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Effects of low-level exposure to sarin and cyclosarin during the 1991 Gulf War on brain function and brain structure in US veterans

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Abstract

Background—Potentially more than 100,000 US troops may have been exposed to the organophosphate chemical warfare agents sarin (GB) and cyclosarin (GF) when a munitions dump at Khamisiyah, Iraq was destroyed during the Gulf War (GW) in 1991. Although little is known about the long-term neurobehavioral or neurophysiological effects of low-dose exposure to GB/GF in humans, recent studies of GW veterans from the Devens Cohort suggest decrements in certain cognitive domains and atrophy in brain white matter occur individuals with higher estimated levels of presumed GB/GF exposure. The goal of the current study is to determine the generalizability of these findings in another cohort of GW veterans with suspected GB/GF exposure.

Methods—Neurobehavioral and imaging data collected in a study on Gulf War Illness between 2002–2007 were used in this study. We focused on the data of 40 GW-deployed veterans categorized as having been exposed to GB/GF at Khamisiyah, Iraq and 40 matched controls. Magnetic resonance images (MRI) of the brain were analyzed using automated and semi-automated image processing techniques that produced volumetric measurements of gray matter (GM), white matter (WM), cerebrospinal fluid (CSF) and hippocampus.

Results—GW veterans with suspected GB/GF exposure had reduced total GM and hippocampal volumes compared to their unexposed peers ($p \leq 0.01$). Although there were no group differences in measures of cognitive function or total WM volume, there were significant, positive correlations between total WM volume and measures of executive function and visuospatial abilities in veterans with suspected GB/GF exposure.

Conclusions—These findings suggest that low-level exposure to GB/GF can have deleterious effects on brain structure and brain function more than decade later.

Keywords

Cognitive functioning; Magnetic resonance imaging; morphometric analysis; Brain; Central nervous system; Chemical warfare agents; Sarin; Cyclosarin; Gulf War veterans

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1. Introduction

More than 100,000 US service members participating in the first 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, 2002). After the war, the Department of Defense (DoD) and the Central Intelligence Agency (CIA) initiated efforts 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 DoD used these unit-level criteria to notify the potentially exposed troops in 1997 The DoD used these unit-level criteria to notify the potentially exposed troops in 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, 1997). Later, the exposure plume models were re-analyzed 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. These efforts resulted in a second round of notification letters mailed to troops whose units were located within the updated hazard areas (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).

Although reviews of the medical records of potentially exposed GW veterans and of published field accounts revealed no clinical indications of GB/GF poisoning at the time of this possible exposure (Riddle et al. 2003), there have since been reports of an elevation in risk for hospitalization due to circulatory diseases (Smith et al. 2003) and a two-fold increase in the incidence of brain cancer deaths more than 4 years after the presumed exposure (Bullman et al. 2005). Investigations of the effects of GB/GF on cognitive function have revealed poorer performance on tasks of short-term memory and attention and tests assessing mood in veterans with self-reported chemical warfare exposure compared to veterans who did not report chemical warfare exposure (White et al. 2001). Using cumulative exposure estimates based on refined models of the Khamisiyah hazard, Proctor et al. (2006) reported decrements in manual dexterity and visuospatial functions while Heaton et al. (2007) reported reduced white matter (WM) and increased lateral ventricle volumes in GW veterans with higher estimated levels of presumed GB/GF exposure.

