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Effects of low-level sarin and cyclosarin exposure on hippocampal microstructure in Gulf War Veterans

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ABSTRACT

In early March 1991, shortly after the end of the Gulf War (GW), a munitions dump was destroyed at Khamisiyah, Iraq. Later, in 1996, the dump was found to have contained the organophosphorus (OP) nerve agents sarin and cyclosarin. We previously reported evidence of smaller hippocampal volumes in GW veterans with predicted exposure to the Khamisiyah plume compared to unexposed GW veterans. To investigate whether these macroscopic hippocampal volume changes are accompanied by microstructural alterations in the hippocampus, the current study acquired diffusion-tensor imaging (DTI), T1-, and T2-weighted images from 170 GW veterans (mean age: 53 ± 7 years), 81 of whom had predicted exposure to the Khamisiyah plume according to Department of Defense (DOD) plume modeling. We examined fractional anisotropy (FA), mean diffusivity (MD), and grey matter (GM) density from a hippocampal region of interest (ROI). Results indicate that, even after accounting for total hippocampal GM density (or hippocampal volume), age, sex, apolipoprotein ε4 genotype, and potential confounding OP pesticide exposures, hippocampal MD significantly predicted Khamisiyah exposure status (model $p = 0.005$, $R^2 = 0.215$, standardized coefficient $\beta = 0.26$, $t = 2.85$). Hippocampal MD was also inversely correlated with verbal memory learning performance in the entire study sample ($p = 0.001$). There were no differences in hippocampal FA or GM density; however, veterans with predicted Khamisiyah exposure had smaller hippocampal volumes compared to unexposed veterans. Because MD is sensitive to general microstructural disruptions that lead to increased extracellular spaces due to neuronal death, inflammation and gliosis, and/or to axonal loss or demyelination, these findings suggest that low-level exposure to the Khamisiyah plume has a detrimental, lasting effects on both macro- and micro-structure of the hippocampus.

1. Introduction

Potentially 100,000 US military personnel were exposed to low-levels of sarin and cyclosarin, organophosphorus (OP) nerve agents, when a munition storage depot at Khamisiyah, Iraq was destroyed by United States (US) combat engineers in March 1991 (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, 1997). Despite the initial skepticism about assumptions made by the Department of Defense (DOD) plume models (Enserink, 2001; Kang and Bullman, 1996), several studies have linked sarin/cyclosarin exposure to central nervous system outcomes. Roberta White's group in Boston was the first to report cognitive (Proctor et al., 2006) and structural brain changes (i.e., smaller white matter volumes on brain imaging) (Heaton et al., 2007) associated with exposure to the Khamisiyah plume in a dose-dependent manner. We have also found associations between predicted exposure to the Khamisiyah plume and structural magnetic resonance imaging (MRI) and behavioral outcomes in GW Veterans. In a 1.5 Tesla (T) MRI study, we found that grey matter (GM) and hippocampal volumes were smaller in Khamisiyah-exposed veterans compared to matched, non-exposed veterans. We also found associations between reduced white matter (WM) volumes and poorer performance on neurobehavioral tests of executive and visuospatial functions (Chao et al., 2010). In a different sample of GW Veterans studied on a 4.0 T MRI, we found that total GM and WM volumes were both significantly reduced in veterans with predicted Khamisiyah exposure (Chao et al., 2011). Subsequently, we extended our findings of structural alterations in the hippocampi of GW Veterans with predicted Khamisiyah exposure to volume changes in the CA2, CA3, and dentate gyrus hippocampal subfields (Chao et al., 2014; Chao et al., 2017). The current study sought to investigate whether these macroscopic changes in hippocampal volume are

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accompanied by changes at the microstructural level.

Diffusion tensor imaging (DTI) is a non-invasive, quantitative imaging technique that can be used to measure the movement of water molecules within tissues (Aung et al., 2013). With DTI analysis it is possible to infer, in each voxel, properties such as the directional preference of diffusion (i.e., fractional anisotropy, FA) and the rate of molecular diffusion (i.e., mean diffusivity, MD) along the main axis of diffusion or along the transverse direction (Soares et al., 2013). To date, all the published DTI studies of GW Veterans have focused on WM regions within the brain (Bierer et al., 2015; Chao et al., 2015; Rayhan et al., 2013). However, there are intrinsic connections in GM (Barbas and Pandya, 1989; Lo et al., 2015; Tardif and Clarke, 2001), including in the hippocampus (Hjorth-Simonsen, 1973). Because changes in microstructural barriers, which normally restrict the Brownian motion of water molecules, can produce measurable differences in the diffusion of water molecules (Ulug et al., 1999), a growing number of investigators have used DTI to investigate microscopic changes in the GM (e.g., breakdown of microstructural barriers in cell membranes and intracellular organelles) (Weston et al., 2015).

Myelinated axons are the predominant components of WM. In myelinated axons, the rate of diffusion along the length of the axon (i.e., axial diffusivity) and perpendicular to the axon (i.e., radial diffusivity) have biological meaning. However, these measures of diffusivity lose their biological meaning in GM, where cell bodies and their processes are the predominant components. Because the net diffusion of water would not necessarily conform to any one specific direction in GM tissues (Chiapponi et al., 2013), assessing the average degree of diffusion in all directions (i.e., MD) may be a better means of studying cortical and subcortical GM. Based on this rationale, we used MD as the primary measure of diffusivity in the current examination of the hippocampal microstructural properties in GW Veterans with and without predicted Khamisiyah exposure. Because FA is one of the most commonly examined DTI measures, we also examined hippocampal FA. However, FA can be fairly non-specific for neuropathological changes, such as changes in the tissue cytoarchitecture, subtle small vessel alterations, axonal demyelination, and possibly gliosis (Li et al., 2017).

