [2]Behavioral Neuroscience Program, Boston University School of Medicine, Boston, MA, USA. [3]Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA. [4]Department of Environmental Health, Boston University School of Public Health, Boston, MA, USA. [5]Massachusetts Veterans Epidemiology Research and Information Center, VA Boston Healthcare System, Boston, MA, USA. [6]Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA. [7]Department of Psychiatry, Boston University School of Medicine, Boston, MA, USA. [8]Military Performance Division, US Army Research Institute of Environmental Medicine, Natick, MA, USA.

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References

1. Yee MK, Zundel CG, Maule AL, Heeren T, Proctor SP, Sullivan K, 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.
2. Zundel CG, Heeren T, Grasso CM, Spiro A III, Proctor SP, Sullivan K, et al. Changes in health status in the Ft. Devens gulf war veterans cohort: 1997-2017. Neuroscience Insights. 2020;15:1–7.
3. 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.
4. RACGWI. Gulf war illness and the health of gulf war veterans: research recommendations. Washington, DC: US Government Printing Office; 2008.
5. DoD. U.S. Department of Defense Office of the Special Assistant to the Undersecretary of Defense (Personnel and Readiness) for Gulf War Illnesses Medical Readiness and Military Deployments. Environmental Exposure Report: Pesticides Final Report, Washington, DC. 2003.
6. Golomb BA, Anthony CR. A review of the scientific literature as it pertains to gulf war illnesses: Pyridostigmine bromide. Santa Monica, CA: RAND Corporation; 1999.
7. Fukuda K, Nisembaum 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.
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. 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.
10. 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).
11. Dursa EK, Barth SK, Porter BW, Schneiderman AI. Health status of female and male gulf war and gulf era veterans: a population-based study. Womens Health Issues. 2019;29(Suppl 1):S39–46.
12. Porter B, Long K, Rull RP, Dursa EK. Millennium cohort study T. prevalence of chronic multisymptom illness/gulf war illness over time among millennium cohort participants, 2001 to 2016. J Occup Environ Med. 2020;62(1):4–10.
13. Gifford EJ, Vahey J, Hauser ER, Sims K, Efird JT, Dursa EK, et al. Gulf war illness in the Gulf war era cohort and biorepository: the Kansas and centers for disease control definitions. Life Sci. 2021;119454.
14. 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.
15. 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).
16. Logroscino G. The role of early life environmental risk factors in Parkinson disease: what is the evidence? Environ Health Perspect. 2005;113(9):1234–8.
17. Giordano G, Costa LG. Developmental neurotoxicity: some old and new issues. ISRN Toxicol. 2012;2012:814795.
18. Langston JW. Predicting Parkinson's disease. NEurology. 1990;40:70–4.
19. McGeer PL, Schwab C, Parent A, Doudet D. Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration. Ann Neurol. 2003;54(5):599–604.
20. Gwini SM, Kelsall HL, Ikin JF, Sim MR, McFarlane AC, Forbes AB. New onset of chronic diseases and changes in lifestyle risk factors among gulf war veterans: a longitudinal comparison of high and low symptom reporters. J Occup Environ Med. 2016;58(8):770–7.
21. Gwini SM, Kelsall HL, Sim MR, Ikin JF, McFarlane AC, Forbes AB. Stability of symptom patterns in Australian gulf war veterans: 10-year longitudinal study. Occup Environ Med. 2016;73(3):195–8.
22. Li B, Mahan CM, Kang HK, Eisen SA, Engel CC. Longitudinal health study of US 1991 gulf war veterans: changes in health status at 10-year follow-up. Am J Epidemiol. 2011;174(7):761–8.
23. 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.
24. Keane TM, Caddell JM, Taylor KL. Mississippi scale for combat-related posttraumatic stress disorder: three studies in reliability and validity. J Consult Clin Psychol. 1988;56(1):85–90.
25. Bartone PT, Ursano RJ, Wright KM, Ingraham LH. The impact of military air disaster on the health of assistance workers. A prosepctive study. J Nerv Ment Dis. 1989;177(6):317–28.

26. Wolfe J, Proctor SP, Davis JD, Borgos MS, Friedman MJ. Healh symptoms reported by Persian gulf war veterans two years after return. Am J Ind Med. 1998;33(2):104–13.
27. Proctor SP, Heeren T, White RF, Wolfe J, Borgos MS, Davis JD, et al. Health status of Persian gulf war veterans: self-reported symptoms, environmn-etal exposures and the effect of stress. Int J Epidemiol. 1998;6:1000–10.
28. 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.
29. Keeler JR, Hurst CG, Dunn MA. Pyridostigmine used as a nerve agent pretreatment under wartime conditions. JAMA. 1991;266(5):693–5.
30. 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.
31. DoD. Directorate for Deployment Health Support of the Special Assistant to the Under Secretary of Defense (Personnel and Readiness) for Gulf War Illness Medical Readiness and Military Deployment. Potential Exposure to sarin form the demolitions at Khamisiyah, Iraq on March 10, 1991 Available at http://www.gulflink.osd.mil. 1997.
32. DoD. Directorate for Deployment Health Support for the Special Assistant to the Under Secretary of Defense (Personnel and Readiness) for Gulf War Illness Medical Readiness and Military Deployments. US demolition operations at the Khamisiyah ammunition point (case narrative). Available at http://www.gulflink.osd.mil/khamisiyahiii. 2002.
33. Graham K, Dipnall J, Van Hooff M, Lawrence-Wood E, Searle A, Ao AM. Identifying clusters of health symptoms in deployed military personnel and their relationship with probable PTSD. J Psychosom Res. 2019;127:109838.
34. Asnaani A, Reddy MK, Shea MT. The impact of PTSD symptoms on physical and mental health functioning in returning veterans. J Anxiety Disord. 2014;28(3):310–7.
35. Fox A, Helmer D, Tseng CL, Patrick-DeLuca L, Osinubi O. Report of autonomic symptoms in a clinical sample of veterans with gulf war illness. Mil Med. 2018;183(3–4):e179–e85.
36. Haley RW, Charuvastra E, Shell WE, Buhner DM, Marshall WW, Biggs MM, et al. Cholinergic autonomic dysfunction in veterans with gulf war illness: confirmation in a population-based sample. JAMA Neurol. 2013;70(2):191–200.
37. Golomb BA. Acetylcholinesterase inhibitors and gulf war illnesses. Proc Natl Acad Sci U S A. 2008;105(11):4295–300.
38. 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.
39. 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.
40. Steele L, Sastre A, Gerkovich MM, Cook MR. Complex factors in the etiology of gulf war illness: wartime exposures and risk factors in veteran subgroups. Environ Health Perspect. 2012;120(1):112–8.

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RESEARCH ARTICLE

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The impact of neurotoxicant exposures on posttraumatic stress disorder trajectories: The Ft. Devens Gulf War Veterans Cohort

Clara G. Zundel[1,2] | **Kathryn Price**[1,3] | **Claudia M. Grasso**[1] | **Avron Spiro III**[5,6,7] | **Timothy Heeren**[8] | **Kimberly Sullivan**[9] | **Maxine H. Krengel**[1,4]

[1] Research Service, VA Boston Healthcare System, Boston, Massachusetts, USA

[2] Behavioral Neuroscience Program, Boston University School of Medicine, Boston, Massachusetts, USA

[3] Department of Psychology, University of Massachusetts–Boston, Boston, Massachusetts, USA

[4] Department of Neurology, Boston University School of Medicine, Boston, Massachusetts, USA

[5] Massachusetts Veterans Epidemiology Research and Information Center, VA Boston Healthcare System, Boston, Massachusetts, USA

[6] Department of Epidemiology, Boston University School of Public Health, Boston, Massachusetts, USA

[7] Department of Psychiatry, Boston University School of Medicine, Boston, Massachusetts, USA

[8] Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA

[9] Department of Environmental Health, Boston University School of Public Health, Boston, Massachusetts, USA

