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

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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 Tesla 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).

Keywords: diffusion tensor imaging, white matter, chemical warfare agents, sarin, cyclosarin, Gulf War veterans

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 methylphosphonoflouridate) 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 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; Chao et al 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; Kumar et al 2012) while changes in radial diffusivity have been associated with morphological changes in myelin (e.g., dys- and de-myelination; Song et al 2003; Song et al 2002). 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; Chao et al 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 Tesla 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; Chao et al 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 Tesla 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 $1.0 \times 1.0 \times 1.0 \text{ mm}^3$ 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 $256 \times 224 \text{ cm}$, 128×112 matrix size, yielding $2 \times 2 \text{ mm}^2$ in-plane resolution, 40 continuous slices each 3 mm thick. One reference image ($b = 0$) and six diffusion-weighted images ($b = 800 \text{ s/mm}^2$, 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 warping protocol in SPM8. The protocol included the following steps: First, each individual FA image was registered to the white matter probabilistic image of the segmented T1-weighted image through affine registration. Next, the

white matter probabilistic images of the entire study sample were registered into Montreal Neurological Institute (MNI) space using standard procedures of the diffeomorphic registration algorithm (DARTEL; Ashburner 2007). Finally, the FA, axial and radial images of all subjects were transferred to MNI space based on transformation parameters of the white matter probabilistic images in DARTEL. We used the DTI atlas from the Laboratory of Brain Anatomical MRI at the Johns Hopkins University (i.e., ICBM-DTI-81 White Matter Atlas (Mori et al 2008); hereinafter referred as the DTI Atlas) as a template to facilitate identification of major white matter structures. All the DTI images and the DTI atlas were warped in MNI space and manually checked to validate registration quality. To avoid inclusion of surrounding gray matter or cerebral spinal fluid, a mask where the white matter probability was $\geq 50\%$ was used to restrict the DTI ROI analyses to white matter regions.

2.4. DTI Regions of Interest (ROIs)

2.4.1. *a priori* Yamasue et al. ROIs

To examine DTI measures from the *a priori* Yamasue et al. ROIs, we extracted FA, axial and radial diffusivity values from parcels in the DTI atlas that corresponded best with the coordinates where Yamasue et al. (2007) reported reduced FA in the sarin attack victims relative to controls. These ROIs included the left parietal lobe (Yamasue et al. peak MNI coordinates: -20, -43, 27), which corresponded with the left posterior corona radiata parcel in the DTI atlas, bilateral temporal stem (Yamasue et al., peak MNI coordinates: 43, -17, -9; -40, -40, -5), which corresponded to the bilateral sagittal straitum in the DTI atlas, and brainstem (Yamasue et al., peak MNI coordinates: 2, -27, -29), which corresponded with the pontine

crossing tract in the DTI atlas. Yamasue et al. (2007) also reported significant correlations between somatic complaints in sarin attack victims and reduced FA in the temporal stem bilaterally (peak MNI coordinates: 34, 0, -15; -38, 2, -16), which corresponded to the bilateral uncinate fasciculus in the DTI atlas, and left subinsula (peak MNI coordinates: -28, -6, 8), which corresponded to the left external capsule in the DTI atlas. For each participant, we extracted the mean FA, axial and radial diffusivity value from each tract using a previously described method (Zhang et al., 2013). To protect against type I error, we reduced the number of measurements by averaging the DTI values from the left and right sagittal straitum and uncinate fasciculus to create one temporal stem ROI. Figure 1 illustrates the *a priori* Yamasue et al. ROIs on coronal views of a group FA map.

2.4.2. Other white matter ROIs

In addition to the *a priori* Yamasue et al. ROIs, we also extracted DTI values from other major white matter tracts and regions throughout the brain. These included bilateral tracts that pass through limbic (fornix, cingulum) and striatal (internal and external capsule) regions, supratentorial tracts (superior and inferior fronto-occipital fasciculus and superior longitudinal fasciculus), the corona radiata, corpus callosum, and superficially located white matter “blades” located in between the cortex and deep white matter. To protect against type I error, we combined homologous DTI atlas parcels across hemispheres, the anterior and posterior parts of the superior cingulum, the anterior and posterior limbs and the retrolenticular part of the internal capsule, the anterior, superior, and posterior parts of the corona radiata, the genu, body, and splenium of the corpus callosum, and the 9 superficial white matter “blades.” This

resulted in eleven white matter ROIs in the fornix, superior and inferior (i.e., hippocampal) cingulum, internal and external capsule, superior and inferior fronto-occipital fasciculus, superior longitudinal fasciculus, corona radiata, corpus callosum, superficial white matter.

Figure 2 illustrates representations of the eleven white matter ROIs on a group FA map.

3. Data Analysis

3.1. Statistical analyses

Statistical analyses of the demographic, clinical, and volumetric measures were conducted using IBM SPSS Statistics, version 22. Demographic and descriptive characteristics were compared across the two exposure groups with Student's *t* tests for continuous variables and chi-square tests for categorical variables.

3.2. Examination of group DTI differences

The main analyses of regional DTI differences by exposure group were performed using multivariate analysis of covariance (MANCOVA), with age, sex, CMI status, trauma exposure, current PTSD, current major depressive disorder, history of alcohol and/or drug abuse or dependence, and total brain gray and white matter volume as covariates. Total brain gray and white matter volumes were adjusted for intracranial volume (ICV) according to the following formula: raw volume - $\beta(\text{ICV} - \text{mean ICV})$, where β is the regression coefficient when the raw volume is regressed against ICV and mean ICV is the group mean. DTI values from the *a priori* Yamasue et al. ROIs were evaluated at $p=0.05$. *P*-values for the other white matter ROIs were adjusted for multiple comparisons according to the number of ROIs ($n=11$) and the average

intercorrelations among the ROIs (Sankoh et al 1997). The average intercorrelation correlation was $r=0.42$ for FA, $r=0.38$ for axial diffusivity, and $r=0.48$ for radial diffusivity. Accordingly, a 2-sided adjusted $p<0.01$ was considered statistically significant for DTI values from the other white matter ROIs.

