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PRINCIPAL INVESTIGATOR: Lea Steele, Ph.D.

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E-Mail: Lea.Steele@bcm.edu

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BAYLOR COLLEGE OF MEDICINE
ONE BAYLOR PLAZA, MS-BCM 310
HOUSTON TX 77030-3411

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14. ABSTRACT

Approximately one third of the nearly 700,000 U.S. military veterans who served in the 1990-1991 Persian Gulf War continue to suffer from Gulf War illness (GWI), a complex of chronic symptoms that includes persistent headaches, memory and cognitive difficulties, widespread pain, unexplained fatigue, gastrointestinal problems, and other difficulties. Multiple findings of significant central nervous system (CNS) involvement have been reported in veterans with GWI, including preliminary findings suggestive of alterations in central dopamine levels and signaling. The current study leveraged existing MRI brain imaging data from a well-characterized sample of 139 Gulf War veterans that included 120 GWI cases (defined by Kansas criteria) and 19 veteran controls. Using high resolution diffusion tensor imaging (DTI), the study provided in-depth assessment of white matter integrity in the substantia nigra (SN), the primary source of central dopamine production, and six thalamic nuclei. Analysis of white matter integrity in regions of interest were based on fractional anisotropy (FA) measures using methods previously developed and validated by study investigators. Results indicate that GWI is associated with significant depletion of white matter integrity affecting the SN, as indicated by significantly reduced mean FA in cases vs. controls ($p<0.0001$), but only minimal changes in thalamic nuclei. Observed SN FA reductions were significantly correlated with greater GWI severity overall and with severity of several symptom domains. Lower SN FA was also significantly associated with two deployment experiences/exposures during the Gulf War, use of skin pesticides and having one or more mild traumatic brain injuries in theater. The observed SN changes would be expected to result in substantial reduction of central dopamine levels and neurotransmission. Study results require replication in a separate sample in order to confirm and expand our understanding of dopaminergic alterations in GWI neuropathology and possible implications for improved GWI diagnosis and treatment.

15. SUBJECT TERMS Gulf War illness, neuroimaging, magnetic resonance imaging, substantia nigra, nigro-striatal circuit, dopamine, diffusion tensor imaging

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Assessment of MRI-Based Marker of Dopaminergic Integrity as a Biological Indicator of Gulf War Illness

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1. INTRODUCTION

Approximately one third of the nearly 700,000 U.S. military veterans who served in the 1990-1991 Persian Gulf War continue to suffer from the serious, often debilitating condition known as Gulf War illness (GWI). GWI is characterized by a complex of chronic symptoms that typically includes persistent headaches, memory and cognitive difficulties, widespread pain, unexplained fatigue, gastrointestinal problems, and other difficulties. Although multiple indicators of significant central nervous system (CNS) alterations and functional decrements have been reported in veterans with GWI, there is still no comprehensive understanding of GWI cerebral neurobiology/neurophysiology and how observed CNS changes are associated with GWI symptoms. In particular, the role of the corticostriatal circuit in GWI has not been well-studied, despite multiple preliminary indications of neuronal dysfunction in this circuit. The current study leverages existing brain imaging data from well-characterized samples of 1990-91 Gulf War veterans to assess brain structures and processes of high interest for understanding GWI. It provides in-depth assessment of the substantia nigra and thalamic nuclei using high resolution diffusion tensor imaging (DTI) in 120 veterans with GWI and 19 healthy Gulf War veteran controls. Detailed analyses characterize the etiologic and clinical correlates of alterations in basal ganglia white matter integrity, including associations of substantia nigra white matter alterations with GWI symptom presentation. Study results will establish a foundation for improved understanding of GWI neuropathology and approaches to diagnosis and therapeutic intervention that include specific targeting of the dopaminergic system.

2. KEYWORDS

Gulf War illness, neuroimaging, magnetic resonance imaging, substantia nigra, nigro-striatal circuit, dopamine, diffusion tensor imaging

3. ACCOMPLISHMENTS: What were the major goals of the project?

Major Project Goals/Tasks and Accomplishments

Task 1. Obtain approvals for award amendments and funding for change in PI, addition of study sites.
All sponsor and institutional approvals were obtained for required project changes prior to initiation of research activities.

Task 2. Finalize subaward agreements and regulatory approvals: all sites.
All administrative and regulatory approvals for subawards and human subjects protocols at all sites were obtained prior to initiation of research activities.

Task 3. Data transfer
Secure transfer of MRI data from the Boston University subaward site to Baylor College of Medicine and the University of Texas subaward site was completed. Data were reviewed and confirmed for receipt of all eligible MRI scans.

Task 4. Analysis of Quality Assurance (QA) data and staff training
Quality assurance data were obtained and reviewed, and staff training completed in conjunction with initiating evaluation and analysis of scanning data

Major Project Goals/Tasks and Accomplishments (cont.)

Task 5. Scanning/rescanning of Gulf War Veterans for reliability-repeatability assessment
No additional MRI scans were done during the project period, owing to pandemic restrictions in recruiting and in-person evaluation of study subjects throughout the final year of the project.

Task 6. Assess alterations in substantia nigra
DTI fractional anisotropy measures in the substantia nigra were completed and all data analyzed. Information on study methods, including specific methods used to obtain measures of the substantia nigra and other regions of interest are provided with this report in Appendix A.

Task 7. Assess alterations in thalamic nuclei
DTI fractional anisotropy measures in all thalamic nuclei (see Appendix A) were completed and all data analyzed.

Task 8. Assess alterations in putamen, caudate, cortex
Volumetric measures were completed in the putamen, caudate, and cortex; diffusion tenor imaging in these areas did not provide sufficient resolution for analysis of white matter integrity.

Task 9. Integration of clinical data, data analyses, finalize manuscripts, data sharing
DTI FA measures obtained for the study were integrated with GWIC clinical data and detailed analyses have been completed. Study results identified significant neuropathology in veterans with GWI in connection with reduced white matter integrity in the substantia nigra, the most critical region for dopamine production in the brain. Observed changes were significantly associated with the severity of GWI symptoms and with exposures during deployment. Detailed study results are included with this report in Appendix B. Study findings will be reported in manuscripts to be finalized and submitted to appropriate journals. Data on DTI measures obtained for the study will also be provided to the Boston University BBRAIN repository to facilitate sharing with other investigators.

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

Nothing to report. The project was not intended or designed to provide training and professional development, other than the training required for project analysts to accurately obtain study measures.

How were the results disseminated to communities of interest?

Data analyses were only recently completed, and have not yet been disseminated via journal publications and presentations to the scientific community, veterans, or the general public.

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

This is not directly applicable for this Final Report. However, after the project performance period we will continue with finalizing and submitting manuscripts and dissemination of results, possibly in conjunction with additional analyses to address questions beyond our initial hypotheses.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

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

No impact yet to report, although we believe our results can potentially stimulate expansion of an important area of Gulf War illness research to improve understanding and potentially diagnosis and treatment of this condition.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

No impact yet to report. However, we believe our results have the potential to further confirm the biological nature and causes of Gulf War Illness, lending support to societal efforts to better address health effects of military exposures.

5. **CHANGES/PROBLEMS:**
Actual or anticipated problems or delays and actions or plans to resolve them

This project has involved multiple challenges and delays throughout the performance period, as detailed in prior project reports. These have included two institutional changes by the original PI, barriers to implementing the original parent study that was to have provided MRI scanning data, a change in the source of MRI data to be used for the study, a change in PI, in-person research and data collection restrictions imposed by the COVID pandemic and, most recently, health and staffing problems affecting key personnel and our ability to obtain project data until after the performance period, necessitating delayed submission of the final report. Despite the many difficulties and delays we are pleased to have been able to address our major project objectives and extremely grateful to CDMRP GWIRP program officers for their support and assistance in advancing this effort.

Changes that had a significant impact on expenditures

Project delays had an impact on timing of expenditures, and the lack of in-person MRI data collection resulted in lower costs than anticipated, allowing unused funds to be returned to the sponsor.

