Evidence of Hippocampal Structural Alterations in Gulf War Veterans With Predicted Exposure to the Khamisiyah Plume

Linda L. Chao, PhD, Morgan R. Raymond, BS, Cynthia K. Leo, BA, and Linda R. Abadjian, PhD

Objectives: To replicate and expand our previous findings of smaller hippocampal volumes in Gulf War (GW) veterans with predicted exposure to the Khamisiyah plume. **Methods:** Total hippocampal and hippocampal subfield volumes were quantified from 3 Tesla magnetic resonance images in 113 GW veterans, 62 of whom had predicted exposure as per the Department of Defense exposure models. **Results:** Veterans with predicted exposure had smaller total hippocampal and CA3/dentate gyrus volumes compared with unexposed veterans, even after accounting for potentially confounding genetic and clinical variables. Among veterans with predicted exposure, memory performance was positively correlated with hippocampal volume and negatively correlated with estimated exposure levels and self-reported memory difficulties. **Conclusions:** These results replicate and extend our previous finding that low-level exposure to chemical nerve agents from the Khamisiyah pit demolition has detrimental, lasting effects on brain structure and function.

According to the Department of Defense (DOD), 100,000 US military personnel were potentially exposed to chemical warfare agents when United States (US) combat engineers destroyed a munition storage depot at Khamisiyah, Iraq in March 1991, after the cease fire that ended the Persian Gulf War had been declared.[1] We previously reported evidence of smaller total hippocampal[2] and hippocampal subfield (ie, CA2, CA3, and dentate gyrus)[3] volumes in two independent cohorts of Gulf War (GW) veterans with predicted exposure to the Khamisiyah plume compared with matched, unexposed GW veterans. However, those earlier studies had several limitations: first, we lacked information about the veterans' apolipoprotein E (ApoE) genotype, which has been associated with smaller total hippocampal[4,5] and smaller CA3/dentate gyrus (DG) volumes in cognitively normal individuals and in patients with Alzheimer's disease (AD).[6] Second, we did not have information about the veterans' history of head injury. There is evidence that physical trauma to the head, either repeated minor injuries or a single major injury, can reduce hippocampal volume.[7–9] Third, although we attempted to match the groups with and without predicted Khamisiyah exposure for Gulf War illness (GWI), or the Centers for Disease Control and Prevention (CDC) chronic multi-symptom illness (CMI) case definition,[10] we did not have information about the severity of veterans' CMI symptoms. Finally, it was curious that we did not find any significant group differences on neuropsychological tests of memory or any significant correlations between hippocampal volume and memory function in veterans with predicted exposure to the Khamisiyah plume given that the hippocampus is an important brain structure for learning and memory processes.[11,12] Therefore, the aims of current study were to: (1) replicate our findings of reduced total and hippocampal subfield volumes in a third, independent cohort of GW veterans with and without predicted exposure to the Khamisiyah plume, this time controlling for variables that we were unable take into account in the previous studies (ie, ApoE genotype, history of head trauma, severity of CMI) and (2) examine the associations between hippocampal volume and performance on a hippocampal-dependent memory task (ie, the California Verbal Learning Test).[13] In exploratory analyses, we also investigated the relationships between estimated cumulative exposure levels, self-reported memory impairment severity, and verbal memory performance.

METHODS

Participants

Participants were all GW veterans recruited from 2014 to 2017 at the San Francisco Veterans Affairs Medical Center (SF VAMC) as part of a VA-funded study on the effects of predicted exposure to the Khamisiyah plume on brain structure and function. The current study focused on 113 GW veterans who: (1) were not part of any of the previous analyses where we found evidence of reduced total hippocampal[2] or hippocampal subfield[3] volume in veterans with predicted exposure to the Khamisiyah plume; (2) had available ApoE genotype information; and (3) had artifact-free structural magnetic resonance imaging (MRI) data. Written informed consent, approved by the University of California, San Francisco and the SF VAMC Institutional Review Boards, was obtained from all study participants.

Study Protocol and Measures

Clinical Assessments

The veterans were evaluated by a Ph.D. level psychologist (L.R.A.) using the Structured Clinical Interview for DSM-IV Diagnosis (SCID),[14] the clinician administered posttraumatic stress disorder (PTSD) scale (CAPS),[15] and an interview version of the life stressor checklist-revised.[16] To capture the veterans' lifetime history of traumatic brain injury (TBI), we used the Ohio State University traumatic brain injury identification (OUS TBI-ID) method.[17,18] A previous study that examined the inter-rater reliability of the SCID for DMS-IV Axis I disorders reported kappa values in the range of 0.61 to 0.83, with a mean kappa of 0.71.[19] The CAPS also has high internal consistency, with an alpha of 0.94 for the total score.[15] Similarly, acceptable to high inter-rater reliability the OSU TBI-ID has been reported for the OSU TBI-ID.[17,18]

Determination of Gulf War Illness (GWI) Case Status

The Kansas Military History and Health Questionnaire[20] was used to query veterans about their GWI symptoms and severity. Subsequently, this information was used to classify veterans as CDC CMI cases[10] and Kansas GWI cases.[20] Although both case definitions have been recommended by the National Academy of Sciences Institute of Medicine (IOM), because the Kansas GWI case definition is more rigorous than the CMI definition, the IOM concluded that the Kansas GWI case definition may be more appropriate for research purposes.[21] For this reason, we used the

From the Center for Imaging of Neurodegenerative Diseases, San Francisco Veterans Affairs Medical Center (Dr Chao, Ms Raymond, Ms Leo, Dr Abadjian); Department of Radiology and Biomedical Imaging (Dr Chao); and Department of Psychiatry (Dr Chao), University of California, San Francisco, San Francisco, California.