3.3. Examination of ability of axial diffusivity from the right inferior fronto-occipital fasciculus to determine CMI status

We used linear regression to examine the ability of axial diffusivity from the right inferior fronto-occipital fasciculus (dependent variable) to predict CMI status (dependent variable) over and above other potentially confounding variables (i.e., age, sex, predicted GB/GF exposure status, trauma exposure, diagnoses of current PTSD and/or major depressive disorder, history of alcohol and/or drug abuse or dependence, and ICV-adjusted whole brain gray and white matter volume).

3.4. Post-hoc analyses

The significant group differences in axial diffusivity from white matter tracts and regions throughout the brain prompted us to further examine the relationship between DTI and neurobehavioral measures. Because some studies have reported increased axial diffusivity in neurodegenerative conditions (e.g., amyotrophic lateral sclerosis (Metwalli et al 2010), Alzheimer's disease (Bosch et al 2010 ; Shu et al 2011), animal model of Parkinson disease (Hikishima et al 2015)) while others have suggested that increased axial diffusivity following brain injury may reflect reorganization in the white matter (e.g., axonal recovery or even

regrowth; Sidaros et al 2008; Voss et al 2006), we reasoned that if higher axial diffusivity in GW veterans with predicted GB/GF exposure were associated with poorer neurobehavioral performance, then may be related to neurodegeneration. On the other hand, if higher axial diffusivity in GW veterans with predicted GB/GF exposure were associated with better neurobehavioral performance, it may be related to white matter reorganization after exposure to GB/GF. We correlated the DTI data with the neurobehavioral measures that we previously reported in our 4 Tesla study of GW veterans (Chao et al. 2011), of which the current cohort is a subset. These included: the Continuous Performance Test (Letz 1991), the Digit Span subtest of the Wechsler Adult Intelligence Scale-III (Wechsler 1997), and the Trail-Making Test, Parts A and B (Reitan and Wolfson 1985), the Short Category Test (Wetzel and Boll 1987), the California Verbal Learning Test-II (Delis et al 2000), and the Grooved Pegboard test (Lafayette Instrument, Lafayette, IN). To protect against type I error, we reduced the number of measurements by averaging FA, axial and radial diffusivity values from the temporal stem and the eleven white matter ROIs to create a composite FA, a composite axial diffusivity and a composite radial diffusivity measure. The relationship between composite DTI measures and the neurobehavioral measures were examined in each group separately with partial correlations that accounted for age, sex, years of education, CMI, trauma exposure, current PTSD and major depressive disorder, history of alcohol and/or drug abuse/dependence. The partial correlations within each group were compared with Fisher's z-test.

Because a previous study reported a positive correlation between axial diffusivity in the posterior limb of the internal capsule, which tractography studies have mapped to the posterior

limb of the internal capsule to motor and premotor cortices (Zarei et al 2007), with psychomotor speed in patients with traumatic brain injury (Kinnunen et al 2011), our finding of a trend for a positive association between axial diffusivity in GW veterans with predicted GB/GF exposure and performance on the Grooved Pegboard test prompted us to further examine axial diffusivity from the posterior limb of the internal capsule. Age was the only potentially confounding variable that was significantly associated performance on the Grooved Pegboard test ($p < 0.001$), as expected from the aging literature (Madden 2001). Therefore, our post-hoc examination of the relationship between axial diffusivity from the posterior limb of the internal capsule and performance on the Grooved Pegboard test only accounted for age. The partial correlation was examined in each group separately and compared using Fisher's z-test.

4. RESULTS

Table 1 summarizes the demographic, military, and clinical data of the GB/GF exposed and unexposed GW veterans. The two groups were matched for age, gender, years of education, and diagnoses of current PTSD, major depressive disorder, and CMI based on criteria described by Fukuda et al. (1998). However, there were group differences in ethnicity ($\chi^2 = 20.67$, $df = 3$, $p < 0.001$) and military status during the Gulf War ($\chi^2 = 8.30$, $df = 2$, $p = 0.02$): There were more Caucasians among the unexposed GW veterans and more Latinos and National Guard personnel among GW veterans with predicted GB/GF exposure.

4.1. Group DTI differences in *a priori* Yamasue et al. ROIs

DTI data from the *a priori* Yamasue et al. ROIs are summarized in Table 2. There was no significant group effect for FA (omnibus MANCOVA: Wilks lambda = 0.96, Pillais approximate $F_{4,103} = 1.19$, $p=0.32$) or radial diffusivity (omnibus MANCOVA: Wilks lambda = 0.96, Pillais approximate $F_{4,103} = 1.18$, $p=0.33$). However, there was a significant group effect for axial diffusivity (omnibus MANCOVA: Wilks lambda = 0.84, Pillais approximate $F_{4,103} = 4.97$, $p=0.001$). The individual ANCOVAs revealed that veterans with predicted GB/GF exposure had significantly higher axial diffusivity in the left parietal cortex ($F_{1,106} = 4.80$, $p<0.03$, Cohen's $d = 0.36$), temporal stem ($F_{1,106} = 15.83$, $p<0.001$, Cohen's $d = 0.99$) and left sub-insular region ($F_{1,106} = 7.88$, $p=0.006$, Cohen's $d = 0.50$) compared to unexposed GW veterans.