Correspondence
Clara G. Zundel, VA Boston Healthcare System, 150 South Huntington Ave. (8A–90), Jamaica Plain, MA 02130.
Email: cgzundel@bu.edu

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Abstract

Gulf War veterans (GWVs) were exposed to neurotoxicants, including sarin nerve gas, anti–nerve agent pills, pesticides, oil well fires, and fumes from unvented tent heaters, all of which have been associated with subsequent adverse health. Posttraumatic stress disorder (PTSD) symptoms have also been associated with GW deployment; however, associations between exposures and PTSD symptoms have not been investigated. We assessed PTSD symptom trajectories and associations with neurotoxicant exposures in Ft. Devens Cohort (FDC) veterans (N = 259) who endorsed trauma exposure during deployment and completed the PTSD Checklist at three follow-ups (1992–1993, 1997–1998, 2013–2017). Results indicate that among veterans with more severe initial PTSD symptoms, symptoms remained significantly higher across follow-ups, Bs = −1.489–1.028, whereas among those with low initial PTSD symptoms, symptom severity increased significantly over time, Bs = 1.043–10.304. Additionally, neurotoxicant exposure was associated with a significant increase in PTSD symptoms, Bs = −1.870–9.003. Significant interactions between time and exposures were observed for PTSD symptom clusters, suggesting that among participants with high initial PTSD symptom, unexposed veterans experienced symptom alleviation, whereas

exposed veterans' PTSD symptoms remained high. In GWVs with low initial PTSD symptoms, both unexposed and exposed veterans experienced PTSD symptom exacerbations over time; however, this occurred at a faster rate among exposed veterans. These findings suggest that in the years following deployment, GWVs who were exposed to both traumatic events and neurotoxicants may experience more severe and chronic PTSD symptoms than those without neurotoxicant exposures.

Compared with nonmilitary populations, veterans report more traumatic experiences, which occur during both military training and deployment. As a result, some veterans develop PTSD (i.e., ~12% of Gulf War veterans; Kang et al., 2003). Veterans who suffer from PTSD report poorer health, lower health-related quality of life, and higher levels of disability compared to those without PTSD (Fang et al., 2015). There has been extensive research within veteran populations on predispositions for PTSD, including biological and psychosocial factors as well as specific characteristics of traumatic events, which has highlighted the heterogeneity of this disorder (see Able and Benedek, 2019, for a review). However, less attention has been given to evaluating change in individual PTSD symptoms and symptom clusters over time and examining which factors may influence chronicity.

Most research on PTSD symptom trajectories in veteran populations has focused on short-term follow-ups (i.e., 1–10 years). Although the results of these studies have been mixed, most have identified a subgroup of veterans who report a worsening or chronic trajectory of PTSD or experience an exacerbation of symptoms over time; however, in these studies, the number of veterans who experience this trajectory has been low (i.e., ~6%–10%; Mota et al., 2019; Vasterling et al., 2016). Few studies have surveyed PTSD symptoms for more than 10 years of follow-up. The findings from these studies suggest that the longer the follow-up period, the more likely an increase in PTSD symptomology is observed (Karstoft, 2013), with one study reporting that 20% of veteran participants exhibited a chronic trajectory of PTSD for over 20 years (Marmar et al., 2015).

Overall, most findings indicate that within short-term follow-up periods conducted in early adulthood, the rates of chronic PTSD symptom trajectories appear to be low. However, in studies with longer follow-up periods that extend into later life, these rates appear much higher, potentially due to the changes associated with aging, such as worsening cognitive function and additional stress (e.g., retirement, bereavement), which have been identified as possible predictors of late-life exacerbated, reactivated, or new-onset clinical PTSD symptomology (Davison et al., 2016; Mota et al., 2016).

Most longitudinal studies assessing PTSD symptomology have been conducted with veterans who served either during the Vietnam War era or in support of the recent conflicts in Iraq and Afghanistan, whereas few studies have assessed PTSD trajectories in 1991 Gulf War (GW) veterans. Overall, the rates of PTSD within GW veterans as assessed immediately following their return home have been shown to be relatively low, ranging from 3.3% to 7.2% (Research Advisory Committee on Gulf War Veterans' Illnesses [RAC-GWVI], 2008). One study of GW National Guard reservists from medical and police units found that PTSD symptoms increased significantly over the span of 2 years, with hyperarousal symptoms reported as the most severe among all measured symptoms at all assessment points (Southwick, 1995). Similarly, in cohorts of GW veterans from Louisiana, PTSD symptoms related to emotional numbing and hyperarousal were shown to significantly increase over the course of 1 year, whereas symptoms of reexperiencing and avoidance showed no change (Benotsch et al., 2000; Thompson et al., 2004). The longest assessment of PTSD symptoms to date within GW veterans (Orcutt, 2004) was conducted over a period of 6 years within the Ft. Devens Cohort (FDC), the longest-running cohort of GW veterans. The author found that PTSD symptoms following the GW were best categorized into two groups: one characterized by low levels of symptoms with little increase over time and one by higher levels of initial symptoms that increased significantly over time. These findings suggest that PTSD symptoms within GW veterans may not be homogenous and may, in fact, be related to other deployment-related factors, such as environmental hazards.

GW veterans are a unique cohort in that their deployment contained multiple potential exposures to neurotoxicants, including sarin nerve gas, pyridostigmine bromide pills (PB), pesticides, and combustion byproducts from oil well fires and unvented tent heaters (White et al., 2016). These exposures have since been associated with chronic

health symptoms that encompass multiple body systems including fatigue; pain; neurological, cognitive, and mood issues; and gastrointestinal, respiratory, and skin problems (Steele, 2000). Although neurotoxicant exposures may be the most notable feature of the 1991 Gulf War, veterans may have been exposed to other harmful experiences, such as mild traumatic brain injury (mTBI) and psychologically traumatic events. Recently, researchers have investigated a "multiple-hit" hypothesis, which posits that exposure to both mTBI and neurotoxicants is associated with increased rates of Gulf War Illness (GWI) and chronic medical conditions, when compared with either exposure alone or no exposure at all (Janulewicz et al., 2018). We similarly hypothesized that exposure to both traumatic events and neurotoxicants during deployment would be associated with increased rates of PTSD symptoms and/or increased symptom severity in a sample of GW veterans. To our knowledge, the present study was the first to investigate trajectories of PTSD symptoms over a 20-year follow-up period in GW veterans. The current study was a longitudinal analysis using the FDC's recent resurvey, conducted more than 20 years postwar (i.e., 2013–2017), and considered the potential impact of GW-specific neurotoxicant exposures on PTSD symptoms and trajectories.

METHOD

Participants and procedure

The FDC of GW veterans is the longest-running cohort of GW veterans and has been described in prior papers (Yee et al., 2020; Zundel et al., 2019). In summary, during the spring of 1991, active duty, reserve, and National Guard U.S. Army personnel who had been deployed to the GW and returned home through Ft. Devens in Massachusetts, were recruited to participate in a survey to assess psychological health and combat exposure. Subsequent questionnaires were mailed to participants in 1992 (Follow-Up 1), 1997–1998 (Follow-Up 2), and 2013–2017 (Follow-Up 3) to assess long-term health, psychological and functional well-being, and GW-specific environmental and combat exposures.

This study utilized a subset of individuals in the FDC who completed the baseline survey and all three follow-up surveys and had complete data for the PTSD Checklist (PCL; Weathers, 1991) at each assessment point. Relevant covariates from the baseline survey included age and gender. Of the 295 veterans who completed all three follow-up surveys, we excluded 19 who reported no traumatic event exposure and 17 who did not have sufficient data (i.e., missing most data points) for the PCL at all three follow-up surveys in which they participated; this resulted in an analytic sample of 259 GW veterans. Of these 259 veterans, 19 had one or two missing data points for the PCL on the follow-up surveys. Missing data points were replaced with the average of the other symptoms within the specific PTSD symptom cluster using a single-imputation method.