This study was supported by VA grant No. CX000798 entitled "Longitudinal Assessment of Gulf War Veterans with Suspected Sarin Exposure."

The authors have no conflicts of interest to report.

Address correspondence to: Linda L. Chao, PhD, 4150 Clement Street, 114M, San Francisco, CA 94121 (linda.chao@ucsf.edu).

DOI: 10.1097/JOM.0000000000001082

Kansas GWI case definition in our statistical analyses. To account for the veterans' present health condition, we also included information about whether veterans had a Kansas GWI exclusionary criterion[20] (ie, diabetes, heart disease other than high blood pressure, lupus, rheumatoid arthritis, seizure disorder, cancer, liver and/or kidney disease, hospitalization within the last 5 years for alcohol or drug dependence, depression, and/or posttraumatic stress disorder) in our analyses.

Image Acquisition

All veterans were scanned at the SF VAMC on a 3 Tesla (T) Siemens Skyra MRI system (Erlangen, Germany) equipped with a 32-channel receiver head coil. The MRI scan protocol included the following: (1) T1-weighted 3D whole brain gradient echo MRI TR/TE/TI = 2500/2.98/1100 ms, $1.0 \times 1.0 \times 1.0\,mm^3$ resolution. (2) T2 weighted turbo spin echo MRI TR/TE 3200/11 ms, $0.9 \times 0.9 \times 3.0\,mm^3$ resolution (for intracranial volume [ICV] estimation), and (3) high resolution T2 weighted fast spin echo sequence for hippocampal subfield volumetry (TR/TE 3990/17 ms, 0.4×0.4 mm in plane resolution, 2 mm slice thickness, 26 interleaved slices, angulated perpendicular to the long axis of the hippocampal formation).

Determination of Total Hippocampal Volume

We used an automated, non-biased atlas-based Bayesian segmentation procedure, applied in FreeSurfer v5.1 (http://surfer.nmr.mgh.harvard.edu/), to derive quantitative estimates of total hippocampal volume from the volumetric T1-weighted MR image.

Determination of Hippocampal Subfield Volumes

We used the automatic segmentation of hippocampal subfields (ASHS)[22] to determine the volumes of the cornu ammonis (CA) subfields CA1, CA2, CA3, dentate gyrus (DG), subiculum, head and tail of the hippocampus, as previously described.[23] The DG label in the current study includes both the granular cell layer of the dentate gyrus and the hilar region, sometimes called CA4. In addition, each hippocampal subfield volume was normalized by the number of slices that spanned the segmentation of that subfield, as previously described.[24]

Intracranial Volume (ICV) Measurement

We used the BET program (FMRIB Image Analysis Group, Oxford University, www.fmrib.ox.ac.uk/fsl) to determine each veterans' intracranial volume (ICV) after checking that all extracranial and skull structures were removed and all intracranial structures were fully preserved in the skull stripped images.

Assessment of Verbal Learning and Memory

We used the California Verbal Learning Test-II (CVLT-II),[13] which has been previously described in detail,[25,26] to assess verbal learning and memory. The dependent variables included in statistical analyses were: number of words correctly recalled following CVLT-II trial 1 (ie, immediate attention and memory), trials 1–5 (ie, total learning), after a short delay (ie, retention), after a longer delay (ie, delayed recall), the number of correctly identified target words in a recognition memory test (ie, ''hits''), and a measure of total recognition discriminability (ie, the extent to which veterans can correctly discriminate target from non-target words in the recognition memory test).

Apolipoprotein E (APOE) Status

As mentioned in the introduction, the APOE ε4 allele has been associated with smaller total hippocampal[4,5] and smaller CA3/DG volumes in cognitively normal individuals and in patients with AD.[6] For this reason, we controlled for APOE genotype in our analyses of hippocampal volume. Veterans with 2/3 or 3/3 combined APOE alleles were classified as APOE ε4-negative while those with 3/4 or 4/4 combined were classified as APOE ε4-positive. Although the APOE ε4 allele is a genetic risk factor for AD,[27,28] the 2/4 combined APOE allele has been associated with a lower risk for AD.[29] Thus, veterans with the 2/4 combined APOE allele were not considered APOE ε4-positive.

Statistical Analysis

IBM SPSS Statistics version 24 (IBM SPSS Statistics for Windows, Version 24.0, IBM Corp, Armonk, NY) was used for statistical analyses. Demographic and descriptive characteristics were compared across the two exposure groups with *t* tests for continuous variables and Fisher exact test for categorical variables. For all analyses, $P < 0.05$ was considered a significant effect unless otherwise described.

We used an analysis of covariance (ANCOVA), with age, sex, ICV, Kansas GWI case and Kansas GWI exclusionary status, current diagnoses of MDD and/or PTSD, current use of psychometric medication (ie, antidepressants, mood stabilizers, and/or anticonvulsants used to control pain), APOE ε2/ε4 status, history of alcohol abuse/dependence, and OUS TBI severity as covariates to examine group differences in total hippocampal volume. Analyses of hippocampal subfield volume were carried out with multivariate analyses of covariance (MANCOVAs) with the same covariates used in analysis of total hippocampal volume. Because we had no hypotheses about laterality effects, the volumes of the left and right total hippocampus and hippocampal subfields were combined to reduce the number of comparisons. Because we hypothesized that we would replicate our previous findings of smaller total and smaller CA2, CA3/DG volumes in veterans with predicted exposure, an alpha value for significance was set at 0.05.