2. Methods

2.1. Study population

The study participants were 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 report focuses on data from 170 GW Veterans who had artifact-free DTI, T1-, and T2-weighted magnetic resonance images (MRI). Forty-four (13 with and 31 without predicted Khamisiyah exposure) veterans had participated in past studies while 126 of the GW Veterans (68 with predicted Khamisiyah exposure) had never been studied before. Written informed consent, approved by the University of California, San Francisco and the SF VAMC Institutional Review Boards, was obtained from all study participants. Table 1 summarizes the demographic and clinical characteristics of the study sample by predicted Khamisiyah exposure status. Table 2 summarizes the demographic and clinical characteristics of veterans by study status (e.g., participated in past studies versus never been studied before).

2.2. Assessments

A detailed description of the determination of predicted Khamisiyah exposure status and exposure dose estimates (Chao et al., 2011; Chao et al., 2010) and the method used to determine Kansas Gulf War Illness (GWI) and Centers for Disease Control and Prevention (CDC) Chronic Multisymptom Illness (CMI) case status has previously been described (Chao, 2016). Briefly, we used the Kansas Military History and Health Questionnaire (Steele, 2000) to query veterans about GWI symptoms and severity and to classify veterans as CDC CMI (Fukuda et al., 1998) and Kansas GWI (Steele, 2000) cases. The Kansas GWI case definition excludes veterans as GWI cases if they report being diagnosed with medical (e.g., diabetes, heart disease other than high blood pressure, cancer) or psychiatric conditions (e.g., schizophrenia, bipolar disorder or manic depression) that could explain their symptoms or interfere with their ability to report them. Although some of the Kansas GWI exclusionary conditions are also exclusionary for the present study (e.g., stroke, seizure disorder, multiple sclerosis, schizophrenia, bipolar disorder or manic depression), other conditions that are not exclusionary for the present study reflect the veterans' co-morbidities (e.g., diabetes, heart disease not including high blood pressure, cancer). For that reason, we included the veterans' Kansas GWI exclusionary status in some of the data analyses as a proxy measure of their current health status.

In addition to low-levels of chemical warfare agents released by the destruction of munition facilities in Khamisiyah, GW military personnel were exposed to a myriad of other potentially hazardous chemicals in the Persian Gulf theater, including extensive spraying and use of pesticides (White et al., 2016). After the GW ended, the Department of Defense (DOD) conducted a health risk assessment to try to ascertain the extent of pesticide exposures during the war (Sullivan et al., 2018; Winkenwerder, 2003). The DOD's Environmental Exposure Report (EER) – Pesticides estimated that 41,000 GW Veterans were likely overexposed to organophosphate (OP) and carbamate pesticides during the war (Winkenwerder, 2003). However, this may be an underestimate because the DOD's assessment did not account for the additive synergist effects of multiple concurrent exposures. The pesticides of potential

Table 1
Demographic and clinical characteristics of study sample by predicted Khamisiyah exposure status.

	Unexposed	Exposed	*t*-Value or χ^2	*p*-Value
N	89	81		
Age (years)	53.5 (6.7)	54.2 (8.3)	0.62	0.54
No. females	18 (20%)	13 (16%)	0.50	0.48
Education (years)	15.0 (2.5)	15.3 (2.5)	1.06	0.29
Ethnicity				
No. Caucasian	63 (71%)	59 (73%)	0.30	0.96
No. African American	8 (9%)	8 (10%)		
No. Latino	11 (12%)	8 (10%)		
No. other	7 (8%)	6 (7%)		
No. officer during GW	24 (27%)	19 (24%)	0.79	0.37
No. CDC CMI cases	66 (74%)	65 (80%)	0.89	0.35
No. Kansas GWI cases	40 (45%)	35 (43%)	0.02	0.88
No. with Kansas GWI exclusions	19 (21%)	27 (33%)	3.09	0.08
No. with trauma exposure	73 (82%)	59 (73%)	2.06	0.15
No. with current PTSD	17 (19%)	9 (11%)	2.09	0.15
CAPS	19.9 (22.2)	22.9 (21.7)	0.78	0.44
No. with current MDD	10 (11%)	9 (10%)	0.01	0.98
BDI	7.9 (7.2)	9.1 (9.5)	1.13	0.26
No. on psychotropic medication	17 (19%)	17 (21%)	0.09	0.76
No. with ETOH history	14 (16%)	26 (32%)	6.31	0.01
No. with substance history	6 (7%)	10 (12%)	1.56	0.21
No. with APOE ε4 allele	24 (27%)	11 (14%)	4.99	0.03
No. with APOE ε2 allele	10 (11%)	12 (15%)	0.41	0.52
History of TBI			3.96	0.27
Improbable	44 (49%)	43 (53%)		
Possible	20 (23%)	23 (28%)		
Mild	25 (28%)	14 (17%)		
Moderate	0 (0%)	1 (1%)		

GW, Gulf War; CDC CMI, Centers for Disease Control and Prevention Chronic Multisymptom Illness; GWI, Gulf War Illness; PTSD, posttraumatic stress disorder; CAPS, Clinician Administered PTSD Scale; MDD, major depressive disorder; BDI, Beck Depression Inventory; ETOH, alcohol; APOE, apolipoprotein; TBI, traumatic brain injury.