Because the first study to investigate PTSD symptom trajectories within the FDC found that the sample was best categorized into two groups (i.e., veterans with low vs. high initial symptoms; Orcutt, 2004), we stratified our analyses to assess whether the effect of neurotoxicant exposures was different for individuals with high levels of initial PTSD symptoms versus those with low levels of initial PTSD symptoms. These low and high initial PTSD symptom groups were determined by their PCL score at Follow-Up 1 such that participants with a score above 35 were considered to have high initial PTSD symptoms (n = 46), and those with a score of 35 or below were considered to have low initial PTSD symptoms (n = 213). These thresholds were implemented following the National Center for PTSD guidelines for *DSM-IV* PCL cutoffs for administration in a general, non–treatment-seeking population (National Center for PTSD, n.d.).

All demographic variables used as covariates in the analyses were taken from the baseline survey, which occurred in 1991. Before administering each survey, participants gave their informed consent for inclusion on each occasion. Prior to each survey distribution, approval was obtained from the Institutional Review Board at Veteran Affairs Boston Healthcare System and, when required, by the Human Research Protection Office at U.S. Army Medical Research & Development Command (USAMRDC).

Measures

PTSD symptoms

Because the FDC began in 1991, PTSD symptoms were defined using the prevailing definition from the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV*; American Psychiatric Association [APA], 1994). To evaluate PTSD symptoms, we used the PCL (Weathers et al., 1991), a 17-item self-report questionnaire reflecting the PTSD criteria outlined in the *DSM-IV*. Participants were asked to rate how much they had been bothered by each symptom during the past month, with responses ranging from 1 (*not at all*) to 5 (*extremely*). Symptom ratings were summed for a total PTSD symptom severity score (range: 17–85), with a score of 36 or higher indicative of probable PTSD (National Center for PTSD, n.d.).

Although the *DSM-IV* contains three symptom clusters, with avoidance and numbing symptoms grouped together, there is growing evidence that these aspects of PTSD may

represent two distinct symptom groups, wherein avoidance is a more strategic and effortful mechanism, and numbing is a more automatic response to hyperarousal (APA, 1994; Asmundson et al., 2000; Foa et al., 1992). Thus, several factor analytic studies have been conducted on the PTSD criteria in the *DSM-IV* and concluded that an individual's symptoms load on to four separate factors: reexperiencing, avoidance, numbing, and hyperarousal (Asmundson et al., 2000; King et al., 1998); the fifth edition of the *DSM* (*DSM-5*) has since incorporated this change (APA, 2013). Therefore, we chose to investigate a four-factor model of PTSD. For the present study, we scored the PCL both for total PTSD symptom severity and for the four factor-based subscales: Reexperiencing (Items 1–5), Avoidance (Items 6–7), Numbing (Items 8–12), and Hyperarousal (Items 13–17). The symptom cluster scores were derived by adding up the individual ratings, ranging from 1 to 5, for each symptom cluster.

GW environmental exposures

FDC veterans were asked about their environmental exposures specific to GW deployment at Follow-Up 2 and Follow-Up 3. To reduce potential recall bias, exposure data were taken from the Follow-Up 2 survey, which was administered 6 years postdeployment. The neurotoxicant exposures of interest were pesticide smell, oil smell, diesel smell, tent heater combustion byproducts, PB pills, and chemical warfare (i.e., number of times on formal alert), based on prior research with the FDC (Proctor et al., 1998; Proctor et al., 2006; White et al., 2016; Wolfe et al., 1998; Wolfe et al., 2002; Yee et al., 2020; Zundel et al., 2019). Participants were asked whether they had experienced a pesticide smell, oil smell, or diesel smell as well as if they had a heater or stove in the area in which they slept; each was coded as a "yes" or "no" response.

Participants were asked to indicate how many anti–nerve gas (i.e., PB) pills they took in the GW, with response options of 0, 1–2, 3–10, 11–21, and more than 21. Responses were coded into whether they took any PB pills ("yes") or never took PB pills ("no"), as previous FDC studies have found significant health effects using this grouping (Yee et al., 2020; Zundel et al., 2019). Additionally, as a separate variable, responses were coded into whether participants took more than 21 PB pills, dichotomized into "yes" (fewer than 21) and "no" (21 or more) responses, as the blister pack given to troops included 21 pills, which was the equivalent of taking the recommended doses for more than 7 days (Keeler et al., 1991; RAC-GWVI, 2008). Additionally, several studies have reported associations between taking more than 21 PB pills and a significantly increased risk for GWI, whereas taking fewer than 21 has been associated with only a modestly increased risk (RAC-GWVI, 2008). Similarly, participants were asked to indicate how many times they were on "formal alert" for a chemical attack (e.g., had to put on full Mission Oriented Protective Posture [MOPP] gear), with response options of 0, 1–2, 3–10, 11–20, and 20 or more times. Responses were coded into whether they were ever on formal alert (i.e., more than 0 times; "yes") or never on "formal alert" (i.e., one or more times; "no"). As a separate variable, if they were on formal alert more than 20 times, which was dichotomized into "yes" for more than 20 times "no" for fewer than 20 times, as the self-reported frequency of chemical alarms has been associated with adverse effects on brain structure, with the strongest effects observed for both 7–30 days and 30 days or more of hearing chemical alarms (Chao et al., 2016).

Data analysis

Descriptive statistics were calculated to compare the demographic, military, and exposure characteristics of the full FDC, after removing the current sample, with the current sample as well as participants with high versus low initial PTSD symptom levels (see Table 1). Repeated linear regressions using the generalized estimation equations (GEE) approach, stratified by low and high initial PTSD symptom status, were used to analyze the PTSD symptom outcomes (i.e., total score, four symptom cluster scores) over time. GEE, introduced by Zeger (1986), is used to analyze repeated-measures data while accounting for the within-subject correlation inherent in these data (Ballinger, 2004). Each model contained time as a categorical variable (Follow-Ups 1, 2, or 3), one of the exposure measures as a factor, and baseline age and gender as covariates. Interaction terms for Follow-Up 2 for each exposure and Follow-Up 3 for each exposure were initially included in each model; nonsignificant terms were removed from final models. In reporting the results, beta values were used to indicate the unstandardized slopes from the regression models. This study utilized a subset of the FDC composed of individuals who completed the baseline survey and all three follow-up surveys and had complete data for the PCL at each survey; thus, there were no missing data. We considered p values less than .05 to be significant. False discovery rate correction was applied for multiple comparisons to the main effect models of the four cluster scores as well as the total score outcomes. Because of sample size issues, we did not perform adjustments for interactions terms; instead, unadjusted p values are presented, as these results are more exploratory. All analyses were performed using SPSS (Version 25).

TABLE 1 Demographic and baselinea characteristics of the full Ft. Devens Cohort (FDC) and the study sample

Characteristic	FDC with study sample removed ($N = 2,690$)		Study sample ($N = 259$)			High initial PTSD symptoms ($n = 46$)		Low initial PTSD symptoms $n = 213$)		
	M	*SD*	*M*	*SD*	*p*[b]	*M*	*SD*	*M*	*SD*	*p*[b]
Age (years)	29.94	8.37	32.43	8.60	< .001	32.09	9.03	32.50	8.53	.770
	n	%	*n*	%		*n*	%	*n*	%	
Male	2467	91.7	226	87.3	.016	36	78.3	190	89.2	.045
Caucasian	2202	81.9	241	93.1	< .001	42	91.3	199	93.4	.613
Activity duty[c]	781	29.0	42	16.2	< .001	5	10.9	37	17.4	.279
Trauma type[d]										
Combat/mopping up	697	25.9	95	36.7	< .001	21	45.7	74	34.7	.161
Exposure to noncombat life-threatening event	121	4.5	19	7.3	.043	3	6.5	16	7.5	.814
Personal domestic violence	647	24.1	49	18.9	.043	7	15.2	42	19.7	.481
Anticipation of life threat	236	8.8	30	11.6	.060	8	17.4	22	10.3	.173
Attributes of war zone environment	219	8.1	19	7.3	.651	0	0	19	8.9	.036
Intraunit hassles/personal performance	455	16.9	47	18.1	.624	7	15.2	40	18.8	.567
No event	307	11.4	0	0	< .001	0	0	0	0	1.000

Note: PTSD = posttraumatic stress disorder.