The veterans studied by Proctor and Heaton were all recruited from the Devens Cohort, 2949 GW-deployed army veterans from the New England area who participated in a longitudinal study assessing psychological and physical health effects of the GW (Proctor et al. 1998). The goal of this study is to determine whether the findings reported by Proctor, Heaton and colleagues reflect something unique about the Devens cohort or is a widespread phenomenon that can be found in all GW veterans with suspected GB/GF exposure. Based on the previous findings of White et al. (2001) and Proctor et al. (2006), our first hypothesis is that that GB/GF exposed veterans would show deficits on measures of short-term memory, attention, visuospatial abilities, and manual dexterity relative to their unexposed peers. Based on the findings of Heaton et al. (2007), our second hypothesis is that GB/GF exposed veterans would have reduced WM and increased ventricular cerebral spinal fluid (CSF) volumes compared to

unexposed veterans. Because Yamasue et al. (2007) recently reported smaller hippocampal volumes in the victims of the 1995 Tokyo subway sarin attack, we also examined hippocampal volumes of GW veterans with and without suspected GB/GF exposure.

Haley et al. (1997) have suggested that the clusters of symptoms that afflict many GW veterans represent discrete factor analysis-derived syndromes that may reflect a spectrum of neurologic injury involving the central, peripheral, and autonomic nervous system. Haley and Kurt (1997) further theorized that Syndrome 2 ("confusion-ataxia") may have resulted from sublethal exposures to chemical nerve agents. Therefore another aim of this study was to examine factor analysis-derived syndromes in GW veterans with and without suspected GB/GF exposure.

2. Materials and Methods

2.1. Description of exposure and exposure dosage estimates

Because measurements of GB/GF exposure levels were not obtained at the time of the demolitions, the DoD and CIA initiated efforts 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). The Office of the Special Assistant for Gulf War Illnesses (OSAGWI) led the effort to estimate the possible hazard areas, which was done using available meteorological data and estimates of atmospheric transport and diffusion. The Naval Research Laboratory, Naval Surface Warfare Center, the Lawrence Livermore National Laboratory, and the Defense Threat Reduction Agency also cooperated in the effort to estimate the possible hazard areas. The plume analyses resulted in four modeled hazards areas, corresponding to each of 4 days (10–13 March 1991) when GB/GF release was considered to be possible. Each model contained the area within the concentration contour of the general population limit (GPL), which reflects the limit below which individuals within the general population could be exposed 24 hours per day for 70 years without experiencing any adverse health effects. The US army and the Centers for Disease Control and Prevention estimate the GPL value to be 0.01296 mg min/m^3 (McNamara and Leitnaker 1971). The plume estimates were superimposed onto geographic maps containing military unit locations and 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 2002, the exposure plume models were re-analyzed 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. In 2007, we requested and received information about exposure status and the cumulative exposure level estimates of 230 GW veterans who participated in our study on the effects of Gulf War Illness from the Deputy Assistant Secretary of Defense for Force Health Protection and Readiness. The exposure level estimates reflect the cumulative exposure level estimate for the unit to which each study participant was assigned from 10 to 13 March 1991. Because the last modeling effort that determined the exposure estimates occurred in 2002, individuals who for some reason were not identified in initial data collections but were later identified as having been with units that were in the hazard area, do not have cumulative exposure level estimates.

2.1. Selection of Participants

Participants were drawn from 230 GW veterans who participated in a study on the effects of Gulf War Illness at the San Francisco Veterans Affairs Medical Center between 2002–2007. Information obtained from the office of the Deputy Assistant Secretary of Defense for Force Health Protection and Readiness, which maintains the database of individuals who are considered to have been in the possible hazard areas, identified 40 GW veterans from this

cohort as having potentially been exposed to GB/GF according to the revised plume modeling data. From the remaining 190 unexposed GW veterans, we selected 40 veterans of similar age, sex, handedness, educational-level and with similar Clinical Administered PTSD Scale (CAPS; Blake et al. 1990) scores with the exposed veterans to serve as controls. The Intuitional Review Boards of the University of California San Francisco and San Francisco VA approved both studies and informed consent was obtained from all participants.

2.2. Study protocol and measures

The complete study protocol included twelve self-report questionnaires about physical and mental health status, a battery of neuropsychological tests, a psychological diagnostic interview, a medical examination, and magnetic resonance imaging (MRI) on a 1.5T scanner. Neuropsychological testing and MRI scans took place on the same day. The current report focuses on the neuropsychological and the structural imaging data.