Significant changes in use or care of human subjects

This project was designed to primarily conduct secondary analyses of previously collected, de-identified data. This involved only minimal contact with human subjects for the six retest/reliability scans. Those were not conducted due to pandemic restrictions. So the only change related to use of human subjects is that we had no direct contact with human subjects for this study.

Significant changes in use or care of vertebrate animals

Not applicable/nothing to report.

Significant changes in use of biohazards and/or select agents

Not applicable/nothing to report.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

Publications, conference papers, and presentations

Journal publications.

Nothing to report.

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

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

Website(s) or other Internet site(s)

A summary description of the project has been posted on Dr. Steele's BCM Veterans Health Research Program Website throughout the project period: www.bcm.edu/vethealth

Veterans or other interested parties can also learn about project activities in real time, how to contact the program, and other information of interest to Gulf War veterans through our Veterans Health Research Program Facebook page: https://www.facebook.com/bcmveteranshealth/

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project (at least one person month per year)?

Name:	Lea Steele
Project Role:	Principal Investigator, Baylor College of Medicine
Nearest person month worked:	2
Contribution to Project:	Study approvals and administrative management, data collation and statistical analyses, preparation of reports
Name:	Deborah M. Little
Project Role:	Site PI and former project PI, University of Texas Health Science Center
Nearest person month worked:	4
Contribution to Project:	Regulatory, image analysis (regions of interest analysis), supervision of technicians
Name:	John Prince
Project Role:	Research analyst
Nearest person month worked:	12
Contribution to Project:	Image analysis (anatomical image analysis/pipeline)
Name:	Matt Price
Project Role:	Research Analyst
Nearest person month worked:	1
Contribution to Project:	Image analysis (anatomical image analysis/pipeline)
Name:	Claire Carson
Project Role:	Research Analyst
Nearest person month worked:	1
Contribution to Project:	Image analysis (regions of interest analysis)

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?.

Dr. Lea Steele: No changes in other active support during the final project year.

Dr. Deborah Little: No changes in other active support during the final project year.

What other organizations were involved as partners?

Project research activities included scientific contributions (supported by subawards) from the University of Texas Health Science Center at Houston (UTHSC-H) and Boston University (BU).

8. SPECIAL REPORTING REQUIREMENTS

Not applicable/Nothing to report.

9. APPENDICES: The following Appendices are provided at the end of the report.

Appendix A. “Final Report: Study Overview and Methods”

Appendix B. “Final Report: Study Results/Research Findings”

10. REFERENCES CITED IN THIS REPORT:

No references were included in the body of the report. References cited in the appendices are listed at the end of Appendix B.

Assessment of MRI-Based Marker of Dopaminergic Integrity as a Biological Indicator of GWI

Appendix A. Final Report: Study Overview and Methods

1. Background and Study Overview

Approximately one in three of the nearly 700,000 U.S. veterans who served in the 1990-1991 Gulf War developed the multisymptom condition known as Gulf War illness (GWI) in the wake of their deployment. More than 30 years after the war, veterans continue to suffer from this complex, often disabling condition (White 2016, Gifford 2021). Multiple clinical studies have reported objective indicators of brain, neurocognitive, immune, and neuroinflammatory pathology in connection with GWI (RAC-GWVI 2008,2014). Yet the exact neurobiological nature of GWI and pathophysiological processes that underlie veterans' symptoms have not been clearly determined. A more precise understanding of GWI neuropathology is essential for formulating useful therapeutic interventions and improving care for veterans with GWI.

In this context, diverse indicators from studies of Gulf War veterans, other human populations, and animal toxicological research suggest GWI may be associated with changes in central dopamine pathways and signaling (e.g. Haley 2000a,b, Torres-Altoro 2011). Such changes are potentially more subtle than the advanced neuronal and dopamine deficits seen in conditions like Parkinson's Disease (PD). Previous findings include indications that toxicant exposures like those encountered during the Gulf War affect brain regions implicated in dopaminergic pathways. And a growing number of studies of conditions that are clinically similar to GWI have identified dopaminergic alterations as potentially important factors. Such findings underscore the importance of evaluating dopaminergic pathways in veterans with GWI and in particular the substantia nigra (SN), given the high levels of dopaminergic neurons and projections from the nigra to the basal ganglia. The current study was developed to address prominent questions concerning possible alterations in dopaminergic pathways in Gulf War veterans in order to improve understanding of GWI pathobiology and possible applications for GWI diagnosis and treatment.

2. Study Hypotheses

The study addressed the following hypotheses:

Hypothesis 1. Veterans with GWI (GWI cases) will show reductions in substantia nigra (SN) integrity, measured by diffusion tensor imaging (DTI) fractional anisotropy (FA), compared to Gulf War veteran controls. This hypothesis was supported.

Hypothesis 2. Veterans with GWI will show alterations in basal ganglia integrity that relate to cognitive alterations, compared to Gulf War veteran controls. This hypothesis was partially supported.

Exploratory Hypothesis 1. DTI Region of Interest/FA measures will differ in relation to neurotoxicant exposures during Gulf War deployment. This hypothesis was supported.

Exploratory Hypothesis 2. Reductions in white matter integrity of the SN and reductions in FA in the subthalamic nuclei will be correlated with scores in specific symptom domains, including pain, neurocognitive, gastrointestinal, and fatigue symptoms. This hypothesis was partially supported.

3. Research Methods

3a. Study Population. The original study plan involved secondary analyses of DTI measures from MRI scans conducted on a Houston-area sample of 130 GWI cases and controls. However, institutional changes and pandemic-related delays required that, with CDMRP approval, we revise the approach to instead utilize MRI scanning data that had recently been collected for the multisite Gulf War Illness Consortium (GWIC) case/control study, for which our team had collected data for the Houston site. This was possible because subject eligibility requirements and specific data collected for the GWIC study were nearly identical to those developed for our original protocol. For the current study, all veteran participants were required to have served in the Gulf War Theater of Operations for any period between August 1990 and July 1991. Gulf War illness cases were required to meet Kansas GWI case criteria (Steele 2000).

Eligible veteran controls had insufficient symptoms to meet Kansas GWI criteria and no history of physician-diagnosed exclusionary conditions. Detailed description of recruitment, sample characteristics, and data collection for the GWIC parent study are provided in a recent publication (Steele 2021). All MRI scans analyzed for the current study were completed on veterans who gave explicit permission for use of their anonymized data in later studies.

Utilizing GWIC MRI data, our final study sample included 139 Gulf War veterans, including 120 GWI cases and 19 Gulf War veteran controls. This included all veterans in the GWIC sample for whom MRI data were collected at the two MRI scanning sites (Houston and Boston) and for whom the clarity of data collected (i.e. sufficient resolution, minimal artifact) allowed replicable measures of regions of interest, as described below. The final sample had a larger number of GWI cases but a considerably smaller number of veteran controls (n=19) than originally planned. This reflected the overall challenges encountered by the GWIC consortium in recruiting Gulf War veteran controls at all three study sites. Still, the overall sample was large in the context of imaging research and provided sufficient power to address our study questions.

3b. MRI Methods

Multiple indicators, from diverse studies and perspectives, suggest that dopaminergic pathways are potentially involved in both the initial development of GWI and the persistent pathobiological processes that underlie veterans' symptoms. The image analyses conducted for this study were designed to evaluate those pathways using three different methods. These include (1) standardized region of interest maps to extract volumes generated using traditional image analysis pipelines using FSL consistent with colleagues in the Gulf War Illness Consortium; (2) calculation of fractional anisotropy (FA) in thalamic nuclei (Little 2010); and (3) calculation of FA in the substantia nigra (Vaillancourt 2009).