The analyses of group differences in CVLT-II scores were also carried out with MANCOVAs with age, sex, years of education and rank in the GW, Kansas GWI case and Kansas GWI exclusionary status, current diagnoses of MDD and/or PTSD, current use of psychometric medication, APOE ε2 and ε4 status, history of alcohol abuse/dependence, and OSU TBI severity as covariates. These analyses were corrected for multiple comparisons according to the number of dependent variables ($n = 6$) and the average intercorrelations among the variables.[30] With an average intercorrelated correlation of $r = 0.60$, a 2-sided adjusted $P < 0.024$ was considered statistically significant. Because delayed recall is contingent on successful learning, we also repeated the analyses for delayed recall using total learning (ie, performance on trials 1–5) as a covariate.

We used Spearman rank correlations to assess the relationship between total hippocampal volume, hippocampal subfield volumes, CVLT-II test performance, and estimated cumulative exposure doses, separately in veterans with and without predicted exposure. Because normal probability plots and the Shapiro-Wilks test indicated that the cumulative exposure level estimates were not normally distributed, the exposure estimates were log-transformed. Two veterans in this study were not identified as having been with units that were in the hazard area after the last DOD modeling effort in 2002. Thus, we did not have cumulative exposure level estimates for these two veterans, despite the fact that they were likely to have been exposed to the Kahmisiyah plume.

Post-hoc Analysis I

Because the left hippocampus has specifically been implicated in verbal memory processing,[31] our findings of significant correlations between total hippocampal volume and some of the CVLT-II measures prompted us to further explore the specific relationship between left hippocampal and left subfield volumes and CVLT-II performance separately in veterans with and without predicted exposure in post-hoc analyses.

TABLE 1. Subject Demographic and Clinical Information

	Non-exposed ($N = 51$)		Exposed ($N = 62$)	
	Mean/No.	Range	Mean/No.	Range
Age (yrs)	52.4 (7.7)	41–67	53.5 (8.0)	42–71
No. (%) female	14 (28%)		12 (19%)	
Education (yrs)	14.5 (2.5)	11–20	15.0 (2.5)	12–20
Ethnicity				
African American	3 (6%)		6 (10%)	
Latino	4 (8%)		3 (5%)	
White	39 (77%)		48 (77%)	
Other	5 (10%)		5 (8%)	
No. (%) married	37 (73%)		50 (81%)	
No. (%) with current major depressive disorder (MDD)	6 (12%)		7 (11%)	
Beck depression inventory (BDI)	9.3 (7.9)	0–42	10.0 (9.9)	0–54
No. (%) with posttraumatic stress disorder (PTSD)	12 (24%)		8 (13%)	
Clinician administered PTSD scale (CAPS)	20.6 (23.2)	0–76	24.6 (22.4)	0–78
No. (%) on psychotropic medication	10 (20%)		12 (19%)	
No. (%) officer during the Gulf War (GW)	15 (29%)		14 (23%)	
No. (%) in the army during the GW[a]	23 (45%)		62 (100%)	
Estimated exposure dose (mg min/m^3)	—		0.39 (0.57)	0.03–2.16
No. (%) who reported hearing chemical alarms during GW	37 (73%)		53 (86%)	
No. (%) CDC CMI cases[*]	40 (78%)		52 (84%)	
No. (%) Kansas GWI cases[†]	25 (49%)		29 (47%)	
No. (%) with Kansas GWI exclusionary criteria[‡]	12 (24%)		20 (32%)	
Kansas GWI symptom severity[§]	23.6 (19.3)	0–84	21.9 (16.7)	0–69
No. (%) with self-reported memory difficulties	34 (67%)		41 (66%)	
No. (%) with Apolipoprotein E (APOE) ε2 allele	7 (14%)		12 (19%)	
No. (%) with APOE ε4 allele[b]	14 (27%)		8 (13%)	
No. (%) with history of alcohol abuse/dependence[a]	9 (18%)		22 (36%)	
No. (%) with history of substance abuse/dependence	2 (4%)		8 (13%)	
No. (%) with no traumatic brain injury (BTI)[?]	10 (20%)		17 (27%)	
No. (%) with improbable TBI[?]	17 (33%)		19 (31%)	
No. (%) with possible TBI[?]	10 (20%)		14 (23%)	
No. (%) with mild TBI[?]	14 (28%)		11 (18%)	
No. (%) with moderate TBI[?]	0 (0%)		1 (2%)	

CDC, Centers for Disease Control and Prevention; CMI, chronic multi-symptom illness; GWI, Gulf War illness; TBI, traumatic brain injury.

[*]According to Fukuda et al.[10]

[†]According to Steele.[20]

[‡]Diabetes, heart disease (other than high blood pressure), stroke, lupus, multiple sclerosis, rheumatoid arthritis, seizure disorder, cancer, liver and kidney disease, hospitalization for PTSD, alcohol, and/or drug dependence since 1990.

[§]Created by summing GWI symptom severity scores from Kansas Gulf War Military History and Health Questionnaire.[20]

[?]According to the Ohio State University Traumatic Brain Injury Identification (OSU TBI-ID) Method.[17,18]

[a]Fisher exact test, $P < 0.05$, 2-sided.

[b]Fisher exact test, $P = 0.06$, 2-sided.