2.3.Neuropsychological battery and clinical assessment

The neuropsychological test battery (Table 1) contained many of the same tests utilized by White et al. (2001) and Proctor et al. (2006). We assessed attention with the Continuous Performance Test (Letz 1991), the Digit Span subtest of the Wechsler Adult Intelligence Scale-III (WAIS-III; Wechsler, 1997), and the Trail Making Test, Part A (Reitan and Wolfson, 1985). Executive function was assessed with the Trail Making Test, Part B, (Reitan and Wolfson, 1985), the Short Category Test (Wetzel and Boll, 1987), and the Controlled Oral Word Association Test (Benton et al. 1994;Loonstra et al., 2001). Memory was assessed with the California Verbal Learning Test-II, (CVLT-II, Delis et al., 2000), the Logical Memory subtest of the Wechsler Memory Scale-III (WMS-III, Wechsler, 1997), and the Brief Visual Memory Test-R (BVMT-R, Benedict, 1997). Manual dexterity was assessed with the Grooved Pegboard (Lafayette Instrument, Lafayette, IN) and the Digit Symbol subtest of the WAIS-III (Wechsler, 1997). Visuospatial abilities were assessed with the Block Design subtest of the WAIS-III (Wechsler, 1997). Crystallized verbal intelligence, an indicator of presumed baseline psychometric intelligence, was estimated with scores from the WAIS-III Verbal Comprehension Index (VCI) and the Wide Range Achievement Test-III (WRAT-III, Wilkinson, 1993) reading subtests. In addition, the Test of Memory Malingering (TOMM; Tombaugh, 1995) was administered to assess tendency to purposefully perform poorly. A TOMM score <90% correct (i.e., <45/50 correct) on trial 2 and/or the retention trial was coded as a TOMM failure. The clinical diagnoses of current major depression (MDD) and current post-traumatic stress disorder (PTSD) were made based on the Structured Clinician Interview for DSM-IV (Spitzer et al., 1992) and the Clinician Administered PTSD Scale (CAPS; Blake et al., 1990), respectively.

2.4. Magnetic resonance imaging

MRI data were acquired on a clinical 1.5-T MR scanner (Vision, Siemens Medical Systems, Iselin NJ). The MRI protocol consisted of a double spin-echo (DSE) sequence, yielding proton density and T2-weighted MR images and a Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence, yielding T1-weighted MR images. Axially angulated contiguous DSE images (TR/TE1/TE2=2500/20/80 ms, 1×1 mm^2 resolution, 3-mm slice thickness, no slice gap) were oriented along the orbital-meatal angle +5°. Coronal MPRAGE (TR/TI/TE=9/300/4 ms, 1×1 mm^2 in-plane resolution, 1.5-mm slabs) were acquired orthogonal to the long axis of the hippocampus. MRI data was unavailable for 3 GB/GF exposed subjects due to claustrophobia.

2.4.1. Tissue segmentation—Individual T1 images were segmented with the default unified segmentation algorithm available in SPM8 (Statistical Parametric Mapping; Institute

of Neurology, London, United Kingdom). This algorithm segments, bias corrects, and spatially normalizes the images all within the same model (Ashburner and Friston, 2005). Maps of gray matter (GM), white matter (WM), and cerebral spinal fluid (CSF) in native space were used to calculate total GM, WM, CSF, and intracranial volumes. Spatially normalized and modulated maps of GM and WM, smoothed with a 10 mm Gaussian kernel, were used in voxel-based morphometry (VBM) analyses. Segmentation data for 1 subject (a GB/GF exposed veteran) was not considered for analysis due to the poor quality of the segmentation.