Fractional anisotropy (FA) is a measure of the degree of water diffusion anisotropy/directional coherence and varies based on tissue density and degree of myelination. When tissue is more dense, water diffusion through the tissue is imposed, leading to directionally dependent (anisotropic) movement in the parallel direction of the tissue, and FA values near 1. When the tissue is less organized as in the case of damaged white matter, water diffusion occurs in more random directions (isotropic) and results in FA values closer to 0. For this study, the diffusion images were reconstructed and FA calculated in DtiStudio (Wakana 2004, Jiang 2006). For all subjects, each DTI image was examined for image quality, artifacts, and in the case of the substantia nigra, coverage of sufficient brain stem anatomy to cover the nigra. A background noise level of 125 (MR units) was applied prior to calculation of pixel-wise FA maps.

Calculation of Thalamic Regions of Interest (DTI). Fiber tracking in DtiStudio was used to assess projection fibers from six thalamic nuclei. Following the calculation of pixel-wise FA and Eigenvector maps, fiber tracking parameters were set to exclude tracking fibers with FA values < 0.20 or a turning angle $> 70°$. Seed voxels were placed within the boundaries of each nucleus in each hemisphere. Two seed voxels (3mm in diameter) were placed in the anterior thalamic nucleus (AN), ventral anterior thalamic nucleus (VA), ventral lateral thalamic nucleus (VL), ventral posterior lateral nucleus and ventral posterior medial nucleus (VPL/VPM), and pulvinar (PU). Given its larger region, three seed voxels of the same diameter were placed in the dorsomedial nucleus (DM). The regional boundaries for placing the seed voxels were based on the methods described in Little et al. 2010, Appendix E-1. The seed voxels were drawn independently by two raters who were blinded to case or control status. Mean FA values were then extracted from the fibers projecting from each seed and the values were recorded. Because of difficulty visualizing the boundaries of VPL from VPM these nuclei were combined. The relative locations on a representative subject are shown in Figure 1. These seed regions and resulting fibers were drawn for both the right and left hemispheres.

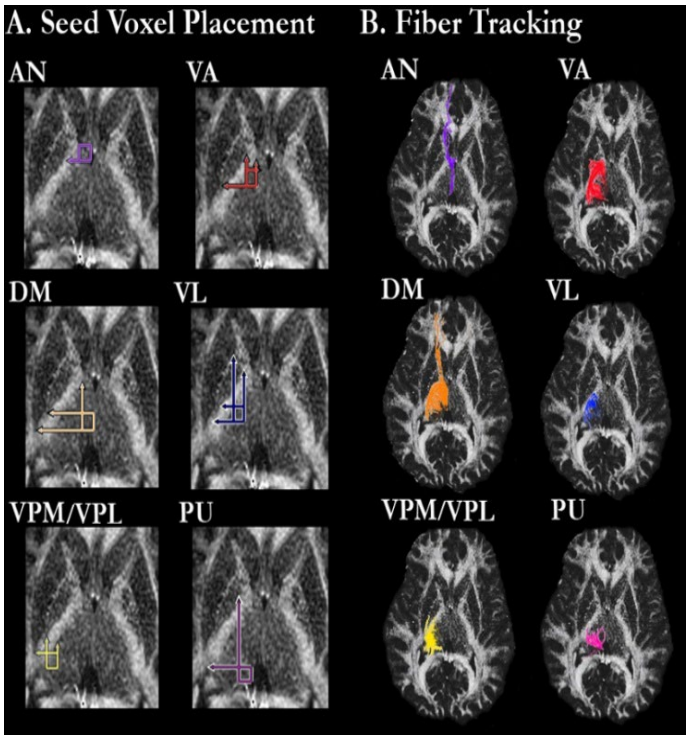

Figure 1. Placement of regions of interest in thalamic nuclei in a representative patient.

Anterior Thalamic Nucleus (AN). The AN seed region was defined laterally by the internal capsule, medially by the edge of the thalamus, and anteriorly by the posterior edge of the caudate head and lateral ventricle. The posterior boundary for the seed voxel was defined by a horizontal line extending from the posterior edge of the anterior limb of the internal capsule. Any fiber that extended to the putamen or globus pallidus was excluded. Furthermore, any fiber that was also identified from seed voxels placed in the VA, VPL, VPM, DM, or PU were excluded.

Ventral Anterior Thalamic Nucleus (VA). The anterior edge of the VA seed voxel was defined by a horizontal line extending from the anterior edge of the internal capsule and extended medially the width of the internal capsule. The VA extended posterior to a horizontal line drawn from the posterior boundary of the putamen. Any fibers that were identified by the AN seed voxel were also excluded.

Ventral Lateral Thalamic Nucleus (VL). The VL seed region was defined medially by a vertical line drawn down from the medial edge of the bend of the internal capsule (the intersection between the anterior limb of the internal capsule and the body of the internal capsule), laterally by the edge of the internal capsule, anteriorly by a horizontal line extending from the posterior edge of the anterior limb of the internal capsule, and posteriorly by a horizontal line extending from the posterior boundary of the putamen. Fibers which were previously identified with either the AN or VA seed voxels were excluded.

Dorsal Medial Thalamic Nucleus (DM). The DM seed region was defined medially by the edge of the thalamic body, laterally by a vertical line extending from the posterior edge of the anterior limb of the medial edge of the internal capsule, posteriorly by a horizontal line extending from the posterior edge of the posterior limb of the internal capsule and anteriorly by a horizontal line extending from the posterior edge of the putamen. Fibers which were previously identified with either the AN, VA, or VL seed voxels were excluded.

Ventral Posterior Lateral and Medial Thalamic Nuclei (VPM/VPL). The VPL/VPM seed region was defined anteriorly by a horizontal line extending from the posterior edge of the putamen, laterally by a vertical line extending from the posterior intersection of the internal capsule and putamen, medially by a vertical line extending the intersection of the anterior limb of the internal capsule with the anterior edge of the putamen and posteriorly by the posterior edge of the thalamus. Fibers that were previously identified with either the AN, VA, VL, or DM seed voxels were excluded.

Pulvinar (PU). A seed region was also placed in the pulvinar. This region was defined medially and posteriorly by the edge of the thalamus, laterally by a vertical line extending from the medial intersection of the anterior limb of the internal capsule and body of the internal capsule and anteriorly by a horizontal line extending from the intersection of the posterior limb of the internal capsule and putamen. Fibers that were previously identified with either the AN, VA, VL, DM, or VPL/VPM seed voxels were excluded.

Calculation of FA in the Substantia Nigra. Regions of interest (ROI) were hand drawn in the substantia nigra based upon the B0 images as shown in Figure 2. The B0 (which is essentially a T2-weighted image) for dorsal and ventral SN are seen in the top two images. Our attempts to differentiate between dorsal and ventral were unsuccessful due to image resolution. As such, we instead drew one region of interest in the substantia nigra. The region of interest drawn in the substantia nigra was 4 voxels in diameter.

We also attempted to draw regions in the subthalamic nucleus and red nucleus. The overall head size of the participants in the study (largely male), introduced too much artifact to reliably identify these structures.

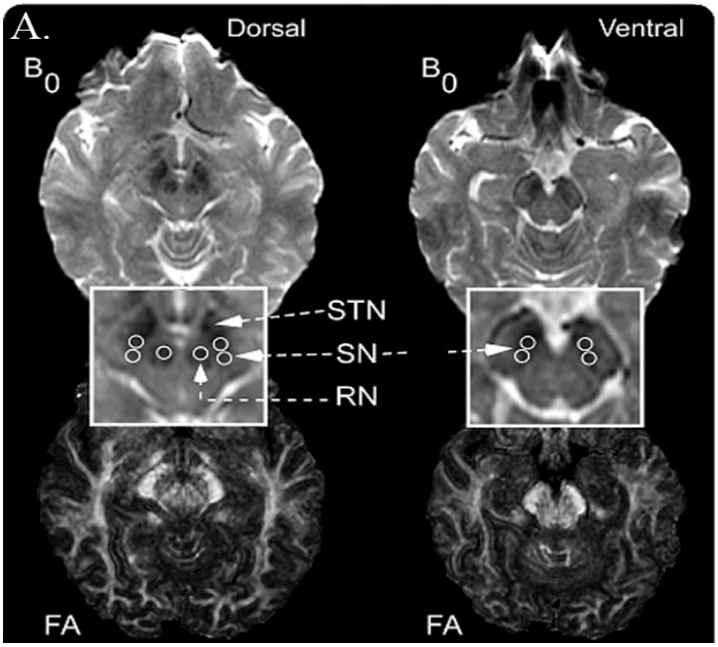

Figure 2. Methods for drawing the regions of interest in the substantia nigra on the B0 image.