[a]As assessed in 1991. [b]For continuous variables an independent samples t-test was used and for nominal variables, a chi-squared test was used to determine statistical significance. [c]Versus reserve or National Guard. [d]Descriptions of each traumatic event category can be found in Wolfe et al. (1993).

RESULTS

Demographic, baseline, and exposure characteristics

Demographic comparisons between the full FDC and the study sample, as well as between participants with high versus low initial PTSD symptom levels, are summarized in Table 1. The study sample differed from the full FDC by age, gender, race, active duty status during the GW, and trauma type. Most participants ($n = 259$) were male (87.3%) and White (93.1%), with 16.2% reporting active duty status at the time of the GW. The mean participant age in 1991 (i.e., baseline survey administration) was 32.43 years. The most commonly reported traumatic event was combat or "mopping up", which entailed suiting up in full MOPP gear in the advent of a chemical attach (~36.7%), followed by personal/domestic (~18.9%). At Follow-Up 2 (i.e., 1997–1998), 68.3% of veterans reported exposures to a diesel smell as well as unvented tent heaters, 70.3% reported exposure to an oil smell, and 33.4% reported exposure to an insecticide or pesticide smell. Moreover, 18.5% of veterans reported taking more than 21 PB pills and 25.1% reported hearing 20 or more chemical alarms. Exposure characteristics are also summarized in Supplementary Table S1.

Change in PTSD symptoms over time

Among participants with high initial PTSD symptom levels (17.8% of the sample), there were no significant changes to the four PTSD symptom cluster scores or the total symptom score across the study period, *p*s = .238–.816, indicating that there was no significant increase in or the resolution of symptoms over time. In veterans with low initial PTSD symptom levels (82% of the sample), both PTSD symptom cluster scores and total scores significantly increased over time, *p*s < .001 except for scores on the Avoidance subscale, which did not increase from Follow-Up 1 to Follow-Up 2, $p = .240$, but did significantly increase from Follow-Up 1 to Follow-Up 3, $p < .001$. Correlations between cluster scores and total scores across follow-ups, stratified initial PTSD symptom level status, are reported in Supplementary Tables S2 and S3.

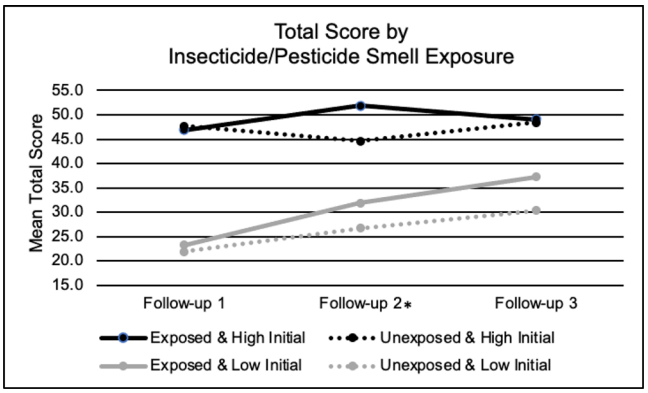

FIGURE 1 Significant Time x Pesticide Smell Exposure interactions for the total score. Note. Significant *p* values represent the difference between groups (exposed vs. unexposed) at that follow-up point. This figure is exemplary of all other significant interactions. $^{*}p < .05$

Longitudinal associations between GW neurotoxicant exposures and PTSD symptom scores

In veterans with high initial PTSD symptom levels, no significant main effects of exposure were observed for diesel smell, oil smell, unvented tent heaters, insecticide or pesticide smell, exposure to more than 20 chemical attack alerts, for any PTSD symptom cluster score or total score, *p*s = .460–.896. A significant main effect of exposure to more than 21 PB pills was observed for the total PTSD symptom score as well as the subscale scores for Reexperiencing, Avoidance, and Hyperarousal. Among veterans who reported exposure to more than 21 PB pills, there was at least one additional symptom reported in each cluster or an increase in severity of one symptom from each cluster, *p*s = .030–.044. No significant main effect of exposure to more than 21 PB pills was observed for the Numbing subscale score, $p = .667$. Additionally, a significant main effect of any PB pill exposure was observed for the Numbing subscale score such that exposed participants reported more than two additional symptoms or increased symptom severity, $B = 2.530$, 95% CI [0.740, 4.320], $p = .030$. No significant main effects of exposure to any PB pills were observed for the Reexperiencing, Avoidance, or Hyperarousal subscales or for the total score, *p*s = .130–474.

Significant interactions between time and insecticide or pesticide smell exposure in veterans with high initial PTSD symptom levels were observed for the reexperiencing cluster, $B = 3.767$, 95% CI [0.670-6.913], $p = .019$, and total score, $B = 7.998$, 95% CI [0.094-15.902], $p = .047$. The total score interactions are displayed in Figure 1. A significant interaction was observed between time and exposure to more than 21 PB pills for the numbing score, $B = 4.105$, 95% CI [1.067, 7.143], $p = .008$. Although a very small number of veterans who had high initial PTSD symptom levels reported no exposure to chemical attack alarms ($n = 4$), those who were exposed appeared to report increases in PTSD symptoms (i.e., all clusters and total score) over time, whereas unexposed participants recovered or appeared to have a decrease in symptoms over time. These data are not reported due to low statistical power. The effects of exposure in participants with high initial PTSD symptom levels are summarized in Table 2.

Among participants with low initial PTSD symptom levels, no significant main effects of exposure were observed for diesel smell, tent heaters, insecticide or pesticide smell, any PB pill ingestion, more than 20 chemical attack alerts, any chemical attack alerts, for all PTSD symptom cluster scores, or PTSD total score, *p*s = .060–.917. Oil smell exposure was associated with a significant score increase on the Avoidance subscale, $B = 0.425$, 95% CI [0.079, 0.772], $p = .030$; Hyperarousal subscale, $B = 0.711$, 95% CI [0.228, 1.194, $p = .020$; and total PTSD symptom score, $B = 1.652$, 95% CI [0.321, 2.984], $p = .030$. No significant associations were observed between oil smell exposure and scores on the reexperiencing or numbing subscales, *p*s = .110–300. Exposure to more than 21 PB pills was associated with a significant score increase on the Reexperiencing, $B = 1.303$, 95% CI [0.409, 2.197], $p = .020$; Avoidance, $B = 0.537$, 95% CI [0.088, 0.985], $p = .038$; or Numbing subscales, $B = 1.100$, 95% CI [0.152, 2.048], $p = .038$. No association was observed for exposure to more than 21 PB pills and Hyperarousal subscale score, $p = .635$.