Exploratory Post-hoc Analysis II

Despite the fact that many GW veterans have subjective memory complaints, there has been controversy in the literature about whether these self-reported memory complaints are related to decrements on objective cognitive tests measures.[32–34] Therefore, in an exploratory post-hoc analysis, we examined the relationship between the severity of self-reported memory difficulties as queried by the Kansas Gulf War Military History and Health Questionnaire (ie, mild, moderate, or severe) and objective performance on the CVLT-II. Because memory impairment can be a symptom of GWI, we used partial correlation to control for the severity of overall GWI symptoms in our exploration of the relationship between CVLT-II scores and the severity of self-reported memory difficulties. To do this, we summed the responses to 29 questions from the symptom portion of the Kansas Gulf War Military History and Health Questionnaire about GWI/CMI symptoms (eg, fatigue, pain, gastrointestinal, respiratory, neurological, mood, and skin problems). The symptom portion of Kansas Gulf War Military History and Health Questionnaire asks participants to indicate if they have a GWI symptom, and if so, how severe it is (ie, 0 = none, 1 = mild, 2 = moderate, 3 = severe). In this respect, it is like a four-point Likert scale. In the current sample, overall GWI symptom severity scores ranged from 0 to 84 (higher scores indicate more and/or more severe symptoms) and had a Cronbach α of 0.95.

RESULTS

Table 1 summarizes the demographic, military, and clinical data of the study sample. The two exposure groups only differed on two demographic/clinical variables: (1) all veterans with predicted Khamisiyah exposure served in the army during the Gulf War whereas only 45% of the veterans without predicted exposure served in the army (Fisher exact test, $P < 0.001$). (2) More veterans with predicted exposure had a history of alcohol abuse/dependence compared with veterans without predicted exposure (Fisher exact test, $P < 0.04$). Although not statistically significant, there was a trend ($P = 0.06$) for there to be more APOE ε4-carriers among veterans without predicted exposure compared with veterans with predicted exposure.

TABLE 2. Least Square Mean Total and Hippocampal Subfield Volumes by Predicted Exposure Group*

	Non-Exposed	Exposed	ANCOVA
Total hippocampus†	8757.18 (815.22)	8308.51 (757.58)	$F_{1,105} = 12.77$, $P = 0.001$
CA1 subfield‡	59.54 (4.91)	57.84 (5.54)	$F_{1,111} = 2.59$, $P = 0.11$
CA2 subfield‡	1.77 (0.35)	1.73 (0.38)	$F_{1,111} = 0.34$, $P = 0.56$
CA3/dentate gyrus subfield‡	41.33 (5.08)	39.00 (4.51)	$F_{1,111} = 6.26$, $P = 0.01$

Volumes are (mm^3) are means and standard deviation (in parentheses).
ANCOVA, analysis of covariance.
*Adjusted for age, sex, ICV, Kansas GWI case status and exclusionary status, current diagnoses of MDD and/or PTSD, current use of psychotropic medication, APOE ε2/ε4 status, history of alcohol abuse/dependence, and OUS TBI severity.
†Determined by Freesurfer v.5.1; data available for 46 veterans without and 60 veterans with predicted Khamisiyah exposure.
‡Determined by ASHS and normalized for number of slices in segmentation; data available for 50 veterans without and 62 veterans with predicted Khamisiyah exposure.

Binary Comparisons Between Predicted Exposure Groups

Table 2 lists the least square mean volumes of the total hippocampus and hippocampal subfields by group. Compared with veterans without predicted exposure, veterans with predicted exposure had smaller total hippocampal volume ($F_{1,105} = 12.77$, $P = 0.001$), even after adjusting for age, sex, ICV, Kansas GWI case status and exclusionary status, current diagnoses of MDD and/or PTSD, current use of psychometric medication, APOE ε2/ε4 status, history of alcohol abuse/dependence, and OUS TBI severity. We obtained similar results when we controlled for CDC CMI case status instead of the Kansas GWI case status. We also observed similar results when we did not control for either Kansas GWI or CDC CMI case status.

There was no main effect of group when the volumes of the CA1, CA2, and CA3/DG subfields were entered in a single omnibus MANCOVA (Wilks Lambda = 0.94, Pillais approximate $F_{3,96} = 2.15$, $P = 0.10$). However, the individual ANCOVAs revealed a significant group effect for CA3/DG subfield volume ($F_{1,111} = 5.75$, $P = 0.02$). In contrast, there were no significant group effects for CA1 ($F_{1,111} = 1.84$, $P = 0.18$) or CA2 ($F_{1,111} = 0.45$, $P = 0.50$) volumes. Again, we obtained similar results when we controlled for CDC CMI case status instead of the Kansas GWI case status and when we did not control for Kansas GWI or CDC CMI case status.

Table 3 lists the least square mean CVLT-II dependent variables by group, accounting for age, sex, years of education, rank during the GW, Kansas GWI case status and exclusionary status, current diagnoses of MDD and/or PTSD, current use of psychometric medication, APOE ε2/ε4 status, history of alcohol abuse/dependence, and OUS TBI severity. We also include raw scores for CVLT trial 1, trials 1–5, short- and long-delay free recall from a large scale normative study that assessed the contribution of influences of ethnicity as well as other demographic factors on CVLT performance.[35]

When all the CVLT-II dependent variables of interest were entered in a single omnibus MANCOVA, there was no main effect of group (Wilks lambda = 0.90, Pillais approximate $F_{6,93} = 1.70$, $P = 0.13$). Although veterans with predicted exposure recognized fewer words than veterans without predicted exposure (individual ANCOVA for recognition hits: $F_{1,112} = 4.60$, $P < 0.04$), this was not statistically significant after corrections for multiple comparisons (with an average intercorrelated correlation of $r = 0.60$, a 2-sided adjusted $P < 0.02$ was considered statistically significant) Table 4.

