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Effects of low-level sarin and cyclosarin exposure on white matter integrity in Gulf War Veterans

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ABSTRACT

Background: We previously found evidence of reduced gray and white matter volume in Gulf War (GW) veterans with predicted low-level exposure to sarin (GB) and cyclosarin (GF). Because loss of white matter tissue integrity has been linked to both gray and white matter atrophy, the current study sought to test the hypothesis that GW veterans with predicted GB/GF exposure have evidence of disrupted white matter microstructural integrity.
Methods: Measures of fractional anisotropy and directional (i.e., axial and radial) diffusivity were assessed from the 4 T diffusion tensor images (DTI) of 59 GW veterans with predicted GB/GF exposure and 59 "matched" unexposed GW veterans (mean age: 48 ± 7 years). The DTI data were analyzed using regions of interest (ROI) analyses that accounted for age, sex, total brain gray and white matter volume, trauma exposure, posttraumatic stress disorder, current major depression, and chronic multisymptom illness status.
Results: There were no significant group differences in fractional anisotropy or radial diffusivity. However, there was increased axial diffusivity in GW veterans with predicted GB/GF exposure compared to matched, unexposed veterans throughout the brain, including the temporal stem, corona radiata, superior and inferior (hippocampal) cingulum, inferior and superior fronto-occipital fasciculus, internal and external capsule, and superficial cortical white matter blades. Post hoc analysis revealed significant correlations between higher fractional anisotropy and lower radial diffusivity with better neurobehavioral performance in unexposed GW veterans. In contrast, only increased axial diffusivity in posterior limb of the internal capsule was associated with better psychomotor function in GW veterans with predicted GB/GF exposure.
Conclusions: The finding that increased axial diffusivity in a region of the brain that contains descending corticospinal fibers was associated with better psychomotor function and the lack of significant neurobehavioral deficits in veterans with predicted GB/GF exposure hint at the possibility that the widespread increases in axial diffusivity that we observed in GW veterans with predicted GB/GF exposure relative to unexposed controls may reflect white matter reorganization after brain injury (i.e., exposure to GB/GF).

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1. Introduction

More than 100,000 US service members participating in the Persian Gulf War (GW) were potentially exposed to low levels of sarin (GB; o-isopropyl methylphosphonofluoridate) and cyclosarin (GF; cyclohexyl methylphosphonoflouridate) following the destruction of an Iraqi munitions storage complex at Khamisiyah, Iraq 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). After the Gulf War ended, the Department of Defense and the Central Intelligence Agency tried to determine the extent of potential human GB/GF exposure by modeling its estimated release from and dispersion around the Khamisiyah site (Persian Gulf War Illness Task Force, 1997). Plume estimates

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were superimposed onto geographic maps containing military unit locations. A soldier was considered potentially exposed to low levels of GB/GF if his or her unit had been located within the modeled hazard area on any of the four target dates in March 1991. The exposure plume models were later reanalyzed and revised using updated troop location and personnel information, improved meteorological modeling, more accurate estimates of the total number of GB/GF-containing rockets destroyed, consideration of the methods used to remove GB/GF from the area, relevant exposure thresholds for GB/GF, and the combined toxicity of these agents (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, 2002).

After the plume model analyses were made public, we and other investigators have reported evidence that GW veterans with predicted GB/GF exposure have reduced gray (Chao et al., 2010, 2011) and white (Heaton et al., 2007; Chao et al., 2011) matter volume compared to unexposed GW veterans. Because loss of tissue integrity in regions of normal-appearing white matter has been associated with atrophy in both gray (Agosta et al., 2011; Steenwijk et al., 2014) and white (Vernooij et al., 2008) matter, the present study sought to test the hypothesis that GW veterans with predicted GB/GF exposure evidence of have disrupted white matter microstructure integrity compared to unexposed GW veterans.

To our knowledge, only one study has used diffusion tensor imaging (DTI) to examine the effects of sarin poisoning on the microstructural structural organization of white matter: Yamasue et al. (2007) reported lower fractional anisotropy (FA), a DTI metric commonly used as a proxy measure for white matter integrity (Alexander et al., 2007), in multiple brain regions in victims of the 1995 Tokyo subway sarin attack compared to healthy controls. Yamasue and colleagues also found significant correlations between somatic complaints and reduced FA in the sarin attack victims. Therefore, the first aim of the study was to examine FA in the brain regions where Yamasue et al. (2007) found reduced FA in the sarin attack victims relative to controls and in the brain regions where reduced FA correlated with somatic complaints in the sarin attack victims (i.e., *a priori* Yamasue et al., regions of interest or ROIs) in GW veterans with and without predicted GB/GF exposure. We hypothesize that GW veterans with predicted GB/GF exposure would have reduced FA in the *a priori* Yamasue et al. ROIs compared to unexposed veterans.