4.2. Group DTI differences in the other white matter ROIs

DTI data from the other white matter ROIs are summarized in Table 3. As with the *a priori* Yamasue et al. ROIs, there was no significant group effect for FA (omnibus MANCOVA: Wilks lambda = 0.90, Pillais approximate $F_{11,96} = 1.02$, $p=0.44$), but there was a significant group effect for axial diffusivity (Wilks lambda = 0.67, Pillais approximate $F_{11,96} = 4.43$, $p<0.001$). Individual ANCOVAs revealed that veterans with predicted GB/GF exposure had higher axial diffusivity in the superior cingulum ($F_{1,106} = 9.48$, $p=0.003$, Cohen's $d = 0.51$), internal capsule ($F_{1,106} = 7.23$, $p=0.008$, Cohen's $d = 0.50$), external capsule ($F_{1,106} = 26.15$, $p<0.001$, Cohen's $d = 0.67$), superior fronto-occipital fasciculus ($F_{1,106} = 23.20$, $p<0.001$, Cohen's $d = 0.78$), inferior fronto-occipital fasciculus ($F_{1,106} = 15.30$, $p<0.001$, Cohen's $d = 0.80$), corona radiata ($F_{1,106} = 15.06$, $p<0.001$, Cohen's $d = 0.50$), and superficial white matter blades ($F_{1,106} = 19.77$, $p<0.001$, Cohen's $d = 0.78$) than unexposed GW veterans. Although axial diffusivity differences in the

inferior/hippocampal cingulum ($F_{1,106} = 6.40, p=0.01$) and the superior longitudinal fasciculus ($F_{1,106} = 5.57, p=0.02$) were not statistically significant after adjustments for multiple comparisons, the effect sizes of the group differences (Cohen's $d = 0.50$ and 0.57) may be considered medium (Cohen, 1988).

There was no overall group effect for radial diffusivity (omnibus MANCOVA: Wilks lambda = 0.86, Pillais approximate $F_{11,96} = 1.48, p=0.17$). However, the individual ANCOVAs revealed that veterans with predicted GB/GF exposure had higher radial diffusivity in the internal capsule ($F_{1,106} = 7.64, p=0.007$, Cohen's $d = 0.57$) and superior fronto-occipital fasciculus ($F_{1,106} = 9.55, p=0.003$, Cohen's $d = 0.50$) compared to unexposed veterans. Although group differences in radial diffusivity in external capsule ($F_{1,106} = 5.34, p=0.02$), superior longitudinal fasciculus ($F_{1,106} = 4.62, p=0.03$), and superficial WM blades ($F_{1,106} = 5.04, p=0.03$) were not statistically significant after adjustments for multiple comparisons, the effect sizes of these radial diffusivity differences (Cohen's $d = 0.50-0.57$) may be considered medium (Cohen, 1988).

4.3. Post-hoc analyses of the relationship between DTI and neurobehavioral measures

Table 4 summarizes the post-hoc partial correlations between the DTI and neurobehavioral data. In unexposed GW veterans, higher FA and lower radial diffusivity values were significantly associated with better performance (e.g., faster response times or fewer errors) on the Continuous Performance test, Trail Making Test Part A, Digit Span (forward and backward) subtest, Short Category test, and recognition memory on the California Verbal Learning Test. In contrast, there was only a trend ($0.05 < p < 0.06$) for higher axial and radial diffusivity values

test in GW veterans with predicted GB/GF exposure to be associated with faster response times on the Grooved Pegboard. Fisher's z-test revealed significant group differences in the correlations between FA and number of omission errors on the Continuous Performance test ($z=-2.03$, $p=0.04$), number of errors on the Short Categories test ($z=2.15$, $p=0.03$), response time on the Trail Making Test A ($z=-3.38$, $p=0.001$), forward ($z=3.16$, $p=0.002$) and backward ($z=2.26$, $p=0.008$) Digit Span, Grooved Pegboard with the non-dominant hand ($z=-2.99$, $p=0.003$), recognition hits on the California Verbal Learning test ($z=3.57$, $p<0.001$) and significant group differences in the correlations between radial diffusivity and response time on the Trail Making Test A ($z=3.26$, $p<0.001$), forward ($z=-3.72$, $p<0.001$) and backward ($z=-2.48$, $p=0.01$) Digit Span, number of errors on the Short Categories test ($z=2.15$, $p=0.03$) and recognition hits on the California Verbal Learning test ($z=-2.19$, $p=0.03$).

4.4. Post-hoc analysis of the relationship between axial diffusivity in the posterior limb of the internal capsule and performance on the Grooved Pegboard test

In veterans with predicted GB/GF exposure, axial diffusivity in the posterior limb of the internal capsule was negatively associated with response time on the Grooved Pegboard test with the dominant ($R_{\text{partial}} = -0.28$, $p=0.03$) and non-dominant ($R_{\text{partial}} = -0.27$, $p=0.04$) hands (i.e., higher axial diffusivity was associated with shorter response times). Axial diffusivity in the posterior limb of the internal capsule was also negatively associated with response time on the Grooved Pegboard test with the dominant ($R_{\text{partial}} = -0.30$, $p=0.02$) in unexposed GW veterans. Fisher's z test revealed no significant group differences in the correlations.

4.5. Ability of axial diffusivity in the right inferior fronto-occipital fasciculus to predict CMI status

After accounting for age, sex, predicted GB/GF exposure status, trauma exposure, diagnoses of current PTSD, major depressive disorder, history of alcohol and/or drug abuse or dependence, and ICV-adjusted gray and white matter volume, axial diffusivity in the right inferior fronto-occipital fasciculus was not significantly associated with CMI status (standardized $\beta = -0.13$, $t = -1.61$, $p = 0.11$). There was still significant association between the axial diffusivity in the right inferior fronto-occipital fasciculus and CMI status (standardized $\beta = -0.08$, $t = -0.88$, $p = 0.38$) even when GW veterans with CMI due to non-fatigue related symptoms (i.e., musculoskeletal pain and mood/cognition) were excluded from the analysis.