There were significant results for the interactions between time and insecticide or pesticide smell exposure among participants with low initial PTSD symptom levels for the Reexperiencing subscale, $B = 0.896$, 95% CI [0.074, 1.718], $p = .033$; Numbing subscale, Follow-Up 2: $B = 1.310$, 95% CI [0.259, 2.361], $p = .015$, Follow-Up 3: $B = 1.677$, 95% CI [0.103, 3.251], $p = .037$; Hyperarousal subscale, Follow-Up 2: $B = 1.221$, 95% CI [0.157, 2.286], $p = .025$, Follow-Up 3: $B = 2.139$, 95% CI [0.724, 3.553], $p = .003$; and total scores, Follow-Up 2: $B = 3.740$, 95% CI [1.072, 6.409], $p = .006$, Follow-Up 3: $B = 5.409$, 95% CI [1.087, 9.730], $p = .014$. The total score interactions are displayed in Figure 1. A significant interaction between time and exposure to more than PB pills was observed for the hyperarousal subscale, $B = 1.625$, 95% CI [0.240, 3.010], $p = .021$, and total scores, $B = 3.870$, 95% CI [0.286, 7.454, $p = .034$. These interactions are displayed in Supplementary Figure S1, Panels B and C. Significant interactions between time and diesel smell exposure were observed for the reexperiencing, $B = 1.871$, 95% CI [0.833, 2.909], $p < .001$; avoidance, Follow-Up 2: $B = 0.486$, 95% CI [0.043, 0.930], $p = .032$, Follow-Up 3: $B = 0.836$, 95% CI [0.211, 1.462, $p = .009$

TABLE 2 Associations between neurotoxicant exposures and Posttraumatic Stress Disorder Checklist symptom cluster scores

	Reexperiencing score		Avoidance score		Numbing score		Hyperarousal score		Total score	
Exposure	**Slope**	***SE***	**Slope**	***SE***	**Slope**	***SE***	**Slope**	***SE***	**Slope**	***SE***
High initial PTSD symptoms ($n = 46$)										
Diesel smell	0.519	1.557	0.869	0.758	0.551	1.414	1.941	1.152	3.879	4.300
Oil smell	0.545	1.735	−.424	0.817	−.167	1.480	1.674	1.295	0.628	4.789
Unvented tent heaters	0.983	1.230	0.807	0.614	0.392	1.032	−.306	1.026	1.876	3.363
Insecticide/pesticide smell	−.870*	1.190	0.367	0.562	0.525	0.995	0.988	0.927	−.846*	2.660
> 21 pyridostigmine bromide pills	**2.969**	**1.400**	**1.262**	**0.599**	0.550*	1.278	**2.212**	**0.920**	**9.003**	**3.275**
Any pyridostigmine bromide pills	1.811	1.259	1.173	0.667	**2.530**	**0.913**	0.814	1.138	6.328	3.256
On alert for chemical attack (> 20)	1.409	1.151	0.186	0.559	0.266	1.043	1.285	0.878	3.146	3.116
Low initial PTSD symptoms ($n = 213$)										
Diesel smell	0.281*	0.207	−.059*	0.155	0.656	0.437	−.113*	0.290	0.344*	0.715
Oil smell	0.209*	0.202	**0.425**	**0.177**	0.765	0.448	**0.711***	**0.246**	**1.652***	**0.679**
Unvented tent heaters	0.492	0.329	−.163*	0.163	0.367	0.459	−.032*	0.311	2.037	1.227
Insecticide/pesticide smell	0.338*	0.239	0.520	0.208	0.203*	0.358	0.537*	0.301	1.324*	0.792
> 21 pyridostigmine bromide pills	**1.303**	**0.456**	**0.537**	**0.229**	**1.100**	**0.484**	0.187*	0.395	1.150*	1.023
Any pyridostigmine bromide pills	0.412	0.342	0.279	0.170	0.603	0.429	0.701	0.396	1.995	1.188
On alert for chemical attack (> 20)	0.870	0.433	0.101	0.206	0.216	0.467	0.698	0.490	1.886	1.441
On alert for chemical attack (any)	−.190	0.535	0.103	0.273	0.672	0.659	0.701	0.501	1.286	1.826

Note: All analyses included baseline age, gender, and time as covariates. Slope refers to the unstandardized slopes of the main effect of exposure from the regression models. Bold font indicates a significant main effect at $p < .05$.

*$p < .05$ for interactions between time and exposure.

and hyperarousal clusters, $B = 1.628$, 95% CI [0.304, 2.952], $p = .016$; as well as for the total score, $B = 5.234$, 95% CI [1.602, 8.865]. These interactions are displayed in Supplementary Figure S2. Significant interactions between time and oil smell exposure were observed for the reexperiencing, $B = 1.960$, 95% CI [.886, 3.035], $p < .001$; hyperarousal, $B = 1.955$, 95% CI [0.668, 3.243], $p = .003$; and total scores, $B = 5.275$, 95% CI [1.559, 8.992], $p = .005$. These interactions are displayed in Supplementary Figure S3. Significant interactions between time and unvented tent heater exposure were observed for the avoidance, $B = 0.566$, 95% CI [0.117, 1.016], $p = .013$, and hyperarousal scores, Follow-Up 2: $B = 1.472$, 95% CI [0.441, 2.503], $p = .005$, Follow-Up 3: $B = 1.635$, 95% CI [0.271,3.000], $p = .019$. These interactions are displayed in Supplementary Figure S4. The effects of exposure in participants with low initial PTSD symptom levels are summarized in Table 2.

DISCUSSION

To our knowledge, this was the first study of its kind to examine changes in PTSD symptom clusters over time in GW veterans and investigate the impact of neurotoxicant exposures on such changes. The results suggest that among veterans with low initial PTSD symptom levels at Follow-Up 1, PTSD symptom cluster scores significantly increased over the span of 25 years but remained below the clinical cutoff suggestive of PTSD. These findings support those from research conducted in other veteran cohorts demonstrating increases in PTSD symptoms over time (Mota et al., 2016; Vasterling et al., 2016). These prior studies have focused solely on veterans with a PTSD diagnosis and have not included veterans who may have subclinical symptom levels. Because the current study included all veterans regardless of whether they report low or high levels of

initial PTSD symptoms, the findings highlight that individual symptoms or symptom clusters may change over time regardless of whether relevant scores exceed a cutoff suggestive of a PTSD diagnosis. This gives credence to the need for consistent follow-up of psychiatric issues in postdeployed veterans.

PTSD symptoms may increase over time for several different reasons. Factors related to aging, such as late-life stressors and life changes contribute to increases in posttraumatic stress symptoms over time (Davison et al., 2016; Mota et al., 2016; Vasterling et al., 2016). Late-life stressors that come with normal aging may include changes in employment, poorer physical health, and illness and death in friends and family. Additionally, with age, many individuals begin to reminisce about their life and may return to memories of their traumatic experiences, causing a subsequent increase in symptoms. Therefore, it appears that GW veterans follow a trajectory similar to those seen in other veteran cohorts wherein PTSD symptoms increase across time.

In contrast, among participants with high initial PTSD symptom levels at Follow-Up 1, PTSD symptom cluster scores did not significantly change over 25 years. In fact, scores remained relatively high and stayed significantly higher than scores in veterans with lower initial symptom levels. Although we did not investigate treatment use, these data suggest that veterans who reported more severe initial PTSD symptoms at Follow-Up 1 did not experience symptom alleviation or resolution over time. Although rates of PTSD for GW veterans are relatively low (Kang et al., 2003), there is still a need for continued screening and clinical evaluation of veterans deemed to be at risk of PTSD in this group.

The present findings suggest that GW veterans, especially those who experienced multiple neurotoxicant exposures during deployment, may be unique as compared to veterans of other conflicts in that participants showed an increase in or chronicity of PTSD symptoms. We found that self-reported neurotoxicant exposures were associated with increased PTSD symptom cluster scores in veterans with both low and high initial PTSD symptom levels at Follow-Up 1. For example, among veterans with more severe symptoms, those with reported PB pill exposure scored, 2.5 points higher, on average, on the Numbing subscale than those who were unexposed, suggesting two additional reported symptoms or a 2-point increase in symptom severity. Among participants with low initial symptom levels, those who reported exposure to 21 or more PB pills scored an average of 1.3 points higher on the Reexperiencing subscale than those who were unexposed, suggesting one additional reported symptom or a 1-point increase in symptom severity. Regardless of initial symptom severity, the findings indicate that these neurotoxicant exposures exert adverse effects on PTSD symptoms. This is similar to observations reported by Janulewicz et al. (2018), who investigated a multiple-hit hypothesis regarding the impact of neurotoxicant exposures and mTBI on increased health-related symptoms and chronic medical conditions.