3c. Health Variables and Data Analyses

Our final study analytic dataset included the MRI measures (described above) that are specific to the current study as well as data obtained as part of the original GWIC study. This included demographic information and veteran-reported data on symptoms, health history and deployment history. It also included physical assessment measures taken at the time of the study, results of standardized health index assessments (Veterans SF36, McGill Pain Index, Multidimensional Fatigue Inventory, Pittsburgh Sleep Quality Index) and data from a battery of neurocognitive tests conducted for the GWIC study (Steele 2021). We also evaluated GWI severity symptom burden within each symptom domain by generating ordinal summary scores for the presence/severity of chronic symptoms consistent with Kansas domain scoring, where 0 = no symptom, 1 = mild symptom, 2 = moderate symptom, and 3 = severe symptom. Summary scores for individual symptom domains were scaled to values 0-10 and overall GWI severity scores (summary index of symptom occurrence and severity in all domains) were scaled to values 0-100.

Data analyses provided initial comparisons between GWI cases and controls on all demographic, military, and baseline health characteristics of interest. Differences were tested for statistical significance using chi square tests for categorical variables and t tests for continuous variables with normal or near normal distributions, with p values determined according to whether variance differed in cases and controls.

Differences in white matter integrity in thalamic nuclei and substantia nigra were evaluated by comparing mean values in cases vs. controls for all FA measures. Assessment of the correlation of FA measures with GWI severity, symptom severity, and scores on standardized health assessments and neurocognitive testing utilized Spearman's correlation coefficients. Analyses to determine bivariate association of white matter integrity with deployment exposures compared mean FA values in exposed vs. unexposed veterans. To control for relevant covariates and possible confounding error stemming from multiple concurrent exposures, we also evaluated the independent association of each exposure of interest with substantia nigra FA using general linear modeling (GLM) procedures for continuous dependent variables. Final models controlled for effects of two exposures and body mass index. All statistical analyses were conducted using SAS, version 9.4

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Appendix B. Final Report: Study Results/Research Findings

Study Sample

As described, the final sample included n=139 Gulf War veterans, 120 GWI cases and 19 veteran controls. Sample characteristics are detailed in Table 1. As shown, there were no significant demographic differences between GWI cases and controls, although cases were, on average, somewhat younger and had a higher proportion of females (22% cases, 5% controls). In addition, cases were significantly more likely than controls to have served in the enlisted ranks during the war (85% cases vs. 58% controls, p=0.005).

Table 1. Demographic, Military, and Deployment Characteristics of Study Sample

	Total Sample (n=139)	GWI Cases (n= 120)	Veteran Controls (n=19)	GWI Case vs. Control Statistical Comparisons
Demographic Characteristics				
Age (years)				
43-49	44 %	45 %	37 %	p = 0.82
50-59	41 %	41 %	42 %	
60-69	12 %	12 %	16 %	
70+	3 %	2 %	5%	
mean age (years)	53.0	52.7	54.8	p = 0.19
Sex				
Female	19 %	22 %	5 %	p = 0.09~
Male	81 %	78 %	95 %	
Race				
Black/African American	7 %	7 %	5 %	p = 0.62
White/Caucasian	84 %	85 %	79 %	
Other/Multiracial	9 %	8 %	16 %	
Ethnicity				
Hispanic	5 %	6 %	0	p = 0.27
Non-Hispanic	95 %	94 %	100 %	
Education level				
High school/GED	5 %	3 %	16 %	p = 0.05~
Some additional training after high school	46 %	47 %	37 %	
4 year college degree	19 %	20 %	11 %	
Advanced degree	31 %	30 %	37 %	
mean education years	15.1	15.0	15.2	p = 0.75
Military Characteristics				
Branch of Service in 1990				
Army	65 %	63 %	79 %	p = 0.50
Navy	12 %	12 %	11 %	
Air Force	6 %	6 %	5 %	
Marines	17 %	18 %	5 %	
Military Rank in 1990				
Enlisted ranks	81 %	85 %	58 %	p < 0.01**
Officer	19 %	15 %	42 %	

GWI = Gulf War illness

~ = trend towards statistical significance, p < 0.10; * = statistically significant, p < 0.05; ** = statistically significant, p < 0.01

Table 2. General Health Characteristics of GWI Cases and Controls in Study Sample

	GWI Cases (n= 120)	Veteran Controls (n=19)	GWI Case vs. Control Statistical Comparisons
Medical History: reported physician diagnoses			
Hypertension	42 %	26 %	p = 0.19
Chronic fatigue syndrome	24 %	0	p = 0.01*
Irritable bowel syndrome	32 %	5 %	p = 0.02*
Posttraumatic stress disorder (PTSD)	52 %	28 %	p = 0.01*
Depression	46 %	21 %	p = 0.04*
Smoking History			
Regular smoker during deployment	32 %	26 %	p = 0.60
Regular smoker at time of study	9 %	5 %	p = 0.63
Health Measures at Time of Study			
Height (mean inches)	69.0	70.3	p = 0.16
Weight (mean pounds)	212.0	211.6	p = 0.97
Body Mass Index (mean)	31.2	30.0	p = 0.22
Pulse (mean beats/min.)	68.7	69.2	p = 0.85
Temperature (mean degrees F)	97.7	97.9	p = 0.25
Resting systolic blood pressure (SBP)	134.6	135.0	p = 0.92
Resting diastolic blood pressure (DBP)	82.4	80.2	p = 0.28
DBP drops 10 or more points on arising	5 %	0	p = 0.32
SBP drops 20 or more points on arising	7 %	0	p = 0.24
Standardized Health Index Assessments			
VR36 PCS: Veterans SF36-Physical Component (mean score, 0-100)	35.5	49.9	p<0.0001**
VR36 MCS: Veterans SF36-Mentall Component (mean score, 0-100)	41.5	52.8	p<0.001**
McGill Pain Index (mean score, 0-78)	31.8	14.4	p<0.0001**
MFI: Multidimensional Fatigue Inventory (mean score, 20-100)	70.4	47.7	p<0.0001**
PSQI: Pittsburgh Sleep Quality Index (mean score, 0-21)	12.7	8.1	p<0.0001**
Neurocognitive Testing Measures			
CVLT-Recall—Short Delay (mean correct, 0-16)	9.5	10.1	p = 0.44
CVLT-Recall—Long Delay (mean correct, 0-16)	9.9	10.9	p = 0.20
DKEFS: Trails A (mean seconds)	33.1	25.3	p<0.0001**
DKEFS: Trails B (mean seconds)	75.0	68.4	p = 0.39
Gulf War Illness Symptom Summary Scores			
Fatigue/Sleep Domain Score (mean, 0-10)	6.6	1.0	p<0.0001**
Pain Domain Score (mean, 0-10)	5.6	1.0	p<0.0001**
Neuro/cognitive/mood Score (mean, 0-10)	4.5	1.3	p<0.0001**
Gastrointestinal Score (mean, 0-10)	3.9	0.2	p<0.0001**
Respiratory Domain Score (mean, 0-10)	2.7	0.5	p<0.0001**
Skin Domain Score (mean, 0-10)	2.7	0.3	p<0.0001**
Overall Symptom Severity Score (mean,1-100)	43.2	7.1	p<0.0001**

GWI = Gulf War illness

~ = trend towards statistical significance, p < 0.10; * = statistically significant, p < 0.05; ** = statistically significant, p < 0.01

Baseline Health Characteristics
General health characteristics of GWI cases and controls are shown in Table 2. Cases were in substantially worse health, overall, than controls as exhibited by medical history, multiple standardized health index scores, and veteran-reported chronic symptoms. For example, general health status of veteran controls, measured by mean Veterans SF36 summary scores, were almost exactly the normal population values of 50 for both physical and mental health, while SF36 scores for GWI cases were substantially below normal values.