Yamasue and colleagues only analyzed FA; however, other DTI parameters can be calculated from the diffusion tensor. Axial diffusivity describes the rate of diffusion along the direction of the semi-major axis (i.e., along the length of the axon) while radial diffusivity describes the rate of diffusion along the semi-minor axes (i.e., perpendicular to the axon). It has been argued that FA may be less sensitive than its component measures (i.e., axial and radial diffusivity) to changes in axonal morphology or myelination (Tyszka et al., 2006). For example, animal studies suggest that alterations in axial diffusivity are associated with morphological changes in the axon (e.g., axonal density or caliber; Kumar et al., 2010, 2012) while changes in radial diffusivity have been associated with morphological changes in myelin (e.g., dys- and de-myelination; Song et al., 2002, 2003). Therefore, the second aim of the study was to examine measures of axial and radial diffusivity in the *a priori* Yamasue et al. ROIs in GW veterans with and without predicted GB/GF exposure.

Yamasue and colleagues observed FA reductions in a *larger* extent of the brain than volume reductions. Because we previously found evidence of *whole brain* gray and white atrophy in GW veterans with predicted GB/GF exposure (Chao et al., 2010, 2011), the third aim of the study was to investigate the effects of predicted GB/GF exposure on DTI measures from major white matter fiber systems through the brain. Finally Rayhan et al. (2013) recently proposed that axial diffusivity in the right inferior fronto-occipital fasciculus may be a biomarker for Gulf War Illness (or Chronic Multisymptom Illness, CMI). Therefore, we examined the ability of axial diffusivity from the right inferior fronto-occipital fasciculus to predict CMI status over and above potentially confounding variables (i.e., age, gender, GB/GF exposure status, and trauma exposure, posttraumatic stress disorder, current major depression).

2. Methods

2.1. Participants

All participants were GW veterans who took part in an imaging study that investigated the effects of Gulf War Illness on the brain on a 4 T MR scanner at the San Francisco Veterans Affairs Medical Center between 2005 and 2010. A detailed description of predicted exposure and exposure dosage estimates has been described previously (Chao et al., 2010, 2011). The current analysis included DTI data from 59 GW veterans with predicted GB/GF exposure and 59 GW veterans selected from a group of 114 unexposed GW veterans to match the GB/GF-exposed veterans for age, sex, level of education, CMI status (Fukuda et al., 1998), diagnoses of posttraumatic stress disorder (PTSD) according to the Clinician-Administered PTSD Scale (Blake et al., 1995), and current major depressive disorder, according to the Structured Clinical Interview for DSM-IV (First et al., 1995).

The Institutional Review Boards of the University of California, San Francisco, the San Francisco Veterans Affairs Medical Center, and the Department of Defense Human Research Protection Office approved both studies. Informed consent was obtained from all participants.

2.2. Image acquisition

All subjects were scanned at the SF Veterans Affairs Medical Center on a Bruker MedSpec 4 T magnetic resonance imaging system equipped with a USA instruments eight-channel array head coil. T1-weighted images were obtained using a 3D volumetric magnetization prepared rapid gradient echo sequence with TR/TE/TI = 2300/3/950 ms, a 7-degree flip angle, and 1.0 mm $\times$ 1.0 mm $\times$ 1.0 mm resolution. In addition, fluid attenuated inversion recovery images with TR/TE/TI = 5000/355/1900 ms were acquired to identify white matter lesions and to improve brain extraction in the segmentation procedure. DTI was acquired using a twice-refocused spin-echo diffusion echo-planar imaging sequence supplemented with twofold parallel imaging acceleration to reduce susceptibility distortions. DTI imaging parameters were TR/TE = 6000/77 ms, field of view 256 cm $\times$ 224 cm, 128 $\times$ 112 matrix size, yielding 2 mm $\times$ 2 mm mm in-plane resolution, 40 continuous slices each 3 mm thick. One reference image ($b = 0$) and six diffusion-weighted images ($b = 800$ s/mm^2, each along 6 non-collinear directions) were acquired.

2.3. DTI image processing

Preprocessing of the DTI data involved skull-stripping, motion and eddy-current correction with FSL package, and geometric distortion correction based on a variational matching algorithm (Tao et al., 2009). After FA, axial and radial diffusivity images were created for each participant (mean diffusivity was not included because it is not independent of measures of axial and radial diffusivity), these images, together with the structural T1-weighted image underwent an automated image segmentation and spatial