5. DISCUSSION

To our knowledge this is the first study to examine white matter microstructure in GW veterans with predicted GB/GF exposure. Contrary to our hypothesis, GW veterans with predicted GB/GF exposure did not have reduced FA in the brain regions where Yamasue et al. (2007) previously found reduced FA in victims of the 1995 Tokyo subway sarin attack. There were also no significant FA differences between GW veterans with and without predicted GB/GF exposure in other major white matter fiber systems throughout the brain. This lack of group FA difference may relate to the fact that FA is derived from the ratio of the three eigenvalues of the diffusion tensor. Therefore an increase (or decrease) in the primary eigenvalue (i.e., axial diffusivity) accompanied by an increase (or decrease) in the other two eigenvalues (which are measured by radial diffusivity) would result in no change in FA. With this respect, it is noteworthy that

previous studies of aging and Alzheimer's disease have found FA to be the least sensitive of all the DTI indices for detecting differences between diagnostic groups (Acosta-Cabronero et al 2010; Clerx et al 2012; Nir et al 2013; Sullivan et al 2010). This was our rationale for examining other DTI parameters in GW veterans with and without predicted GB/GF exposure in the present study.

Examination of axial and radial diffusivity in the *a priori* Yamasue et al. ROIs revealed significant group differences in axial diffusivity in the temporal stem bilaterally, the left parietal, and left subinsular regions. Specifically, veterans with predicted GB/GF exposure had higher axial diffusivity in these regions compared to unexposed GW veterans. Our investigation of other white matter ROIs also revealed higher axial diffusivity in the superior cingulum, internal and external capsule, superior and inferior fronto-occipital fasciculus, corona radiata, and superficial white matter blades of veterans with predicted GB/GF exposure compared to unexposed controls. According to the guidelines proposed by Cohen (1988), the effect sizes of these group differences may be considered medium to large.

It was not clear whether the increased axial diffusivity that we observed in GW veterans with predicted GB/GF exposure relative to unexposed veterans was associated with neurodegeneration or white matter reorganization because increased axial diffusivity has been linked with both (e.g., amyotrophic lateral sclerosis (Metwalli et al 2010), Alzheimer's disease (Bosch et al 2010 ; Nir et al 2013; Shu et al 2011), animal models of Parkinson disease (Hikishima et al 2015), white matter reorganization due to axonal recovery or even regrowth

(Sidaros et al 2008; Voss et al 2006)). For this reason, we conducted post-hoc analyses to examine the association between DTI measures and neurobehavioral performance. In unexposed GW veterans, we found significant correlations between higher FA and lower radial diffusivity values and better performance on measures of attention, executive function, and recognition memory. This makes sense because healthier white matter tracts would be expected to be associated with better neurobehavioral function and in healthy white matter tracts, water molecules diffuse more freely along the axonal fibers (which would be measured as high FA) but are relatively restricted perpendicular to the fibers due to tightly packed axons and myelin sheets that surround them (which would be measured as low radial diffusivity; Basser and Pierpaoli, 1996). In contrast, in GW veterans with predicted GB/GF exposure there was only a trend for increased axial and radial diffusivity to be associated with faster response times on the Grooved Pegboard test.

Because increased axial diffusivity in the posterior limb of the internal capsule has been associated with faster psychomotor speed (Kinnunen et al 2011) and better functional outcome after traumatic brain injury (Sidaros et al 2008), this prompted us to further investigate the relationship between axial diffusivity in the posterior limb of the internal capsule with performance on the Grooved Pegboard test. This second post-hoc analysis revealed a positive correlation between axial diffusivity in the posterior limb of the internal capsule, which maps to the posterior limb of the internal capsule to motor and premotor cortices (Zarei et al 2007), and response time on the Grooved Pegboard test in veterans with predicted GB/GF exposure. Although there have been reports of psychomotor slowing in GW veterans with predicted

GB/GF exposure (Proctor et al 2006) and in victims of the Tokyo subway sarin attack (Miyaki et al 2005; Yokoyama et al 1998), it is noteworthy that we did not find any evidence of psychomotor slowing in the current cohort of GW veterans with predicted GB/GF exposure (Chao et al. 2011). Instead, this cohort of GW veterans with predicted GB/GF exposure actually performed *faster* than unexposed GW veterans on the Trail Making Test A and the Grooved Pegboard test with the non-dominant hand. Moreover, compared to unexposed GW veterans, there were no major neurobehavioral impairments in this cohort of GW veterans with predicted GB/GF exposure other than subtle attention deficits (i.e., more omission errors on the Continuous Performance Test; Chao et al 2011). We similarly found no evidence of significant neurobehavioral deficits in an earlier study of a different cohort of GW veterans with predicted GB/GF exposure compared to unexposed veterans. Thus these findings, together with the absence of correlations between DTI measures and neurobehavioral performance GW veterans with predicted GB/GF exposure hints at the possibility of white matter reorganization in GW veterans with predicted GB/GF exposure. However, this interpretation should be viewed with caution because the biophysical basis of changes in axial diffusivity in the brain are still not completely understood (Budde et al 2009; Englund et al 2004) and because measures of axial and radial diffusivities may be problematic when crossing fibers make eigenvalue directions between subjects uncertain (Wheeler-Kingshott and Cercignani 2009).

If increased the axial diffusivity that we observed in GW veterans with predicted GB/GF exposure may be a sign of white matter reorganization after GB/GF exposure, then why would there also be a significant correlation between axial diffusivity in the posterior limb of the

internal capsule and performance on the Grooved Pegboard test in unexposed GW veterans?

Although the plume model analysis suggests that these veterans were probably not exposed to GB/GF, GW military personnel were likely exposed to a variety of other potentially hazardous chemicals, including pyridostigmine bromide pills given prophylactically as a protective measure against nerve agents and extensive use of pesticides (Research Advisory Committee on Gulf War Veterans' Illness, 2008; U.S. Department of Defense 2003; Fricker et al 2000; Steele et al 2012). Therefore, it is reasonable to hypothesize that there may also be some degree of white matter reorganization after exposure to other potentially hazardous chemicals in unexposed GW veterans.