Despite major differences in health status, there were no significant case/control differences in vital signs or other objective physical measures at the time of data collection. This included data obtained on positional changes in blood pressure that are suggestive of orthostatic hypotension. This problem has been associated with a number of neurological disorders including Parkinson's Disease and dysautonomia but affected very few veterans in our sample.

MRI Measures: Brain Structures
For baseline comparison purposes, we obtained measures of intracranial volume and volume of several prominent brain structures including brainstem, corpus callosum, hippocampus, caudate, putamen, basal ganglia, and thalamus. Mean intracranial volume (ICV) was somewhat less in cases vs. controls (p=0.09), and individual ICV was controlled for when assessing all brain measures. Mean hippocampus volume in GWI cases (8030 mm^3) was significantly less than in controls (8944 mm^3, p=0.001), but no other significant case-control volumetric differences were identified.

Diffusion Tensor Imaging (DTI): White Matter Fractional Anisotropy (FA) Measures
Thalamic nuclei. We examined white matter integrity in six thalamic nuclei by determining FA in defined regions, as previously detailed. Results are provided in Table 3. As shown, mean FA values were similar for cases and controls in the pulvinar (PU) and ventral postmedial and postlateral nuclei (VMP/VPL). DTI measures indicated a lower mean FA in GWI cases vs. controls that approached significance in two thalamic nuclei: the anterior nucleus (AN) (p = 0.054) and the dorsomedial nucleus (DM) (p=0.073)

Table 3. Diffusion Tensor Imaging:
Mean Fractional Anisotropy (FA) Values in Thalamic Nuclei and Substantia Nigra: GWI Cases vs. Controls

	Mean Fractional Anisotropy (FA) Values		
Thalamic Nuclei	GWI Cases n=120	Veteran Controls n=19	Case vs.Control Statistical Comparisons
AN: Anterior Nucleus	0.336	0.366	p = 0.05~
VA: Ventral Anterior Nucleus	0.337	0.351	p = 0.11
DM: Dorsomedial Nucleus	0.320	0.351	p = 0.07~
VL: Ventral Lateral Nucleus	0.332	0.345	p = 0.17
VPM/VPL: Ventral Postmedial + Ventral Postlateral Nucleus	0.351	0.357	p = 0.78
PU: Pulvinar	0.282	0.293	p = 0.62
SN: Substantia Nigra	0.405	0.523	p<0.001**

GWI = Gulf War illness
~ = trend towards statistical significance, p < 0.10; * = statistically significant, p < 0.05; ** = statistically significant, p < 0.01

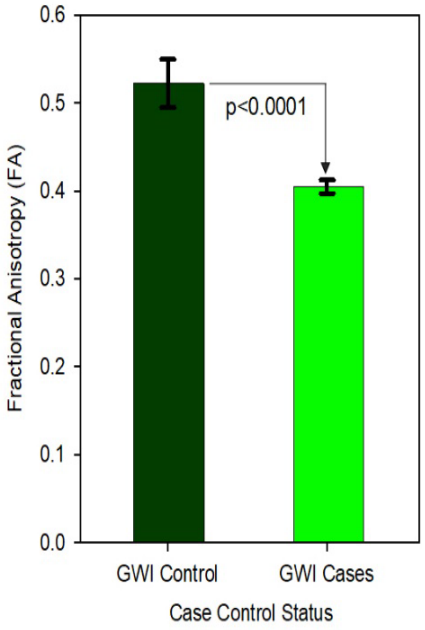

Figure 1. Mean fractional anisotropy in the substantia nigra (SN) of GWI cases and controls. Error bars represent ± 1 SEM.

Substantia Nigra. The primary structure of interest for the current study is the substantia nigra (SN), a region in the midbrain and part of the basal ganglia. The SN is the most critical region for dopamine production in the brain and it therefore affects multiple central nervous functions that include movement control, cognitive executive function, and aspects of mood and emotion. The most studied effect of dopaminergic neuron loss in the SN is that it leads to Parkinson's Disease (PD), and MRI DTI measures have been investigated as possible biomarkers for PD and other neurodegenerative conditions.

As seen in Table 3 and graphed in Figure 1, we identified a highly significant difference in DTI FA between GWI cases (mean = 0.405) and controls (mean = 0.523) in the SN ($p<0.0001$). This indicates substantially reduced white matter integrity in the SN of study veterans with GWI, with veteran controls exhibiting 29% greater SN FA than GWI cases.

DTI Fractional Anisotropy: Additional Exploratory Findings. Although not shown in the table, DTI data collected at the GWIC Houston site provided sufficient resolution to allow us to determine FA in the cortical spinal tract (CST) in a subsample of 32 GWI cases and 4 controls for whom data quality was minimally affected by movement or artifact. Despite the small number of subjects, we noted a significant case-control difference, with CST FA significantly lower in GWI cases (mean=0.540) vs. controls (mean=0.658), p=0.012. And, although not specifically interpretable with data collected for the current study, we also noted a significant correlation between SN FA and hippocampus size in the full study sample (n=139). That is, reduced white matter integrity in the substantia nigra was significantly correlated (r=0.23,p=0.013) with smaller hippocampus volume.

Evaluation of Reduced White Matter Integrity in Relation to Veterans' Health Characteristics and Gulf War Exposures

Due to the prominent association of GWI case status with reduced FA in the substantia nigra and possible implications for GWI etiology, clinical presentation, and prognosis, we undertook detailed analyses to determine the degree to which SN FA was associated with veterans' symptoms and illness severity, standardized health index metrics, and exposures during the Gulf War.

Association of White Matter Integrity with Veterans' Symptoms. As previously described, a summary symptom score was determined for each of the six Kansas-defined GWI symptom domains, as well as an overall GWI symptom severity score. We evaluated the degree to which DTI FA values in thalamic nuclei and the substantia nigra correlated with symptom severity in each of the six GWI symptom domains and with overall GWI severity. Results are presented in Table 4. For ease of interpretation, details are provided only for statistically significant correlations.

As shown, among all six thalamic nuclei and six symptoms domains, only three significant correlations between FA values and symptom severity were identified. These included significant negative correlations between fatigue severity and FA in both the anterior nucleus (AN) and ventral anterior nucleus (VN), and a significant negative correlation of VN FA with pain severity. That is, lower AN and VN FA were associated with greater symptom severity in these domains.