2.4.3. Hippocampal Volumetry—Hippocampal boundaries were traced semi-automatically on MPRAGE images using a high dimensional brain-warping algorithm (Medtronic Surgical Navigation Technologies, Louisville, CO) using previously described methods (Hsu et al., 2002). The hippocampal boundaries were visually inspected and manual corrections were made in cases where misregistrations occurred. Because we had no a priori hypothesis about laterality, volumes of the right and left hippocampus were combined to reduce the number of measurements.

2.5. Statistical analyses

Statistical analyses of the demographic, clinical, neuropsychological, and volumetric measures were conducted using Statistical Package for the Social Sciences (SPSS), version 17 and R (http://www.r-project.org). Demographic and descriptive characteristics were compared across the two dichotomous exposure groups with Kruskal-Wallis tests for continuous variables and chi-square tests for categorical variables. Self-report questionnaires and principle component analysis software, generously provided by Dr. Robert Haley, were used to derived syndromes from factor analysis described in Haley et al. (1997). Kruskal-Wallis tests were used to compare factor analysis-derived syndromes across the two exposure groups. Three sets of analyses were carried out to examine the association between GB/GF exposure and neuropsychological performance. The first analysis involved binary comparisons between exposed and unexposed GW veterans with the Kruskal-Wallis tests. The second analysis used Spearman's correlation coefficients to assess the relationship between individual unit-level dose-estimates and neuropsychological test performance in GB/GF exposed veterans. The third set of analyses used linear trend models with the individual unit-level dose-estimates as the independent variable. Similarly, three sets of analyses were conducted to examine the association between GB/GF exposure and volumetric MRI measures. First, analyses of covariance (ANCOVA) were used to examine group differences in the volumetric measures with age, sex, and intracranial volume (ICV, defined as the sum of the GM, WM, and CSF) as confounding covariates. Second, partial correlations, controlling for age, sex, and ICV, were used to assess the relationship between unit-level dose-estimates and total GM, WM, CSF, and hippocampal volumes. Lastly, linear trend models with individual unit-level dose-estimates as the independent variable and adjustments for age, sex, and ICV. Significance level was set at $p < 0.05$ for all analyses, unless otherwise stated. We also used voxel-based morphometry (VBM) to examine the effects of GB/GF exposure on GM density regionally (i.e., voxel-by-voxel). Group differences were assessed with t-tests using the general linear model with age, sex, and ICV as confounding covariates. Because a voxel-wise statistical parametric map (SPM) comprises the result of many statistical tests, is necessary to correct for these multiple dependent comparisons. Therefore, the VBM results were corrected using the Family Wise Errors method ($p<0.05$ FWE corrected). This tests the hypothesis that the probability of obtaining at least one cluster with k voxels or more somewhere in the search volume is less than 0.05 (Ashburner and Friston 2000; Friston et al. 1993). Finally, Spearman's correlation coefficients were used to assess the relationship between neuropsychological test performance and total GM, WM, and hippocampal volumes

2.5.1. Haley Factor analyses—In a previous analysis of symptoms in Gulf War veterans, Haley et al., (1997) identified 6 syndromes suggesting dysfunction of the central, peripheral, and autonomic components of the nervous system. In order to identify Gulf War veterans who met the factor analytic criteria for the syndromes in the current study, we used the same standardized survey booklet as Haley et al. (1997) and scripts generously provided by Dr. Haley that call the FACTOR, LOGISTIC, and FREQ procedures in SAS (version 9.2, SAS Institute, Cary, NC) to perform a 2-stage factor analysis to disentangle ambiguous symptoms and to identify syndromes. We focused attention on Syndrome 2 because Haley and Kurt (1997) previously found that veterans with this syndrome exhibited significantly more evidence of central nervous system (CNS) dysfunction and theorized that this syndrome may have resulted from sublethal exposures to chemical nerve agents.