Because delayed recall is contingent on successful learning, we repeated the analyses for delayed recall using total learning (ie, performance on trials 1–5) as a covariate. Total learning was a significant covariate ($F_{1,97} \geq 138.23$, $P < 0.001$) for both short- and long-delay free recall. However, there was still no significant over all group difference for short- or long-delay free recall after accounting for total learning.

Relationship Between Total Hippocampal and Subfield Volumes, CVLT-II Performance, and Estimates of Cumulative Exposure Levels

Among veterans with predicted exposure, there were positive correlations between total hippocampal volume and performance on CVLT-II trial 1 (ie, immediate attention and memory; Spearman $\rho = 0.28$, $P = 0.03$), recognition hits (Spearman $\rho = 0.32$, $P = 0.01$),

TABLE 3. Least Square Mean CVLT-II Scores by Predicted Exposure Group*

CVLT-II Measures	Non-Exposed ($N = 51$; mean age: 52.4 ± 7.7)	Exposed ($N = 62$; mean age: 3.5 ± 8.0)	$F_{1,112}$	Norm Base Sample† Age 40–50 ($N = 184$)	Norm Validation Sample† Age 40–50 ($N = 48$)	Norm Base Sample† Age 60+ ($N = 266$)	Norm Validation Sample† Age 60+ ($N = 96$)
Trial 1 (raw)	6.3 (1.5)	5.9 (1.8)	1.70	7.3 (2.1)	8.2 (2.2)	6.3 (2.1)	6.4 (2.4)
Trials 1–5 (raw)	51.0 (9.9)	48.9 (8.5)	1.33	52.4 (10.2)	54.6 (10.7)	46.4 (11.1)	46.3 (12.1)
Short delay (raw)	11.0 (3.1)	10.4 (2.7)	0.95	10.8 (3.1)	10.6 (3.3)	9.0 (3.1)	8.9 (3.6)
Long delay (raw)	11.0 (3.3)	10.9 (3.0)	0.04	11.0 (3.1)	11.9 (3.2)	9.4 (3.3)	9.3 (3.9)
Recognition hits (raw)	14.8 (1.6)	14.1 (1.8)	4.60[a]	—	—	—	—
Total recognition discriminability (d')	3.2 (0.8)	2.9 (0.7)	3.69[b]	—	—	—	—

Means and standard deviation (in parentheses) are reported.
*Data from Norman et al.[35]
†Accounting for age, sex, years of education, rank during the GW, Kansas GWI case status and exclusionary status, current diagnoses of MDD and/or PTSD, current use of psychotropic medication, OUS TBI severity, APOE ε2/ε4 status, and past history of alcohol abuse/dependence.
[a]$P < 0.04$.
[b]$P = 0.06$.

TABLE 4. Results of Post-hoc Correlations between CVLT-II Performance, the Severity of Self-Report Memory Impairment, and Overall GWI Symptoms

	Non-Exposed ($n = 51$)		Exposed ($n = 62$)	
CVLT-II Measures	**Memory Impairment**	**Overall GWI Symptom**	**Memory Impairment**	**Overall GWI Symptom**
Trial 1	−0.04	−0.05	−0.18	−0.17
Trials 1–5	−0.14	−0.09	−0.32[b]	−0.25
Short-delay free recall	−0.23	−0.10	−0.29[a]	−0.12
Long-delay free recall	−0.14	0.06	−0.35[b]	−0.20
Recognition hits	−0.11	−0.05	−0.39[b]	−0.27[a]
Total recognition discriminability	−0.07	0.04	−0.22	−0.19

Spearman rho reported. CVLT-II, California Verbal Learning Test-II; GWI, Gulf War illness.
[a] $P \leq 0.05$.
[b] $P \leq 0.01$.

and total recognition discriminability (Spearman $\rho = 0.26$, $P < 0.05$) in veterans with predicted exposure. In contrast, there were no significant correlations between hippocampal volume and CVLT-II performance in veterans without predicted exposure.

Because the left hippocampus has been implicated in verbal memory processing,[31] we further examined the relationship between left total hippocampal and left subfield volumes and CVLT-II performance in post-hoc analyses. Among veterans with predicted exposure, left hippocampal volume correlated positively with performance on CVLT-II trial 1 (Spearman $\rho = 0.30$, $P = 0.02$), trials 1–5 (Spearman $\rho = 0.28$, $P = 0.03$), short-delay free recall (Spearman $\rho = 0.26$, $P < 0.05$), recognition hits (Spearman $\rho = 0.32$, $P = 0.01$), and total recognition discriminability (Spearman $\rho = 0.28$, $P = 0.03$). In contrast, there were no significant correlations between left hippocampal subfield volumes and CVLT-II performance among veterans without predicted exposure.

Among veterans with predicted exposure, we also found an inverse correlation between the log-transformed exposure level estimates and total recognition discriminability (Spearman $\rho = -0.27$, $P = 0.04$).

Post-hoc Exploratory Analyses of Self-Reported Memory Difficulties and CVLT-II Performance

Among veterans with predicted exposure, the self-reported severity of memory difficulties was significantly correlated with CVLT-II performance (trials 1–5: Spearman $\rho = -0.32$, $P = 0.01$; short-delay free recall: Spearman $\rho = -0.29$, $P = 0.03$; long-delay free recall: Spearman $\rho = -0.35$, $P = 0.006$, and recognition hits: Spearman $\rho = -0.39$, $P = 0.002$). Even after controlling for overall GWI symptom severity, which, as expected, was significantly correlated with the severity of self-reported memory difficulties (Spearman $\rho = 0.78$ in veterans with predicted exposure), self-reported memory difficulties still correlated negatively with performance on CVLT-II short-delay free recall (partial correlation coefficient = −0.33, $P = 0.009$), long-delay free recall (partial correlation coefficient = −0.30, $P = 0.02$), and recognition hits (partial correlation coefficient = −0.25, $P < 0.05$). In contrast, there were no significant correlations between the self-reported severity of memory difficulties and CVLT-II performance among veterans without predicted exposure.