Table 4. Fractional Anisotropy (FA) in Thalamic Nuclei and Substantia Nigra: Correlation with GWI Symptom Summary Scores

	Anterior Nucleus FA	Ventral Anterior Nucleus FA	Dorsomedial Nucleus FA	Ventral Lateral Nucleus FA	Ventral Postmedial +Postlateral Nucleus FA	Pulvinar FA	Substantia Nigra FA
Symptom Summary Measure	r[1] p value	r[1] p value	r[1] p value	r[1] p value	r[1] p value	r[1] p value	r[1] p value
Fatigue/Sleep Domain Score							
All Veterans	-0.186 (p=0.049)	-0.190 (p=0.044)	ns	ns	ns	ns	-0.278 (p=0.003)
Cases Only	ns	ns	ns	ns	ns	ns	ns
Pain Domain Score							
All Veterans	ns	-0.298 (p=0.001)	ns	ns	ns	ns	-0.343 (p<0.001)
Cases Only	ns	-0.252 (p=0.012)	ns	ns	ns	ns	-0.241 (p=0.014)
Neuro/Cognitive/ Mood Domain Score							
All Veterans	ns	ns	ns	ns	ns	ns	-0.306 (p=0.001)
Cases Only	ns	ns	ns	ns	ns	ns	-0.195 (p=0.048)
Gastrointestinal Domain Score							
All Veterans	ns	ns	ns	ns	ns	ns	ns
Cases Only	ns	ns	ns	ns	ns	ns	ns
Respiratory Domain Score							
All Veterans	ns	ns	ns	ns	ns	ns	-0.236 (p=0.011)
Cases Only	ns	ns	ns	ns	ns	ns	ns
Skin Domain Score							
All Veterans	ns	ns	ns	ns	ns	ns	-0.305 (p=0.001)
Cases Only	ns	ns	ns	ns	ns	ns	-0.224 (p=0.023)
Overall GWI Symptom Severity Score:							
All Veterans	ns	ns	ns	ns	ns	ns	-0.335 (p<0.001)
Cases Only	ns	ns	ns	ns	ns	ns	-0.207 (p=0.036)

[1] r = Spearman's Correlation coefficient; GWI = Gulf War illness; ns = no significant correlation

In contrast, lower FA in the substantia nigra was significantly correlated with multiple symptom severity indicators, both when all veterans in the sample were evaluated together, and when GWI cases were considered separately. The magnitude of SN FA correlations were also generally greater than those observed for the thalamic nuclei, in relation to both GWI severity overall and individual symptom domains. Among all veterans combined, lower SN FA significantly correlated with greater severity of pain, fatigue, neurocognitive, respiratory, and skin symptoms. A similar pattern was identified when evaluating SN FA in GWI cases alone, although correlation coefficients were somewhat reduced. This indicates that reduced FA in the substantia nigra is associated not only with GWI case status overall, but also with the degree of symptom severity among GWI cases. In GWI cases, lower FA in the substantia nigra was most strongly correlated with pain severity. Correlations with GWI severity overall and neurocognitive symptoms were less pronounced but also significant.

Table 5. Substantia Nigra Fractional Anisotropy (FA): Correlation with Individual Symptoms

	Correlation with Substantia Nigra FA	
	All Veterans	GWI Cases Only
Symptom Presence/Severity (0-4)	r[1] p value	r[1] p value
Fatigue/Sleep Domain symptoms		
Fatigue	-0.270 (p=0.004)	ns
Not feeling rested after sleep	-0.242 (p=0.009)	ns
Feeling unwell after exercise/exertion	-0.242 (p=0.009)	ns
Neuro/Cognitive/Mood Domain symptoms		
Feeling down or depressed	-0.326 (p<0.001)	-0.260 (p=0.008)
Feeling irritable/have angry outbursts	-0.293 (p=0.002)	-0.217 (p=0.028)
Eyes very sensitive to light	-0.270 (p=0.004)	ns
Difficulty remembering recent information	-0.268 (p=0.004)	-0.194 (p=0.050)
Difficulty concentrating	-0.251 (p=0.007)	ns
Night sweats	-0.247 (p=0.008)	-0.215 (p=0.029)
Numbness/tingling in extremities	-0.239 (p=0.010)	-0.205 (p=0.039)
Pain Domain symptoms		
Pain in joints	-0.330 (p<0.001)	-0.215 (p=0.030)
Body pain, hurt all over	-0.287 (p=0.002)	-0.206 (p=0.037)
Pain in muscles	-0.278 (p=0.003)	-0.192 (p=0.051)
Skin Domain Symptoms		
Other skin problems	-0.265 (p=0.004)	-0.201 (p=0.039)
Skin rashes	-0.264 (p=0.010)	ns

[1] r = Spearman's Correlation coefficient; GWI = Gulf War Illness; ns = no significant correlation

We also evaluated individual symptoms in domains most correlated with SN FA, to determine which symptoms appeared to be most strongly associated with the identified reduction in SN FA. As shown in Table 5, lower SN FA appeared to most strongly correlate with joint pain, feelings of depression and irritability, and generalized pain throughout the body when all veterans were considered together, as well as among GWI cases alone.

Lastly, we explored possible correlation of lower SN FA with standardized health index measures and neurocognitive test results, as shown in Table 6. Similar to the symptom severity indicators, lower SN FA was most strongly correlated with pain severity, as measured by the McGill Pain Index, but was also significantly associated with overall health status (VR36 PCS) and fatigue severity, as measured by the

Multidimensional Fatigue Inventory. Lower SN FA was also significantly correlated with reduced performance on the CVLT long delay recall test, suggesting a possible association with poorer memory retention.

Table 6. Correlation of Substantia Nigra Fractional Anisotropy (FA) Values with Standardized Health Index and Cognitive Measures

Physical and Mental Health Index Scores	**Correlation with Substantia Nigra FA** r[1] p value
Veterans SF 36: Physical Component Summary Score	0.215 (p=0.024)~
Veterans SF 36: Mental Component Summary Score	0.196 (p=0.040)*
McGill Pain Index Score (0–78)	-0.280 (p=0.004)**
Multidimensional Fatigue Inventory Score (20-100)	-0.236 (p=0.013)*
Pittsburgh Sleep Quality Index Score (0-21)	-0.165 (p=0.087)~
Neurocognitive Testing Measures	
CVLT-Recall—Short Delay	0.170 (p=0.070)~
CVLT-Recall—Long Delay	0.200 (p=0.032)*
DKEFS: Trails A	-0.038 (p=0.684)
DKEFS: Trails B	-0.157 (p=0.095)~

[1] r = Spearman's Correlation coefficient
~ = trend towards statistical significance, p < 0.10; * = statistically significant, p < 0.05; ** = statistically significant, p < 0.01

Association of White Matter Integrity with Veterans' Experiences and Exposures During the Gulf War

We also examined the degree to which FA in thalamic nuclei and the substantia nigra were associated with veteran-reported exposures during Gulf War deployment. Prominent exposures of interest included those most consistently associated with GWI across all studies of Gulf War veterans (extended use of pesticides and pyridostigmine bromide pills). We also evaluated effects of other prominent Gulf War experiences and exposures of concern (oil well fires, direct participation in ground combat, and being in areas where chemical alarms sounded, as a proxy for possible exposure to nerve agents). In addition, we evaluated possible association of reduced FA with having one or more mild traumatic brain injuries (mTBIs) in theater, which has recently been reported as a risk factor for GWI in the GWIC cohort (Janulewicz 2018, Cheng 2020, Steele 2021). Significant associations of white matter integrity with exposures were determined for each region by comparing mean FA values between exposed and not exposed veterans. Bivariate results for all exposures and regions of interest are provided in Table 7a.

Table 7a. Association of Deployment Exposures with Mean Fractional Anisotropy (FA) Values in Thalamic Nuclei and Substantia Nigra
All Veterans: (Bivariate, Unadjusted)

Experience/Exposure	Exposed?	Mean Anterior Nucleus FA	Mean Ventral Anterior Nucleus FA	Mean Dorsomedial Nucleus FA	Mean Ventral Lateral Nucleus FA	Mean Ventral Postmedial +Postlateral Nucleus FA	Mean Pulvinar FA	Mean Substantia Nigra FA
Saw smoke from oil fires ≥ 7 days	Yes	0.3352 ns	0.3378 ns	0.3304 ns	0.3307 ns	0.3574 ns	0.2762 ns	0.4070 ns
	No	0.3488	0.3403	0.3158	0.3373	0.3460	0.2844	0.4368
Heard chemical alarms sounded ≥ 7 days	Yes	0.3229 **	0.3375 ns	0.3182 ns	0.3335 ns	0.3519 ns	0.2865 ns	0.3958 *
	No	0.3537	0.3397	0.3338	0.3328	0.3542	0.2774	0.4387
Directly involved in ground combat	Yes	0.3402 ns	0.3419 ns	0.3159 ns	0.3211 ~	0.3567 ns	0.2807 ns	0.3994 ~
	No	0.3401	0.3361	0.3325	0.3429	0.3503	0.2819	0.4317
Used pesticides cream/spray on skin ≥ 7 days	Yes	0.3373 ns	0.3393 ns	0.3252 ns	0.3319 ns	0.3463 ns	0.2790 ns	0.3994 **
	No	0.3447	0.3377	0.3247	0.3351	0.3645	0.2853	0.4478
Used PB (NAPP) pills ≥ 7 days	Yes	0.3356 ns	0.3370 ns	0.3261 ns	0.3334 ns	0.3489 ns	0.2908 *	0.4026 *
	No	0.3456	0.3408	0.3238	0.3328	0.3584	0.2700	0.4356
Experienced one or more mTBIs in theater	Yes	0.3277 ns	0.3294 ns	0.3274 ns	0.3339 ns	0.3343 ~	0.2890 ns	0.3755 **
	No	0.3438	0.3440	0.3232	0.3342	0.3621	0.2808	0.4354