Rayhan and colleagues (2013) recently suggested that axial diffusivity in the right inferior fronto-occipital fasciculus may be a potential biomarker for CMI. However we did not find axial diffusivity from the right inferior fronto-occipital axial diffusivity to be significantly associated with CMI status after accounting for potentially confounding variables. One possible reason for the discrepant findings may be a difference in study samples: All the GW veterans with CMI in the Rayhan et al. study also met the clinical criteria for Chronic Fatigue Syndrome (Fukuda et al 1994). In contrast, we did not assess GW veterans for Chronic Fatigue Syndrome. Some the GW veterans with CMI in the current study did not have symptoms of fatigue because the (Fukuda et al 1998) case definition of CMI only requires symptoms in 2/3 domains. However, we still did not observe a significant association between right inferior fronto-occipital fasciculus axial diffusivity and CMI status even after excluding veterans with CMI due to non-fatigue-related

symptoms from the analysis. Therefore other reasons (e.g., 3T vs. 4T MR scanner, different imaging parameters, different pre- and post-processing of the DTI data) may account for why we were unable to replicate Rayhan et al.'s findings.

The present findings should be considered in the context of several study limitations: First, the DTI data had low (6 directions) angular resolution. Second, we did not have information about the severity of the symptoms associated with CMI, smoking status, or history of head injury (although history of head injury associated with prolonged loss of consciousness was exclusionary for the study). Third, it may be possible that military personnel more likely to have been in the GB/GF exposure areas are different from those in low or no exposure areas. Unfortunately, we are not able to address this question in the current study. Fourth, because the GB/GF exposure estimates were calculated at the unit rather than individual level, there is no definitive way of knowing if an individual soldier was with his or her unit on the four target dates the model. Moreover, the modeled exposure estimates may be subject to misclassification (United States General Accounting Office, 2004). However, general misclassification errors are likely to be random, therefore would have limited influence on the present results. Fifth, as mentioned earlier, it has been suggested that measures of axial and radial diffusivities may be problematic when crossing fibers make eigenvalue directions between subjects uncertain (Wheeler-Kingshott and Cercignani 2009). Finally, the cross-sectional study design did not allow us to address issues pertaining to longitudinal changes in white matter microstructure. Therefore future longitudinal DTI studies will be needed to resolve whether the white matter alterations that we observed in GW veterans with predicted

GB/GF exposure represent white matter reorganization associated with adaptive axonal recovery or neurodegeneration.

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REFERENCES

- Acosta-Cabronero J, Williams GB, Pengas G, Nestor PJ. Absolute diffusivities define the landscape of white matter degeneration in Alzheimer's disease. *Brain* 2010; 133:529-39.
- Agosta F, Pievani M, Sala S, et al. White matter damage in Alzheimer disease and its relationship to gray matter atrophy. *Radiology* 2011; 258:853-63.
- Alexander AL, Lee JE, Lazar M, et al. Diffusion tensor imaging of the brain. *Neurotherapeutics* 2007; 3:316-29.
- Ashburner J. A fast diffeomorphic image registration algorithm. *Neuroimage* 2007; 38:95-113.
- Basser PJ, Pierpaoli C. 1996. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson B* 111:209-19
- Blake DD, Weathers FW, Nagy LM, et al. The development of a Clinican-Administered PTSD Scale. *J Trauma Stress* 1995; 8:75-90.
- Bosch B, Arenaza-Urquijo EM, Rami L, et al. Multiple DTI index analysis in normal aging, amnesic MCI and AD. Relationship with neuropsychological performance. *Neurobiol Aging* 2010 ; 33:61-74.
- Budde MD, Xie M, Cross AH, et al. Axial diffusivity is the primary correlate of axonal injury in the EAE spinal cord: a quantitative pixelwise analysis. *J Neurosci* 2009; 29:2805-13.
- Chao LL, Rothlind JC, Cardenas VA, et al. 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.

Chao LL, Abadjian L, Hlavin J, et al. Effects of low-level sarin and cyclosarin exposure and Gulf War Illness on brain structure and function: a study at 4 Tesla. *Neurotoxicology* 2011; 32:814–22.

Clerx L, Visser PJ, Verhey F, et al. New MRI markers for Alzheimer's disease: a meta-analysis of diffusion tensor imaging and a comparison with medial temporal lobe measurements. *J. Alzheimers Dis.* 2012; 29:405-29.

Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Hillsdale, New Jersey: Laurence Erlbaum Associates; 1988.

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. Available from: <http://www.gulflink.osd.mil>; 1997.

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. US demolition operations at the Khamisiyah ammunition point (case narrative). Available at: <http://www.gulflink.osd.mil/khamisiyahiii>; 2002.

Delis DC, Kramer JH, Kaplan E, et al. 2000. California Verbal Learning Test.

Englund E, Sjobeck M, Brockstedt S, et al. Diffusion tensor MRI post mortem demonstrated cerebral white matter pathology. *J Neurol* 2004; 251:350-2.

First M, Spitzer R, Gibbon M, et al. *Structured Clinical Interview for DMS-IV*. New York: New Work State Psychiatric Institute Biometrics Research Department; 1995.