DISCUSSION

In the current study we replicated our previous findings of smaller total hippocampal[2] and smaller CA3/DG hippocampal subfield volumes[3] in a third, independent sample of GW veterans identified by the DoD exposure models as having possible exposure to nerve gas agents from the demolition operation in Khamisiyah, Iraq. Furthermore, we extended these previous findings by demonstrating that the effect of predicted exposure to the Khamisiyah plume on total hippocampal and CA3/DG subfield volumes is independent of several factors that may affect hippocampal volume, such as the veterans' ApoE4 genotype,[5,6,36] TBI severity,[7–9] diagnoses of PTSD[37–39] and clinical depression, current use of psychotropic medication,[40–44] history of alcohol abuse/dependence,[45,46] and a the presence of other medical conditions that may influence hippocampal volume (eg, Kansas GWI exclusionary criterion such as cardiovascular disease and diabetes mellitus).[47]

Despite the initial skepticism about the assumptions made by the DoD plume models,[48,49] our current and previous findings of smaller total hippocampal[2] and CA3/DG[3] volumes in GW veterans with predicted exposure to the Khamisiyah plume are in line with several previous rodent studies on the effects organophosphate poisoning on the hippocampus[50,51] and the effects of subclinical (ie, levels that do not cause any overt clinical signs even after multiple days of exposure) sarin exposure on the dentate gyrus, CA4 and CA3 hippocampal subfields.[52,53] It is also noteworthy that reduced hippocampal volume has been reported in surviving victims of the 1995 Tokyo sarin subway attack.[54]

Although memory differences between veterans with and without predicted exposure on the CVLT-II did not survive corrections for multiple comparisons in the current study, we did observe positive correlations between total hippocampal volume, particularly on the left side, and CVLT-II performance in veterans with predicted exposure. We also found an inverse correlation between memory performance and the DoD's estimated cumulative exposure levels and an inverse association between the severity of self-reported memory difficulties and memory performance in GW veterans with predicted exposure to the Khamisiyah plume. The fact that we found this relationship, 25 years after the demolition at Khamisyah may be related, in part, to the Henderson et al[53] finding that rats with subclinical exposures to sarin had subtle biological and neurochemical changes in the hippocampus that that persist long after the exposure.

In the aging-dementia literature, it has been suggested that individuals with subjective memory complaints may be at increased risk for developing Alzheimer's disease (AD).[55] Evidence in support of this comes from findings of significant correlations between subjective memory complaints and decreased performance on objective memory measures,[56] faster rates of decline on immediate recall and related psychometric measures,[57,58] and findings of reduced entorhinal cortex,[59] hippocampal, anterior cingulate, and precuneus volumes,[60] and higher amyloid-β deposition[56,61,62] in individuals with subjective memory complaints compared with healthy controls. Memory complaints are common among GW veterans, particularly those with GWI.[32,63,64] We recently reported evidence that subjective memory complaints predicted CVLT-II performance (short-and long-delay free recall) over and above potentially confounding demographic and clinical variables in 272 GW veterans with CDC CMI.[65]

In the current study, we found an inverse association between the severity of self-reported memory difficulties and CVLT-II memory performance in GW veterans with predicted exposure to the Khamisiyah plume, independent of CDC CMI or GWI status. This, together with our findings of reduced hippocampal volume, which has been associated with the development of late-life dementia[66,67] in three, separate groups of GW veterans with predicted exposure to the Khamisiyah plume, suggest that regular follow-up of these veterans, particularly as they approach old age, may be warranted.