Note: Statistical tests compare mean FA values in exposed vs. not exposed, accounting for differences in variance between the 2 groups
ns = not statistically significant; ~ = trend towards statistical significance, $p < 0.10$
* = statistically significant, $p < 0.05$
** = statistically significant, $p < 0.01$

As previously observed in relation to case status and symptoms, Gulf War exposures were generally *not* associated with FA measures of white matter integrity in thalamic nuclei. Only two significant associations were identified in bivariate analyses—one each in the anterior nucleus (AN) and pulvinar (PU). Veterans who were in areas where chemical alarms had sounded for 7 days or longer had significantly reduced AN FA. And veterans who used pyridostigmine bromide (PB) pills for 7 days or more had significantly *better* mean FA in the pulvinar, raising the possibility of a protective effect.

In contrast, all exposures evaluated were associated with *lower* FA in the substantia nigra, with four significant exposure-FA associations identified in bivariate analyses. Most prominently, veterans who reported having one or more mTBIs during deployment had significantly lower mean FA values in the substantia nigra ($p<0.001$) compared to veterans who did not report mTBIs in theater. Similarly, veterans who reported using skin pesticide cream or sprays for 7 days or longer had significantly reduced mean FA in the substantia nigra ($p=0.006$) compared to unexposed veterans. In addition, veterans who reported using PB pills or being in areas where chemical alarms sounded for 7 days or longer had significantly lower SN FA than unexposed veterans in bivariate analyses.

A standard issue for all Gulf War exposure assessments is the potential for confounding error due to multiple overlapping exposures in theater. In order to determine the independent association of each exposure with SN FA, we utilized multivariable models that controlled for significant concurrent exposures, as well as body mass index (bmi) which was also associated with SN FA in bivariate analysis. Final results are shown in Table 7b and indicate that in our sample, only 2 Gulf War experiences/ exposures were significantly associated with lower white matter integrity in adjusted analyses—having one or more mTBIs during deployment and use of skin pesticides for seven days or longer.

Table 7b. Adjusted[1] Association of Substantia Nigra Fractional Anisotropy (FA) with Deployment Exposures

	Association of Exposure with Mean Substantia Nigra FA	
Exposure	unadjusted	adjusted
Saw smoke from oil fires ≥ 7 days	p = 0.100	p = 0.310
Heard chemical alarms sounded ≥ 7 days	p = 0.022 *	p = 0.150
Directly involved in ground combat	p = 0.059~	p = 0.200
Used pesticides cream/spray on skin ≥ 7 days	p = 0.006 **	p = 0.026 *
Used PB (NAPP) pills ≥ 7 days	p = 0.054 *	p = 0.287
Experienced one or more mTBIs in theater	p = 0.001 **	p = 0.011 *

[1] GLM models compare mean FA value in exposed vs. not exposed veterans, adjusted for BMI, mTBI in theater, use of skin pesticides ≥ 7 days
~ = trend towards statistical significance, $p < 0.10$; * = statistically significant, $p \leq 0.05$; ** = statistically significant, $p < 0.01$

Exploratory Analyses: Potential utility of DTI FA measures as biomarkers for identifying GWI case status and/or GWI subgroups of interest

Our observation that reduced FA in the substantia nigra was consistently associated with GWI case status, severity of prominent GWI symptoms, and GWI severity overall raised the question of whether a threshold value of SN FA might be useful, clinically or for research purposes, for differentiating GWI cases from controls. We therefore evaluated the distribution of SN FA values to empirically identify a value to be used in exploratory analyses to address this question. Of course, identified metrics cannot be considered definitive, owing to the exploratory nature of the assessment and sample limitations.

In our sample, 90% of cases had SN FA values ≤ 0.50, but only 4 controls did. Use of the SN FA value range of ≤ 0.50 provided a positive predictive value (PPV) of 0.96 , i.e., this value correctly predicted 96% of GWI cases. In addition, use of this value as a candidate screening or diagnostic indicator provided 90% sensitivity, but only 67% specificity for correctly identifying GWI case status. This approach was preliminary, but could be considerably refined and potentially validated using more detailed measures in an appropriate sample. For example, the substantia nigra is not a single uniform structure. It is possible that a particular SN area is more sensitive to GWI risk factors and/or pathobiological processes and would provide more precise screening metrics. Still, we believe our exploratory findings provide a clear example and insights concerning the potential use of SN FA, and possibly other FA measures, for identifying GWI case status, and/or important GWI subgroups, for research and clinical purposes.

Additional Results and Observations from Collected Data

Unexpected MRI Findings of Brain Pathology

Although not specifically a component of our study question, a key unexpected finding that has not previously been indicated in the literature, was that veterans in the study showed significant pathology on anatomical imaging. This pathology largely took the form of atrophy and both white and gray matter lesions. A total of 13 veterans demonstrated lesions in the brainstem. Beyond the relevance of this finding on its own, this made analysis of the substantia nigra invalid in these veterans. Additionally, a total of 39 veterans had lesions in the corpus callosum. The observation of atrophy and lesions are in addition to three veterans who were excluded for MRI evidence of overt stroke (n=2) and abnormal cerebrospinal fluid flow (n=1).

In terms of atrophy, most studies have relied solely upon calculation of regional atrophy using normalized, processed images. This is not the same approach as is used in clinical diagnostic imaging. Although not yet validated, we commonly observed visible atrophy around the corpus callosum and anterior and parietal lobules. Our group isfunded to validate these visual observations in a current study (award W81XWH-21-1-0963).

Study Limitations and Scanning Data Issues

It is important to note a number of study limitations that may have affected study results. As described, the unexpected but large amount of pathology limited our ability to obtain clear measures in some veterans. In addition, study participants were evaluated at two different sites, which is in most respects a study strength. Still, differences in resolution of the DTI sequences across scanners and between upgrades affected our ability to differentiate thalamic nuclei boundaries in some veterans. We also lacked the level of scanning resolution needed to pinpoint where a specific region or arm of the SN was more prominently associated with our outcomes. Large head size of some veteran subjects relative to head coil size also limited how much of the brain stem we could cover. Lastly, while the final sample size was large (n=139) in the context of imaging studies, it included a relatively low percentage of veteran controls. This would be expected to affect the study's power to detect some differences between GWI cases and controls.

Discussion and Implications of Research Findings

The primary finding of this study is that GWI is associated with significant depletion of brain white matter integrity affecting the substantia nigra (SN), as demonstrated by reduced fractional anisotropy (FA). The observed SN effects were significantly associated with GWI case status and overall severity, with greater severity in some symptom domains, and with specific exposures during Gulf War deployment. The observed SN changes would be expected to result in substantial reduction of central dopamine levels and neurotransmission. Reductions in FA and associations with GWI symptoms and Gulf War exposures were observed almost exclusively in the SN. Although reduction in dopamine function might also be expected to reduce input into the thalamus, we found only limited effects on fibers in the thalamic nuclei. However, our findings are preliminary and require confirmation in a separate sample. This would ideally include more detailed high resolution evaluation of specific SN regions and associated tracts.