- Fricker RD, Jr., Reardon E, Spektor DM, et al. Pesticide Use During the Gulf War: A Survey of Gulf War Veterans., Arlington, VA; 2000.
- Fukuda K, Niesenbaum R, Stewart G, et al. Chronic multisymptom illness affecting Air Force veterans of the Gulf War. JAMA 1998; 280:981-8.
- Fukuda K, Straus SE, Hickie I, et al. The chronic fatigue syndrome: a comprehensive approach to its definition and study International Chronic Fatigue Syndrome Study Group. Ann Intern Med. 1994; 12:953-9.
- Heaton KJ, Palumbo CL, Proctor SP, et al. Quantitative magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. Neurotoxicology 2007; 28:761-9.
- Hikishima K, Ando K, Yano R, et al. Parkinson disease: Diffusion MR Imaging to detect nigrostriatal pathway loss in a marmoset model treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Radiology 2015; 15:140601. [Epub ahead of print]
- Kinnunen KM, Greenwood R, Powell JH, et al. White matter damage and cognitive impairment after traumatic brain injury. Brain 2011; 134:449-63.
- Kumar R, Macey PM, Woo MA, et al. Rostral brain axonal injury in congenital central hypoventilation syndrome. J Neurosci Res. 2010; 88:2146-54.
- Kumar R, Nguyen HD, Macey PM, et al. Regional brain axial and radial diffusivity changes during development. J Neurosci Res. 2012; 90:346-55.
- Letz RL. 1991. NES2 User's Manual.
- Madden DJ. Speed and timing of behavioral processes. In Birren JE, Schaie KW, editors Handbook of the psychology of aging. San Diego: Academic Press; 2001. p.288-312.

Metwalli NS, Benatar M, Nair G, et al. Utility of axial and radial diffusivity from diffusion tensor MRI as markers of neurodegeneration in amyotrophic lateral sclerosis. *Brain Res* 2010; 1348:156-64.

Miyaki K, Nishiwaki Y, Maekawa K, et al. Effects of sarin on the nervous system of subway workers seven years after the Tokyo subway sarin attack. *J Occup Health* 2005; 47:299–304.

Mori S, Oishi K, Jiang H, et al. Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template. *Neuroimage* 2008; 40:570-82.

Nir TM, Jahanshad N, Villalon-Reina JE, et al. Effectiveness of regional DTI measures in distinguishing Alzheimer's disease, MCI, and normal aging. *Neuroimage* 2013; 3:180-95.

Persian Gulf War Illness Task Force. *Khamisiyah: a historical perspective on related intelligence*. Washington, DC: Central Intelligence Agency; 1997.

Proctor SP, Heaton KJ, Heeren T, et al. Effects of chemical warfare agent exposure on central nervous system functioning in US army veterans of the 1991 Gulf War. *Neurotoxicology* 2006; 27:931–9

Research Advisory Committee on Gulf War Veterans' Illness (RAC-GWVI). *Gulf War Illness and the Health of Gulf War Veterans*, Washington, DC; 2008.

Rayhan RU, Stevens BW, Timbol CR, et al.. Increased brain white matter axial diffusivity associated with fatigue, pain and hyperalgesia in Gulf War illness. *PLoS One* 2013; 8:e58493.

Reitan RM, Wolfson D.. *The Halstead-Reitan Neuropsychological Test Battery: Theory and Interpretation*; 1985.

- Sankoh AJ, Huque MF, et al. Some comments on frequently used multiple endpoint adjustment methods in clinical trials. *Statistics in Medicine* 1997; 16:2529–42.
- Shu N, Wang Z, Qi Z, et al. Multiple diffusion indices reveals white matter degeneration in Alzheimer's disease and mild cognitive impairment: a tract-based spatial statistics study. *J Alzheimers Dis*. 2011;26:275-85.
- Sidaros A, Engberg A, Sidaros K, et al. Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study. *Brain* 2008; 131:559-72.
- Song SK, Sun SW, Ju WK, et al. Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *NeuroImage* 2003; 20:1714-22.
- Song SK, Sun SW, Ramsbottom M, et al. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage* 2002; 17:1429-36.
- Steele L, Sastre A, Gerkovich MM, et al. Complex Factors in the Etiology of Gulf War Illness: Wartime Exposures and Risk Factors in Veteran Subgroups. *Environmental Health Perspect* 2012; 120:112-8.
- Steenwijk MD, Daams M, Pouwels PJ, et al. What explains gray matter atrophy in long-standing multiple sclerosis? *Radiology* 2014; 272:832-42
- Sullivan EV, Rohlfing T, Pfefferbaum A. Quantitative fiber tracking of lateral and interhemispheric white matter systems in normal aging: relations to timed performance. *Neurobiol Aging* 2010; 31:464-81.

- Tao R, Fletcher PT, Gerber S, et al. A variational image-based approach to the correction of susceptibility artifacts in the alignment of diffusion weighted and structural MRI. *Inf Process Med Imaging* 2009; 21:651-63.
- Tyszka JM, Readhead C, Bearer EL, et al. Statistical diffusion tensor histology reveals regional dysmyelination effects in the shiverer mouse mutant. *Neuroimage* 2006; 29:1058-65.
- United States Department of Defense. Environmental Exposure Report: Pesticides: Final Report., Washington, DC; 2003.
- United States General Accounting Office. DOD's conclusions about U.S. troops' exposure cannot be adequately supported. Washington, D.C.: United States General Accounting Office; 2004.
- Vernooij MW, de Groot M, van der Lugt A, et al. White matter atrophy and lesion formation explain the loss of structural integrity of white matter in aging. *Neuroimage* 2008; 43:470-7.
- Voss HU, Ulug AM, Dyke JP, et al. Possible axonal regrowth in late recovery from the minimally conscious state. *J Clin Invest.* 2006; 116:2005-11.
- Wechsler D. The Wechsler Adult Intelligence Scale. San Antonio, TX: Psychological Corporation
- Wetzel L, Boll T. 1987. Short Category Test, Booklet Format; 1997.
- Wheeler-Kingshott CAM, Cercignani M. About "axial" and "radial" diffusivities. *Magn Reson Med.* 2009; 61:1255-60.
- 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.