Importantly, our results are also consistent with those related to the survivors of the 1995 Tokyo sarin terrorist attack. Sarin nerve gas is an acetylcholinesterase inhibitor and, thus, depletes levels of cholinesterase, leading to excess acetylcholine in synapses and a chronic state of hyperexcitability (Golomb et al., 2008). Research on this population has found that survivors exhibit persistent patterns of PTSD symptoms, with hyperarousal symptoms the most frequently reported, and that these patterns are correlated with serum cholinesterase levels as measured shortly after the attack (Araki et al., 2005; Ohtani et al., 2004; Tochigi et al., 2005). Tochigi et al. (2005) concluded that these findings could have been due to higher degrees of psychological trauma in individuals who experience severe situations associated with more exposure to sarin or that higher levels of sarin exposure cause more severe damage to the brain, leading to more severe posttraumatic stress symptoms.

Researchers have recently hypothesized that environmental and occupational exposures, such as those experienced during the GW prime individuals to react adversely to traumatic events via an increased proinflammatory state, thus increasing the risk for and symptoms of PTSD (Georgopoulos et al., 2018; Walker et al., 2016). This is similar to hypotheses posited in the mTBI literature suggesting that neuroinflammation resulting from brain injury underlies the high rates of comorbid mTBI and PTSD (Kaplan et al., 2018). In fact, elevated levels of proinflammatory cytokines and chemokines have been observed in GW veterans. For example, Butterick et al. (2019) observed elevated levels of interleukin-6 and C-reactive proteins, markers of proinflammatory processes, in blood samples of GW veterans obtained nearly 30 years postwar. Recently, evidence of in vivo neuroinflammation, as assessed using a positron emission tomography radioligand that binds to active microglial and astrocytes, was observed in GW veterans, suggesting active inflammatory processes occurring nearly 30 years after potential neurotoxicant exposure (Alshelh et al., 2020). Therefore, it is possible that GW veterans exposed to neurotoxicants and traumatic events during deployment may be experiencing a multiple hit of proinflammatory responses, which, in turn, increases the severity or longevity of PTSD symptoms.

We observed several significant interactions between neurotoxicant exposures and time on PTSD symptom cluster scores. These interactions were observed at Follow-Up 2 and Follow-Up 3, which occurred 6–7 years and 20 years, respectively, after the war. This suggests that as time goes

on, exposed veterans exhibit distinct trajectories of PTSD symptoms as compared to unexposed veterans. Among participants with high initial PTSD symptom levels, unexposed veterans were shown to experience a decrease in PTSD symptom severity over time, whereas exposed veterans experienced no significant change but maintained a relatively high symptom level. For example, among veterans with more severe initial symptoms, those who reported exposure to more than 21 PB pills endorsed nearly five more symptoms or an increase in symptom severity on the PCL Numbing subscale by end of the follow-up period as compared to those who did not report this exposure. In participants with low less severe initial symptom levels, both exposed and unexposed veterans experienced an increase in PTSD symptoms over time; however, exposed veterans showed a much steeper and earlier increase in symptom severity. For example, among veterans with low initial PTSD symptom levels, those who reported insecticide or pesticide smell exposure endorsed more than two symptoms or an increase in symptom severity on the PCL Hyperarousal subscale by the end of the follow-up period as compared to those who did not report this exposure.

GW neurotoxicant exposures have been hypothesized to induce accelerated aging patterns (Proctor et al., 2006; Zundel et al., 2019). Accelerated aging is the phenomenon in which individuals may experience symptoms of aging, including medical conditions like diabetes and high blood pressure or symptoms like sleep dysregulation or joint pain, much earlier than their same5aged peers (Proctor et al., 2006; Zundel et al., 2019). The apparent increase in PTSD symptoms seen in the later years of follow-up among participants with low initial PTSD symptom levels who reported exposure to neurotoxicants may, in part, be due to an accelerated aging pattern. This could explain why the increase in PTSD symptoms commonly observed in other veteran cohorts over time appeared to be occurring at a faster rate in exposed groups versus unexposed groups.

The present findings suggest that neurotoxicant exposures may play a role in increased PTSD symptoms over time within a GW veteran cohort. Although exposure to traumatic events or neurotoxicants alone has been shown to induce inflammatory responses and the subsequent risk of posttraumatic stress or health-related symptoms, together, these two insults may result in both symptom exacerbation and a prolonged course of symptoms. Therefore, clinicians who treat veterans should be aware of other possible neurobiological factors, such as neurotoxicant exposure, that may impact their patients' experience with PTSD. Doing so may benefit treatment plans for mood disturbances and other chronic health issues reported by GW veterans.

Study limitations should be noted. First, study dropout and selection bias are inherent in longitudinal studies, as individuals with more health problems may be more likely to remain in the study than healthy individuals; alternatively, longitudinal studies may exclude the sickest veterans, who are either too ill to participate or may have died during the course of the study. Thus, the study sample differed from the full FDC on several key demographic characteristics and, as such, the reported findings may not be generalizable to the full FDC nor to the entire GW veteran population. Second, although we examined PTSD symptoms using a self-report measure, clinical PTSD diagnosis cannot be confirmed. Third, it is unknown whether the posttraumatic stress symptoms observed in this study resulted from wartime trauma or index traumatic events that occurred after deployment. Additionally, the results of the study may be confounded by the fact that veterans who experienced neurotoxicant exposures may have also experienced more traumatic events during or after deployment, which may independently lead to more severe or chronic PTSD trajectories. Fourth, information regarding participants' involvement in psychological or physical health–related treatments were not included in the surveys. It is possible that treatment could have affected both how symptoms changed and symptom trajectories over time. Additionally, patients with unexplained illnesses may have an increased incidence of PTSD due to the poor treatment they receive by the medical community as well as the stigma surrounding these illnesses and a general lack of support (Weir et al., 2014). This may, in part, explain why PTSD symptoms have been found to increase for many GW veterans who continue to be told that their chronic symptoms are psychosomatic (National Academies of Sciences, Engineering, and Medicine, 2016). Next, self-reported exposures to neurotoxicant exposures were used, as no objective measures of war-related exposures are available. However, recall bias was minimized by using exposure data from Follow-Up 2, which was less than 5 years following participants' return from the GW and before many exposure-outcome reports had been made public. Further, the survey's neurotoxicant data were dichotomized into "yes" and "no" responses, limiting our ability to investigate dose–response relationships. Additionally, it is unknown whether neurotoxicant exposure before or after the GW may have influenced the current findings. Future studies should include lifetime neurotoxicant exposure history.

Further research should focus on examining the mechanisms by which neurotoxicant exposure impacts PTSD symptoms. Specifically, studies examining the association between cognitive dysfunction and PTSD symptoms are needed, as elevated neuroinflammatory responses can also affect cognitive processes, including deficits in executive function, attention, and learning and memory, which may interfere with trauma-exposed individuals' ability to

process their experience, leading to an increased risk of developing PTSD symptoms (Quinones et al., 2020). Further, future studies should investigate normal aging stressors (e.g., retirement, declines in physical health) and their impact on PTSD symptoms over time, which would enable the nature of the effects in the current study to be further delineated (i.e., increases in symptoms due to normal aging or due to neurotoxicants). Additionally, future researchers should investigate whether there are specific treatment-resistant PTSD symptoms (e.g., irritability) that are prominent within neurotoxicant-exposed populations, and additional studies examining PTSD trajectories between those who do and do not receive treatment are warranted. Moreover, the interaction between time and PTSD symptom cluster scores suggests that longitudinal studies should be conducted to examine the trajectories of reported symptoms in all neurotoxicant-exposed populations, not just within GW veterans.