Although specific results in the current study are novel, they are consistent with early findings that suggested the potential for dopaminergic alterations in the brain to underlie and/or contribute to GWI symptoms. Specifically, Haley and colleagues (Haley, 2000a) reported initial evidence for significant neuronal alterations in the basal ganglia in symptomatic Gulf War veterans and associated findings indicative of alterations in central dopamine levels (Haley 2000b). Current results also parallel findings from animal studies that have demonstrated persistent alterations in central dopamine metabolism and signaling following low-dose exposure to toxicants associated with Gulf War deployment (Torres-Altoro 2011, Oswal 2013). Dysregulation of dopaminergic neurotransmission has also been demonstrated in civilian patients with conditions such as fibromyalgia and chronic fatigue syndrome, and investigated as a possible target for therapeutic intervention (Homan 2005, Wood 2007, Miller 2014, Albrecht 2016, Ledermann 2016,2021).

Dr. Little's previous collaborative work across groups led to the identification and validation of methods to characterize integrity of the SN in humans using DTI. This work, developed in the context of early Parkinson's Disease (PD) literature, used DTI to provide the first in-vivo assessment of age-related degeneration in specific segments of the SN of humans and also provided a marker for early stage PD (Vaillancourt 2009, 2012) using FA measures in the SN. This provided critical support for methods used in the current study for evaluating SN white matter integrity in veterans with GWI.

Our study hypothesis that the substantia nigra is impacted in veterans with Gulf War illness was supported. We interpret this, based upon the literature and our own past findings, to indicate that there is a reduction in dopaminergic cells in the substantia nigra. If true, the impact functionally would potentially be a gross reduction in dopamine signaling and dopamine throughout the central nervous system. In the brain, this could have impacts most directly on pain gating, somatosensory and motor function and frontal lobe function.

References

1. Albrecht DS, MacKie PJ, Kareken DA, Hutchins GD, Chumin EJ, Christian BT, Yoder KK. Differential dopamine function in fibromyalgia. Brain Imaging Behav. 2016: 0(3):829-39.

2. Cheng CH, Koo BB, Calderazzo S, Quinn E, Aenlle K, Steele L, Klimas N, Krengel M, Janulewicz P, Toomey R, Michalovicz LT, Kelly KA, Heeren T, Little D, O'Callaghan JP, Sullivan K. Alterations in high-order diffusion imaging in veterans with Gulf War Illness is associated with chemical weapons exposure and mild traumatic brain injury. Brain Behav Immun. 2020 Oct;89:281-290.

3. Gifford EJ, Vahey J, Hauser ER, Sims KJ, Efird JT, Dursa EK, Steele L, Helmer DA, Provenzale D. Gulf War illness in the Gulf War Era Cohort and Biorepository: The Kansas and Centers for Disease Control definitions. Life Sci. 2021 Aug 1;278:119454.

4. Haley RW, Marshall WW, McDonald GG, Daugherty MA, Petty F, Fleckenstein JL. Brain abnormalities in Gulf War syndrome: evaluation with 1H MR spectroscopy. Radiology. 2000a:215(3):807-17.

5. Haley RW, Fleckenstein JL, Marshall WW, McDonald GG, Kramer GL, Petty F. Effect of basal ganglia injury on central dopamine activity in Gulf War syndrome: correlation of proton magnetic resonance spectroscopy and plasma homovanillic acid levels. Arch Neurol. 2000b: 57(9):1280-5.

6. Holman AJ, Myers RR. A randomized, double-blind, placebo-controlled trial of pramipexole, a dopamine agonist, in patients with fibromyalgia receiving concomitant medications. Arthritis Rheum. 2005:52(8):2495-505.

7. 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. The Multiple hit hypothesis for Gulf War Illness: Self-reported chemical/biological weapons exposure and mild traumatic brain injury. Brain Sci. 2018: 8(11):198.

8. Jiang H, van Zijl PC, Kim J, Pearlson GD, Mori S. DtiStudio: Resource program for diffusion tensor computation and fiber bundle tracking. Comput Methods Programs Biomed. 2006;81(2):106-16.

9. Ledermann, K. 2021. "Understanding and "Restoring Dopaminergic Function in Fibromyalgia Patients (FIBRODOPA)" Details at ClinicalTrials.gov:: https://clinicaltrials.gov/ct2/show/NCT04451564

10. Ledermann K, Jenewein J, Sprott H, Hasler G, Schnyder U, Warnock G, Johayem A, Kollias S, Buck A, Martin-Soelch C. Relation of dopamine receptor 2 binding to pain perception in female fibromyalgia patients with and without depression--A [^{11}C] raclopride PET-study. Eur Neuropsychopharmacol. 2016;26:320-330.

11. Little DM, Kraus MF, Joseph J, Geary EK, Susmaras T, Zhou XJ, Pliskin N, Gorelick PB. Thalamic integrity underlies executive dysfunction in traumatic brain injury. Neurology. 2010 Feb 16;74:558-64.

12. Miller AH, Jones JF, Drake DF, Tian H, Unger ER, Pagnoni G. Decreased basal ganglia activation in subjects with chronic fatigue syndrome: association with symptoms of fatigue. PLoS One. 2014:23: e98156.

13. Oswal DP, Garrett TL, Morris M, Lucot JB. Low-dose sarin exposure produces long term changes in brain neurochemistry of mice. Neurochem Res. 2013: 38:108-16.

14. Research Advisory Committee on Gulf War Veterans' Illnesses . Gulf War Illness and the Health of Gulf War Veterans: Scientific Findings and Recommendations. U.S. Government Printing Office; Washington, DC, USA: 2008.

15. Research Advisory Committee on Gulf War Veterans' Illnesses . Gulf War Illness and the Health of Gulf War Veterans: Research Update and Recommendations, 2009–2013. U.S. Government Printing Office; Washington, DC, USA: 2014

16. Steele L, Klimas N, Krengel M, Quinn E, Toomey R, Little D, Abreu M, Aenlle K, Killiany R, Koo BB, Janulewicz P, Heeren T, Clark AN, Ajama J, Cirillo J, Buentello G, Lerma V, Coller JK, Sullivan K. Brain-Immune Interactions as the Basis of Gulf War Illness: Clinical Assessment and Deployment Profile of 1990-1991 Gulf War Veterans in the Gulf War Illness Consortium (GWIC) Multisite Case-Control Study. Brain Sci. 2021;11:1132.

17. 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.

18. Torres-Altoro MI, Mathur BN, Drerup JM, Thomas R, Lovinger DM, O'Callaghan JP, Bibb JA. Organophosphates dysregulate dopamine signaling, glutamatergic neurotransmission, and induce neuronal injury markers in striatum. J Neurochem. 2011;119:303-13.

19. Vaillancourt DE, Spraker MB, Prodoehl J, Abraham I, Corcos DM, Zhou XJ, Comella CL, Little DM. High-resolution diffusion tensor imaging in the substantia nigra of de novo Parkinson disease. Neurology. 2009; 72:1378-84.

20. Vaillancourt DE, Spraker MB, Prodoehl J, Zhou XJ, Little DM. Effects of aging on the ventral and dorsal substantia nigra using diffusion tensor imaging. Neurobiol Aging. 2012;33:35-42.

21. Wakana S, Jiang H, Nagae-Poetscher LM, van Zijl PC, Mori S. Fiber tract-based atlas of human white matter anatomy. Radiology. 2004 Jan;230:77-87.

22. White RF, Steele L, O'Callaghan JP, Sullivan K, Binns JH, Golomb BA, Bloom FE, Bunker JA, Crawford F, Graves JC, Hardie A, Klimas N, Knox M, Meggs WJ, Melling J, Philbert MA, Grashow R. 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.

23. Wood PB, Patterson JC 2nd, Sunderland JJ, Tainter KH, Glabus MF, Lilien DL. Reduced presynaptic dopamine activity in fibromyalgia syndrome demonstrated with positron emission tomography: a pilot study. J Pain. 2007; 8:51-8.