- Yokoyama K, Araki S, Murata K, et al. Chronic neurobehavioral effects of Tokyo subway sarin poisoning in relation to posttraumatic stress disorder. *Arch Environ Health* 1998; 53:249–56.
- Zarei M, Johansen-Berg H, Jenkinson M, et al. Two-dimensional population map of cortical connections in the human internal capsule. *J Magn Reson Imaging* 2007; 25:48-54.
- Zhang YZ, Schuff N, Camacho M, et al. MRI markers for mild cognitive impairment: comparisons between white matter integrity and gray matter volume measurements *PLoS One* 2013; 8:e663687.

Figure Legend:

Figure 1: Representation of *a priori* Yamasue et al. ROIs overlaid on coronal sections of a group FA map. Top row shows left subinsula ROI; second row shows left subinsula and bilateral temporal stem ROIs; third row shows left subinsula, bilateral temporal stem, left parietal cortex, and brainstem ROIs; bottom row shows left parietal and bilateral temporal stem ROIs.

Figure 2: Representation of eleven other white matter ROIs overlaid on coronal, axial, and sagittal sections of a group FA map. (A) corpus callosum = red, fornix = light blue; superior cingulum = green; corona radiata = yellow; internal capsule = dark blue; external capsule = magenta; (B) inferior fronto-occipital fasciculus = pink ; superior fronto-occipital fasciculus = orange; superior longitudinal fasciculus = lavender; inferior (hippocampal) cingulum = green (C) superficial WM blades = cyan.

Figure1

Figure2

Table 1. Demographic, military, and clinical characteristics GB/GF exposed and unexposed GW veterans

	<u>Exposed</u>	<u>Unexposed</u>
N	59	59
Age (years)	48.5 (7.6)	48.4 (7.2)
No. females (%)	6 (10%)	6 (10%)
Ethnicity ^a		
African American (%)	8 (14%)	3 (5%)
Caucasian (%)	26 (44%)	42 (71%)
Latino (%)	19 (32%)	9 (15%)
Other (%)	6 (10%)	5 (8%)
Education (years)	15.1 (2.2)	15.6 (2.2)
Military status during Gulf War ^a		
Active Duty (%)	43 (73%)	52 (88%)
National Guard (%)	11 (19%)	2 (3%)
Reserves (%)	5 (9%)	5 (9%)
No. with service-connected disability	25 (42%)	34 (58%)
Average % disability (range)	21 (5-80)	34 (5-100)
No. with CMI (%)	36 (61%)	36 (61%)
No. with current PTSD diagnosis (%)	4 (7%)	4 (7%)
Current CAPS score	9.8 (18.9)	9.5 (16.7)
No. with current MDD diagnosis (%)	5 (8%)	7 (12%)
HAM-D Score	5.2 (4.8)	6.1 (6.5)
No. on psychotropic medication	9 (15%)	15 (25%)
No. with history of alcohol abuse/dependence	21 (36%)	28 (47%)
Current average no. drinks/month	22.5 (25.8)	20.5 (33.7)
No. with history of drug abuse/dependence	7 (12%)	6 (10%)

Means (SD) or N (%) reported

CMI, Chronic multisymptom Illness; PTSD, posttraumatic stress disorder; CAPS, Clinician Administered PTSD Scale; MDD, major depressive disorder; HAM-D, Hamilton Depressive Scale

^aSignificantly different between the two groups, $p < 0.05$

Table 2. Least Squares Mean DTI values^a by group from the Yamasue et al. a priori ROIs

DTI index	Yamasue et al. ROI	DTI atlas ROI	Unexposed	Exposed	F(1,106)	Cohen's <i>d</i>
FA	L. parietal	L. posterior corona radiata	0.42 (0.03)	0.43 (0.03)	0.93	-0.33
	Bilateral temporal stem	L & R sagittal striatum & uncinate	0.52 (0.05)	0.51 (0.06)	0.53	0.18
	brainstem	Pontine crossing tract	0.48 (0.08)	0.48 (0.09)	0.03	0.00
	L. subinsula	L. external capsule	0.40 (0.05)	0.40 (0.04)	0.10	0.00
AD	L. parietal	L. posterior corona radiata	1.19 (0.05)	1.21 (0.06)	4.80*	-0.36
	Bilateral temporal stem	L& R sagittal striatum & uncinate	1.35 (0.04)	1.39(0.04)	15.83***	-0.99
	brainstem	Pontine crossing tract	1.15 (0.18)	1.11 (0.18)	1.22	0.22
	L. subinsula	L. external capsule	1.13 (0.04)	1.15 (0.04)	7.88**	-0.50
RD	L. parietal	L. posterior corona radiata	0.61 (0.05)	0.62 (0.04)	0.62	-0.22
	Bilateral temporal stem	L & R sagittal striatum & uncinate	0.57 (0.05)	0.59 (0.09)	3.04	-0.27
	brainstem	Pontine crossing tract	0.53 (0.10)	0.51 (0.09)	1.99	0.21
	L. subinsula	L. external capsule	0.61 (0.05)	0.63 (0.05)	1.83	-0.40

Means and standard deviations (in parentheses) are reported.

^acontrolling for age, sex, total brain GM and WM volume, CMI-status, trauma exposure, diagnoses of current PTSD and/or major depressive disorder, history of alcohol and/or drug abuse/dependence.