REFERENCES

1. 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 Deployments. Potential exposure to sarin from the demolitions at Khamisiyah, Iraq on March 10, 1991. Vol August 1997. Falls Church, VA. Available at: http://www.gulflink.osd.mil. Accessed January 16, 1998.
2. Chao LL, Rothlind JC, Cardenas VA, Meyerhoff DJ, Weiner MW. Effects of low-level exposure to sarin and cyclosarin during the 1991 Gulf War on brain function and brain structure in US veterans. *Neurotoxicology.* 2010;31:493–501.
3. Chao LL, Kriger S, Buckley S, Ng P, Mueller SG. Effects of low-level sarin and cyclosarin exposure on hippocampal subfields in Gulf War Veterans. *Neurotoxicology.* 2014;44:263–269.
4. Lehtovirta M, Laakso MP, Soininen H, et al. Volumes of hippocampus, amygdala and frontal lobe in Alzheimer patients with different apolipoprotein E genotypes. *Neuroscience*. 1995;67:65–72.
5. O'Dwyer L, Lamberton F, Matura S, et al. Reduced hippocampal volume in healthy young ApoE4 carriers: an MRI study. *PLoS One*. 2012;7:e48895.
6. Mueller SG, Schuff N, Raptentsetsang S, Elman J, Weiner MW. Selective effect of Apo e4 on CA3 and dentate in normal aging and Alzheimer's disease using high resolution MRI at 4 T. *Neuroimage*. 2008;42:42–48.
7. Bigler ED, Blatter DD, Anderson CV, et al. Hippocampal volume in normal aging and traumatic brain injury. *AJNR Am J Neuroradiol*. 1997;18:11–23.
8. Ariza M, Serra-Grabulosa JM, Junqué C, et al. Hippocampal head atrophy after traumatic brain injury. *Neuropsychologia*. 2006;44:1956–1961.
9. Beauchamp MH, Ditchfield M, Maller JJ, et al. Hippocampus, amygdala and global brain changes 10 years after childhood traumatic brain injury. *Int J Dev Neurosci*. 2011;29:137–143.
10. Fukuda K, Nisenbaum R, Stewart G, et al. Chronic multisymptom illness affecting Air Force veterans of the Gulf War. *JAMA*. 1998;280:981–988.
11. Dickerson BC, Eichenbaum H. The episodic memory system: Neurocircuitry and disorders. *Neuropsychopharmacology.* 2010;35:86–104.
12. Jarrard LE. On the role of the hippocampus in learning and memory in the rat. *Behav Neural Biol*. 1993;60:9–26.
13. Delis DC, Kramer JH, Kaplan E, Ober BA. *California Verbal Learning Test.* 2nd ed. San Antonio, TX: Psychological Corporation; 2000.
14. First M, Spitzer R, Gobbon M, Williams J. *Structured Clinical Interview for DMS-IV.* New York: New Work State Psychiatric Institute Biometrics Research Department; 1995.
15. Blake DD, Weathers FW, Nagy LM, et al. The development of a Clinican-Administered PTSD Scale. *J Trauma Stress*. 1995;8:75–90.
16. Wolfe J, Kimerling R, Brown P, Chresman K, Levin K. Psychometric review of the life stressor checklist-revised. In: Stamm BH, editor. *Instrumentation in Stress, Trauma, and Adaptation*. Lutherville, MD: Sidran Press; 1996. p. 144–151.
17. Corrigan JD, Bogner JA. Initial reliability and validity of the OSU TBI identification method. *J Head Trauma Rehabil*. 2007;22:318–329.
18. Bogner JA, Corrigan JD. Reliability and validity of the OSU TBI identification method with prisoners. *J Head Trauma Rehabil*. 2009;24:279–291.
19. Lobbestael J, Leurgans M, Arntz A. Inter-rater reliability of the structured clinical interview for DSM-IV Axis I Disorders (SCID I) and Axis II Disorders (SCID II). *Clin Psychol Psychother.* 2011;18:75–79.
20. Steele L. Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. *Am J Epidemiol*. 2000;152:992–1002.
21. Institute of Medicine. *Gulf War and Health: Volume 8—Health Effects of Serving in the Gulf War.* Washington, D.C.: National Academies Press; 2010
22. Yushkevich PA, Wang H, Pluta J, et al. Nearly automatic segmentation of hippocampal subfields in in vivo focal T2 weighted MRI. *Neuroimage*. 2010;53:1208–1224.
23. Chao LL. Associations between the self-reported frequency of hearing chemical alarms in theater and visuospatial function in Gulf War Veterans. *J Occup Environ Med*. 2016;58:1014–1020.
24. Pluta J, Yushkevich P, Das S, Wolk D. In vivo analysis of hippocampal subfield atrophy in mild cognitive impairment via semi-automatic segmentation of T2-weighted MRI. *J Alzheimers Dis*. 2012;31:85–99.
25. Yehuda R, Golier JA, Halligan SL, Harvey PD. Learning and memory in holocaust survivors with posttraumatic stress disorder. *Biol Psychiatry.* 2004;55:291–295.
26. Pohlack ST, Meyer P, Cacciaglia R, Liebscher C, Ridder S, Flor H. Bigger is better! Hippocampal volume and declarative memory performance in healthy young men. *Brain Sruct Funct*. 2014;219:255–267.
27. Harold D, Abraham R, Hollingworth P, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet*. 2009;41:1088–1093.
28. Lambert JC, Heath S, Even G, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet*. 2009;41:1094–1099.
29. Corder EH, Saunders AM, Risch NJ, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet*. 1994;7:180–184.
30. Sankoh AJ, Huque MF, Dubey SD. Some comments on frequently used multiple endpoint adjustment methods in clinical trials. *Stat Med*. 1997; 16:2529–2542.
31. Kelley WM, Miezin FM, McDermott KB, et al. Hemispheric specialization in human dorsal frontal cortex and medial temporal lobe for verbal and nonverbal memory encoding. *Neuron*. 1998;20:927–936.
32. Vasterling JJ, Bremner JD. The impact of the 1991 Gulf War on the mind and brain: findings from neuropsychological and neuroimaging research. *Philos Trans R Soc Lond B Biol Sci*. 2006;361:593–604.
33. White RKS. Response to is Gulf War illness ''Real'': The jury is still out by D.R. Orme. *Fed Pract;*. 2011;13–22.
34. Binns J, Steele L. The 'Reality' of Gulf War illness. *Fed Pract*. 2011;28:37.
35. Norman MA, Evans JD, Miller WS, Heaton RK. Demographically corrected norms for the California Verbal Learning Test. *J Clin Exp Neuropsychol*. 2000;22:80–94.
36. Mueller SG, Weiner MW. Selective effect of age, Apo e4, and Alzheimer's disease on hippocampal subfields. *Hippocampus*. 2009;19:558–564.
37. Gurvits TV, Shenton ME, Hokama H, et al. Magnetic resonance imaging study of hippocampal volume in chronic, combatrelated posttraumatic stress disorder. *Biol Psychiatry.* 1996;40:1091–1099.
38. Bremner JD, Randall P, Scott TM, Vermetten E, Staib L, Bronen RA. Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse—a preliminary report. *Biol Psychiatry.* 1997;41:23–32.
39. Bonne O, Brandes D, Gilboa A, et al. Longitudinal MRI study of hippocampal volume in trauma survivors with PTSD. *Am J Psychiatry.* 2001;158:1248–1251.
40. Videbech P, Ravnkilde B. Hippocampal volume and depression: a meta-analysis of MRI studies. *Am J Psychiatry.* 2004;161:1957–1966.
41. Wrench JM, Wilson SJ, Bladin PF, Reutens DC. Hippocampal volume and depression: insights from epilepsy surgery. *J Neurol Neurosurg Psychiatry.* 2009;80:539–544.
42. Zou K, Deng W, Li T, et al. Changes of brain morphometry in first-episode, drug-naive, non-late-life adult patients with major depression: an optimized voxel-based morphometry study. *Biol Psychiatry.* 2010;67:186–188.
43. Sheline YI, Gado MH, Kraemer HC. Untreated depression and hippocampal volume loss. *Am J Psychiatry.* 2003;160:1516–1518.
44. Malykhin NV, Carter R, Seres P, Coupland NJ. Structural changes in the hippocampus in major depressive disorder: contributions of disease and treatment. *J Psychiatry Neurosci*. 2010;35:337–343.
45. Agartz I, Momenan R, Rawlings RR, Kerich MJ, Hommer DW. Hippocampal volume in patients with alcohol dependence. *Arch Gen Psychiatry.* 1999; 56:356–363.
46. Lee J, Im SJ, Lee SG, et al. Volume of hippocampal subfields in patients with alcohol dependence. *Psychiatry Res*. 2016;258:16–22.
47. Fotuhi M, Do D, Jack C. Modifiable factors that alter the size of the hippocampus with ageing. *Nat Rev Neurol*. 2012;8:189–202.
48. Enserink M. Congress explores the scientific fringe. *Science*. 2001;291:814.
49. Kang HK, Bullman TA. Mortality among U.S. veterans of the Persian Gulf War. *N Engl J Med*. 1996;335:1498–1504.
50. Abdel-Rahman A, Shetty AK, Abou-Donia MB. Acute exposure to sarin increases blood brain barrier permeability and induces neuropathological changes in the rat brain: dose-response relationships. *Neuroscience*. 2002;113:721–741.
51. Pazdernik TL, Emerson MR, Cross R, Nelson SR, Samson FE. Soman-induced seizures: limbic activity, oxidative stress, and neuroprotective proteins. *J Appl Toxicol*. 2001;21(Suppl):S87–S94.