OPEN PRACTICES STATEMENT

The study reported in this article was not formally preregistered. Neither the data nor the materials have been made available on a permanent third-party archive; Department of Veterans Affairs (VA) privacy and data security policies and regulatory constraints provide that only aggregate summary data may be removed from the VA for publication. The authors have provided detailed results of these analyses in the paper. These restrictions are in place to maintain patient privacy and confidentiality. Access to these data can be granted to persons who are not an employee of the VA; however, there is an official protocol that must be followed for doing so. The authors invite individuals who wish to access the raw data that were used for this analysis to contact Dr. Maxine Krengel (Maxine.Krengel@va.gov) to discuss the details of the VA data access approval process.

AUTHOR NOTE

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ORCID

Clara G. Zundel https://orcid.org/0000-0002-1736-2695

REFERENCES

Able, M. L., & Benedek, D. M. (2019). Severity and symptom trajectory in combat-related PTSD: a Review of the literature. *Current Psychiatry Reports*, *21*(7), 58. https://doi.org/10.1007/s11920-019-1042-z

Alshelh, Z., Albrecht, D. S., Bergan, C., Akeju, O., Clauw, D. J., Conboy, L., Edwards, R. R., Kim, M., Lee, Y. C., Protsenko, E., Napadow, V., Sullivan, K., & Loggia, M. L. (2020). In-vivo imaging of neuroinflammation in veterans with Gulf War illness. *Brain, Behavior, and Immunity*, *87*, 498–507. https://doi.org/10.1016/j.bbi.2020.01.020

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders* (4th ed.). Author.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders*: (5th ed.). Author. https://doi.org/10.1176/appi.books.9780890425596

Araki, T., Kasai, K., Yamasue, H., Kato, N., Kudo, N., Ohtani, T., Nakagome, K., Kirihara, K., Yamada, H., Abe, O., & Iwanami, A. (2005). Association between lower P300 amplitude and smaller anterior cingulate cortex volume in patients with posttraumatic stress disorder: A study of victims of Tokyo subway sarin attack. *Neuroimage*, *25*(1), 43–50. https://doi.org/10.1016/j.neuroimage.2004.11.039

Asmundson, G. J., Frombach, I., McQuaid, J., Pedrelli, P., Lenox, R., Stein, M.B. (2000). Dimensionality of posttraumtic stress symptoms: a confirmatory factor analysis of *DSM-IV* symptom clusters and other symptom models. *Behaviour, Research, and Therapy*, *38*(2), 203–214. https://doi.org/10.1016/s0005-7967(99)00061-3

Ballinger, G. A. (2004). Using generalized estimating equations for longitudinal data analysis. *Organizational Research Methods*, *7*(2), 127–150. https://doi.org/10.1177/1094428104263672

Benotsch, E. G., Brailey, K., Vasterling, J. J., Uddo, M., Constans, J. I., & Sutker, P. B. (2000). War zone stress, personal and environmental resources, and PTSD symptoms in Gulf War Veterans: A longitudinal perspective. *Journal of Abnormal Psychology*, *109*(2), 205–213. https://doi.org/10.1037/0021-843x.109.2.205

Butterick, T. A., Trembley, J. H., Hocum Stone, L. L., Muller, C. J., Rudquist, R. R., & Bach, R. R. (2019). Gulf War Illness-associated increases in blood levels of interleukin 6 and C-reactive protein: Biomarker evidence of inflammation. *BMC Research Notes*, *12*(1), 816. https://doi.org/10.1186/s13104-019-4855-2

Chao, L. L., Reeb, R., Esparza, I. L., & Abadjian, L. R. (2016). Associations between the self-reported frequency of hearing chemical alarms in theater and regional brain volume in Gulf War Veterans. *Neurotoxicology*, *53*, 246–256. https://doi.org/10.1016/j.neuro.2016.02.009

Davison, E. H., Kaiser, A. P., Spiro, A., 3rd, Moye, J., King, L. A., & King, D. W. (2016). From late-onset stress symptomatology to later-adulthood trauma reengagement in aging combat veterans: Taking a broader view. *Gerontologist*, *56*(1), 14–21. https://doi.org/10.1093/geront/gnv097

Fang, S. C., Schnurr, P. P., Kulish, A. L., Holowka, D. W.X, B. P., Keane, T. M., & Rosen, R. (2015). Psychosocial functioning and health-related quality of life associated with posttraumatic stress

disorder in male and female Iraq and Afghanistan War veterans: The VALOR Registry. *Journal of Women's Health*, *24*(12), 1038–1046. https://doi.org/10.1089/jwh.2014.5096

Foa, E. B., Zinbarg, R., Rothbuam, B.O. (1992). Uncontrollability and unpredictability in post-traumatic stress disorder: An animal model. *Psychological Bulletin*, *11*(2), 218–238. https://doi.org/10.1037/0033-2909.112.2.218

Georgopoulos, A. P., James, L.M., Christova, P., Engdahl, B.E. (2018). A two-hit model of the biological origin of posttraumtic stress disorder (PTSD). *Journal of Mental Health & Clinical Psychology*, *2*(5), 9–14.

Golomb, B. A. (2008). Acetylcholinesterase inhibitors and Gulf War illnesses. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(11), 4295–4300. https://doi.org/10.1073/pnas.0711986105

National Academies of Sciences, Engineering, and Medicine. (2016). *Gulf War and health: Volume 10: Update of health effects of serving in the Gulf War, 2016*. National Academies Press. https://doi.org/10.17226/21840

Janulewicz, P., Krengel, M., Quinn, E., Heeren, T., Toomey, R., Killiany, R., Zundel, C., Ajama, J., O'Callaghan, J., Steele, L., Klimas, N., & Sullivan, K. (2018). The multiple-hit hypothesis for Gulf War illness: Self-reported chemical/biological weapons exposure and mild traumatic brain injury. *Brain Sciences*, *8*(11), 198. https://doi.org/10.3390/brainsci8110198

Kang, H. K., Natelson, B. H., Mahan, C. M., Lee, K. Y., & Murphy, F. M. (2003). Post-traumatic stress disorder and chronic fatigue syndrome-like illness among Gulf War veterans: Aa population-based survey of 30,000 veterans. *American Journal of Epidemiology*, *157*(2), 141–148. https://doi.org/10.1093/aje/kwf187

Kaplan, G. B., Leite-Morris, K. A., Wang, L., Rumbika, K. K., Heinrichs, S. C., Zeng, X., Wu, L., Arena, D. T., & Teng, Y. D. (2018 15). Pathophysiological bases of comorbidity: Traumatic brain injury and post-traumatic stress disorder. *Journal of Neurotrauma*, *35*(2), 210–225. https://doi.org/10.1089/neu.2016.4953

Karstoft, K. I., Armour, C., Elklit, A., Solomon, Z. (2013). Long-term trajectories of posttraumatic stress disorder in veterans: The role of social resources. *The Journal of Clinical Psychiatry*, *74*(12), 1163–1168. https://doi.org/10.4088/JCP.13m08428

Keeler, J. R., Hurst, C. G., & Dunn, M. A. (1991). Pyridostigmine used as a nerve agent pre-treatment under wartime conditions. *Journal of the American Medical Association*, *266*(5), 693–695. https://doi.org/10.1001/jama.1991.03470050093029

King, D. W., Leskin, G.A., King, L.A., Weathers, F.W. (1998). Confirmatory factor analysis of the Clinician-Administered PTSD Scale: Evidence for the dimensionality of posttraumatic stress disorder. *Psychological Assessment*, *10*(2), 90–96. https://doi.org/10.1037/1040-3590.10.2.90

Marmar, C. R., Schlenger, W., Henn-Haase, C., Qian, M., Purchia, E., Li, M., Corry, N., Williams, C. S., Ho, C. L., Horesh, D., Karstoft, K. I., Shalev, A., & Kulka, R. A. (2015). Course of posttraumatic stress disorder 40 years after the Vietnam War: Findings from the National Vietnam Veterans Longitudinal Study. *Journal of the American Medical Association Psychiatry*, *72*(9), 875–881. https://doi.org/10.1001/jamapsychiatry.2015.0803