*p<0.05, **p<0.01, ***p<0.001

Table 3. Least Squares DTI values^a by group from the other white matter ROIs

DTI index	ROI	Unexposed	Exposed	F(1,106)	Cohen's d
FA	Fornix	0.53 (0.08)	0.52 (0.10)	0.38	0.11
	Superior cingulum	0.44 (0.04)	0.44 (0.04)	0.85	0.00
	Inferior (hippocampal) cingulum	0.43 (0.04)	0.43 (0.04)	0.81	0.00
	Internal capsule	0.59 (0.03)	0.58 (0.04)	0.12	0.28
	External capsule	0.41 (0.04)	0.41 (0.03)	0.62	0.00
	Superior fonto-occipital fasciculus	0.45 (0.04)	0.45 (0.04)	0.82	0.00
	Inferior fronto-occipital fasciculus	0.47 (0.05)	0.48 (0.06)	0.69	-0.18
	Superior longitudinal fasciculus	0.48 (0.03)	0.47 (0.03)	0.13	0.33
	Corona Radiata	0.42 (0.02)	0.42 (0.02)	0.99	0.00
	Corpus Callosum	0.59 (0.04)	0.58 (0.06)	0.50	0.20
	Superficial WM blades	0.45 (0.02)	0.45 (0.03)	0.45	0.00
AD	Fornix	2.26 (0.14)	2.28 (0.16)	0.68	-0.13
	Superior cingulum	1.18 (0.05)	1.22 (0.10)	9.48**	-0.51
	Inferior (hippocampal) cingulum	1.14 (0.06)	1.17 (0.05)	6.40*	-0.54
	Internal capsule	1.30 (0.04)	1.32 (0.04)	7.23**	-0.50
	External capsule	1.13 (0.03)	1.15 (0.03)	26.15***	-0.67
	Superior fonto-occipital fasciculus	1.12 (0.04)	1.16 (0.06)	23.20***	-0.78
	Inferior fronto-occipital fasciculus	1.30 (0.05)	1.34 (0.05)	15.30***	-0.80
	Superior longitudinal fasciculus	1.18 (0.03)	1.20 (0.04)	5.57*	-0.57
	Corona Radiata	1.17 (0.04)	1.19 (0.04)	15.06***	-0.50
	Corpus Callosum	1.54 (0.05)	1.55 (0.04)	1.02	-0.22
	Superficial WM blades	1.17 (0.02)	1.19 (0.03)	19.77***	-0.78
RD	Fornix	1.01 (0.23)	1.06 (0.24)	1.34	-0.22
	Superior cingulum	0.58 (0.04)	0.60 (0.09)	3.37	-0.29
	Inferior (hippocampal) cingulum	0.58 (0.06)	0.59 (0.06)	1.68	-0.17
	Internal capsule	0.46 (0.03)	0.48 (0.04)	7.64**	-0.57
	External capsule	0.60 (0.04)	0.62 (0.04)	5.34*	-0.50

Superior fronto-occipital fasciculus	0.55 (0.04)	0.57 (0.04)	9.55**	-0.50
Inferior fronto-occipital fasciculus	0.60 (0.06)	0.61 (0.07)	0.97	-0.15
Superior longitudinal fasciculus	0.55 (0.04)	0.57 (0.04)	4.62*	-0.50
Corona Radiata	0.60 (0.03)	0.61 (0.03)	3.73	-0.33
Corpus Callosum	0.55 (0.07)	0.56 (0.08)	0.56	-0.13
Superficial WM blades	0.58 (0.03)	0.60 (0.04)	5.04*	-0.57

Means and standard deviations (in parentheses) are reported.

^acontrolling for age, sex, total brain GM and WM volume, CMI-status, trauma exposure, diagnoses of current PTSD and/or major depressive disorder, history of alcohol and/or drug abuse/dependence.

*p<0.05, **p<0.01, ***p<0.001

Table 4. Post-hoc partial correlations between DTI and neurobehavioral data by group

	<u>Unexposed</u>			<u>Exposed</u>		
	FA	Axial diffusivity	Radial diffusivity	FA	Axial diffusivity	Radial diffusivity
Attention						
CPT response time to targets ^{a,b}	-0.39 ^e	-0.26	0.32 ^d	-0.01	0.19	0.03
CPT no. omission errors ^b	-0.36 ^d	-0.07	0.33 ^d	0.04	0.14	0.03
CPT no. commission errors ^b	0.04	-0.07	-0.10	0.03	-0.22	-0.05
Trail Making Test A ^a	-0.50 ^f	-0.10	0.05 ^e	0.09	-0.04	-0.13
WAIS-III Digit Spans (forward)	0.46 ^e	-0.22	-0.51 ^f	-0.10	0.12	0.14
Executive function						
Trail Making Test B ^a	-0.25	0.09	0.27	0.11	-0.14	-0.14
Short Category Test, total errors ^a	-0.34 ^d	0.01	0.36 ^d	-0.02	-0.06	-0.03
WAIS-III Digit Spans (backward)	0.41 ^e	0.08	-0.37 ^d	-0.06	0.17	0.08
Psychomotor function (Grooved Pegboard test)						
Dominant hand ^a	-0.20	-0.17	0.14	0.24	-0.28 ^g	-0.27 ^g
Non-dominant hand ^a	-0.30 ^d	-0.03	0.28	0.25	-0.21	-0.27 ^g
Memory (California Verbal Learning Test)						
Short-delay free recall ^c	-0.02	-0.01	0.02	-0.10	-0.07	0.05
Long-delay free recall ^c	0.01	0.15	0.05	-0.04	0.08	0.03
Recognition hits ^c	0.38 ^d	0.05	-0.35 ^d	-0.27	0.09	0.05

Partial correlation coefficients, controlling for age, sex, education, Chronic Multisymptom Illness, trauma exposure, posttraumatic stress disorder, major depressive disorder, history of alcohol and/or drug abuse/dependence.

CPT, Continuous Performance Test; WAIS, Wechsler Adult Intelligence Scale

^aLower scores indicate better performance

^bdata available for 50 unexposed and 51 exposed GW veterans

^cdata available for 59 unexposed and 58 exposed GW veterans

^d $p < 0.05$

^e $p \leq 0.01$

^f $p < 0.001$

^g $0.05 < p < 0.06$