52. Veronesi B, Jones K, Pope C. The neurotoxicity of subchronic acetylcholinesterase (AChE) inhibition in rat hippocampus. *Toxicol Appl Pharmacol*. 1990;104:440–456.
53. Henderson RF, Barr EB, Blackwell WB, et al. Response of rats to low levels of sarin. *Toxicol Appl Pharmacol*. 2002;184:67–76.
54. Yamasue H, Abe O, Kasai K, et al. Human brain structural change related to acute single exposure to sarin. *Ann Neurol*. 2007;61: 37–46.
55. Schultz SA, Oh JM, Koscik RL, et al. Subjective memory complaints, cortical thinning, and cognitive dysfunction in middle-age adults at risk of AD. *Alzheimers Dement*. 2015;1:33–40.
56. Amariglio RE, Becker JA, Carmasin J, Wadsworth LP, Lorius N, Sullivan C. Subjective cognitive complaints and amyloid burden in cognitively normal older individuals. *Neuropsychologia*. 2012;50:2880–2886.
57. Reisberg B, Shulman MB, Torossian C, Leng L, Zhu W. Outcome over seven years of healthy adults with and without subjective cognitive impairment. *Alzheimers Dement*. 2010;6:11–24.
58. Hohman TJ, Beason-Held LL, Lamar M, Resnick SM. Subjective cognitive complaints and longitudinal changes in memory and brain function. *Neuropsychology*. 2011;25:125–130.
59. Jessen F, Feyen L, Freymann K, Tepest R, Maier W, Heun R. Volume reduction of the entorhinal cortex in subjective memory impairment. *Neurobiol Aging*. 2006;27:1751–1756.
60. Hafkemeijer A, Altmann-Schneider I, Oleksik AM, van de Wiel L, Middelkoop HA, van Buchem MA. Increased functional connectivity and brain atrophy in elderly with subjective memory complaints. *Brain Connect*. 2013;3:353–362.
61. Rodda J, Okello A, Edison P, Dannhauser T, Brooks DJ, Walker Z. (11)C-PIB PET in subjective cognitive impairment. *Eur Psychiatry*. 2010;25:123–125.
62. Perrotin A, Mormino EC, Madison CM, Hayenga AO, Jagust WJ. Subjective cognition and amyloid deposition imaging: A Pittsburgh compound B positron emission tomography study in normal elderly individuals. *Arch Neurol*. 2012;69:223–229.
63. Murphy FM, Kang H, Dalager NA, et al. The health status of Gulf War veterans: lessons learned from the Department of Veterans Affairs health registry. *Mil Med*. 1999;164:327–331.
64. Joseph SC, Team CCPE. A comprehensive clinical evaluation of 20,000 Persian Gulf War veterans. *Mil Med*. 1997;162:149–155.
65. Chao LL. Evidence of objective memory impairments in deployed Gulf War veterans with subjective memory complaints. *Mil Med*; 2017 (in press).
66. Steffens DC, Payne ME, Greenberg DL, et al. Hippocampal volume and incident dementia in geriatric depression. *Am J Geriatr Psychiatry*. 2002; 10:62–71.
67. den Heijer T, van der Lijn F, Koudstaal PJ, et al. A 10-year follow-up of hippocampal volume on magnetic resonance imaging in early dementia and cognitive decline. *Brain*. 2010;133:1163–1172.