Mota, N. P., Cook, J. M., Smith, N. B., Tsai, J., Harpaz-Rotem, I., Krystal, J. H., Southwick, S. M., & Pietrzak, R. H. (2019). Posttraumatic stress symptom courses in U.S. military veterans: A seven-year, nationally representative, prospective cohort study. *Journal of Psychiatric Research*, *119*, 23–31. https://doi.org/10.1016/j.jpsychires.2019.09.005

Mota, N., Tsai, J., Kirwin, P. D., Harpaz-Rotem, I., Krystal, J. H., Southwick, S. M., & Pietrzak, R. H. (2016). Late-life exacerbation of PTSD symptoms in U.S. veterans: Results from the National Health and Resilience in Veterans Study. *The Journal of Clinical Psychiatry*, *77*(3), 348–354. https://doi.org/10.4088/JCP.15m10101

Ohtani, T., Iwanami, A., Kasai, K., Yamada, H., Kato, T., Sasaki, T., & Kato, N. (2004). Post-traumatic stress disorder symptoms in victims of Tokyo subway attack: A 5-year follow-up study. *Psychiatry and Clinical Neurosciences*, *58*(6), 624–629. https://doi.org/10.1111/j.1440-1819.2004.01313.x

Orcutt, H. K., Erickson, D.J., Wolfe, J. (2004). The course of PTSD symptoms among Gulf War veterans: A growth mixture modeling approach. *Journal of Traumatic Stress*, *17*(3), 195–202. https://doi.org/10.1023/B:JOTS.0000029262.42865.c2

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 U.S. army veterans. *Neurotoxicology*, *27*(6), 931–939. https://doi.org/10.1016/j.neuro.2006.08.001

Proctor, S. P., Heeren, T., White, R. F., Wolfe, J., Borgos, M.S., Davis, J. D., Pepper, L., Clapp, R., Sutker, P. B., Vasterling, J. J., & Ozonoff, D. (1998). Health status of Persian Gulf War veterans: Self-reported symptoms, environmental exposures, and the effect of stress. *International Journal of Epidemiology*, *27*(6), 1000–1010. https://doi.org/10.1093/ije/27.6.1000

Quinones, M. M., Gallegos, A. M., Lin, F. V., & Heffner, K. (2020). Dysregulation of inflammation, neurobiology, and cognitive function in PTSD: An integrative review. *Cognitive, Affective & Behavioral Neuroscience*, *20*(3), 455–480. https://doi.org/10.3758/s13415-020-00782-9

Research Advisory Committee on Gulf War Veterans' Illnesses. (2008). *Gulf War illness and the health of Gulf War veterans: Research update and recommendations*. U.S. Government Printing Office.

Southwick, S. M., Morgan, III, C. A., Darnell, A., Bremner, D., Nicholaou, A. L., Nagy, L. M., & Charney, D. S. (1995). Trauma-related symptoms in veterans of Operation Desert Storm: A 2-year follow-up. *The American Journal of Psychiatry*, *152*(8), 1150–1155. https://doi.org/10.1176/ajp.152.8.1150

Steele, L. (2000). Prevalence and patterns of Gulf War illness in Kansas veterans: Association of symptoms with characteristics of person, place, and time of military service. *American Journal of Epidemiology*, *152*(10), 992–1002. https://doi.org/10.1093/aje/152.10.992

Thompson, K. E., Vasterling, J. J., Benotsch, E. G., Brailey, K., Constans, J., Uddo, M., & Sutker, P. B. (2004). Early symptom predictors of chronic distress in Gulf War veterans. *The Journal of Nervous and Mental Disease*, *192*(2), 146–152. https://doi.org/10.1097/01.nmd.0000110286.10445.ab

Tochigi, M., Otani, T., Yamasue, H., Kasai, K., Kato, T., Iwanami, A., Kato, N., & Sasaki, T. (2005). Support for relationship between serum cholinesterase and post-traumatic stress disorder: 5-year follow-ups of victims of the Tokyo subway sarin poisoning. *Neuroscience Research*, *52*(2), 129–131. https://doi.org/10.1016/j.neures.2005.03.012

National Center for PTSD. (n.d.). *Using the PTSD Checklist for DSM-IV (PCL)*. https://www.ptsd.va.gov/professional/assessment/documents/PCL_handoutDSM4.pdf

Vasterling, J. J., Aslan, M., Proctor, S. P., Ko, J. X, B P., Jakupcak, M., Schnurr, P. P., Gleason, T., Huang, G. D., & Concato, J. (2016). Longitudinal examination of posttraumatic stress disorder as a long-term outcome of Iraq War deployment. *American Journal of Epidemiology*, *184*(11), 796–805. https://doi.org/10.1093/aje/kww151

Walker, A., McKune, A., Ferguson, S., Pyne, D. B., & Rattray, B. (2016). Chronic occupational exposures can influence the rate of PTSD and depressive disorders in first responders and military personnel. *Extreme Physiology & Medicine*, *5*(1), 8. https://doi.org/10.1186/s13728-016-0049-x

Weathers, F. W., Blake, D. D., & Litz, B. T. (1991, August). *Reliability and validity of a new structured interview for PTSD* [Conference presentation]. Annual meeting of the American Psychological Association, San Franciso, CA.

Weir, K. (2014). Beyond tired. *Monitor on Psychology*, *45*(9), 66. https://www.apa.org/monitor/2014/10/beyond-tired

White, R. F., Steele, L., O'Callaghan, J. P., Sullivan, K., Binns, J. H., Golomb, B. A., Bloom, F. E., Bunker, J. A., Crawford, F., Graves, J. C., Hardie, A., Klimas, N., Knox, M., Meggs, W. J., Melling, J., Philbert, M. A., & Grashow, R. (2016). Recent research on Gulf War illness and other health problems in veterans of the 1991 Gulf War: Effects of toxicant exposures during deployment. *Cortex*, *74*, 449–475. https://doi.org/10.1016/j.cortex.2015.08.022

Wolfe, J., Brown, P. J., & Kelley, J. M. (1993). Reassessing war stress: Exposure and the Persian Gulf War. *Journal of Social Issues*, *49*(4), 15–31. https://doi.org/10.1111/j.1540-4560.1993.tb01179.x

Wolfe, J., Proctor, S. P., Davis, J. D., Borgos, M. S., & Friedman, M. J. (1998). Health symptoms reported by Persian Gulf War veterans two years after return. *American Journal of Industrial Medicine*, *33*(2), 104–113. https://doi.org/10.1002/(SICI)1097-0274(199802)33:2⟨104::AID-AJIM2⟩3.0.CO;2-Y

Wolfe, J., Proctor, S. P., Erickson, D. J., & Hu, H. (2002). Risk factors for multisymptom illness in U.S. Army veterans of the Gulf War. *Journal of Occupational and Environmental Medicine*, *44*(3), 271–281. https://doi.org/10.1097/00043764-200203000-00015

Yee, M. K., Zundel, C. G., Maule, A. L., Heeren, T., Proctor, S. P., Sullivan, K. A., & Krengel, M. H. (2020). Longitudinal assessment of health symptoms in relation to neurotoxicant exposures in 1991 Gulf War Veterans: The Ft. Devens Cohort. *Journal of Occupational and Environmental Medicine*, *62*(9), 663–668. https://doi.org/10.1097/JOM.0000000000001910

Zeger, S. L., & Lian, K. Y. (1986). Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*, *42*(1), 121–130. https://doi.org/10.2307/2531248

Zundel, C. G., Krengel, M. H., Heeren, T., Yee, M. K., Grasso, C., Mulewicz Lloyd, P. A., Coughlin, S. S., & Sullivan, K. (2019). Rates of chronic medical conditions in 1991 Gulf War veterans compared to the general population. *International Journal of Environmental Research and Public Health*, *16*(6), 949. https://doi.org/10.3390/ijerph16060949

SUPPORTING INFORMATION

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