3. Results

As expected, there was no significant difference between the potentially exposed and unexposed GW veterans in gender, age, education, handedness, PTSD symptomatology as assessed by the CAPS, diagnoses of PTSD, MDD, or chronic multi-symptom illness (CMI) based on previously described criteria (Fukuda et al. 1998) (Table 2). There were also no significant group differences in factor analysis-derived syndromes (Table 3).

3.1. Neuropsychological testing results

The neuropsychological data are summarized in Table 4. There were no significant group differences on measures of general verbal intelligence, attention, executive function, or manual dexterity. There was a trend for GB/GF exposed veterans to perform more poorly than unexposed veterans on tests of visuospatial memory (BVMT total recall, p=0.07) and visuospatial abilities (WAIS-III block design, p=0.17). However, there was also a significant (p=0.01) group difference on Trial 2 of the Test of Memory Malingering (TOMM). Careful examination of the TOMM data revealed that four GB/GF exposed veterans failed (i.e., scored lower than 45 on trial 2 and the retention trial). Because this may be an indication that these individuals deliberately showed inadequate effort on the memory, and possibly other neuropsychological tests, we reanalyzed the neuropsychological data without these subjects. Removing these subjects from analysis also eliminated the slight group differences in visuospatial memory and visuospatial abilities.

Because different numbers of subjects had missing neuropsychological data, we reanalyzed the neuropsychological data in the subset of subjects (32 exposed and 26 nonexposed subjects) with complete data. This analysis, revealed a significant group difference in CVLT long delay free recall (p=0.03). However, the group difference was no longer significant after excluding the GB/GF exposed veterans who failed the TOMM from the analysis.

Spearman's correlations revealed no significant relationships between unit-level dose-estimates and neuropsychological data in the GB/GF exposed veterans. Linear trend analyses revealed no significant dose–response relationships between GB/GF exposure and neurobehavioral functioning

3.2. Structural imaging results

Figure 1 shows examples of the high-resolution anatomical images and the resulting GM, WM, and CSF maps derived from the automated segmentation procedure implemented in SPM8. Relative to unexposed veterans, those with suspected GB/GF exposure had less total GM ($F_{1,75}$=7.68, p=0.007) and hippocampal ($F_{1,74}$=6.09, p=0.016) volumes. In contrast, there were no significant group differences in total WM or ventricular CSF volume, as hypothesized (Table 5). Partial correlations, controlling for ICV, age, and sex, revealed no significant

relationships between unit-level dose-estimates and total GM, WM, CSF or hippocampal volume in the GB/GF exposed veterans. Linear trend analyses revealed no significant dose–response relationships between GB/GF exposure and total GM, WM, CSF or hippocampal volume.

The unexpected difference in total GM volume prompted us to use voxel-based morphometry (VBM) to further examine regionally specific group difference in GM density. There were no significant group differences at threshold of $p < 0.05$ after the FEW-correction for multiple comparisons (Ashburner and Friston 2000; Friston et al. 1993). However, an exploratory analysis of statistical trends (at uncorrected threshold of p=0.001) revealed regions of reduced GM density in the frontal, parietal, and occipital cortices in GB/GF exposed compared with unexposed veterans (see Fig 2).

3.3. Relationship between Neuropsychological and Structural imaging data

Table 6 lists the Spearman's correlation coefficients between hippocampal, total GM and WM volumes and neuropsychological test performance in GB/GF exposed and unexposed veterans. In GB/GF exposed veterans, hippocampal volume correlated positively with Verbal Comprehension Index (VCI) scores while total GM volume correlated positively with performance on the COWAT (r=0.61, p<0.0001) and Block Design test (r=0.42, p=0.01) and negatively with time to complete the Trail-making test A (r=−0.35, p=0.04) and time to place all pegs in the Grooved pegboard with the non-dominant hand (r=−0.36, p=0.03). In GB/GF exposed veterans, total WM volume also correlated positively with performance on the COWAT (r=0.43, p=0.01) and the Block Design test (r=0.34, p=0.04). In unexposed veterans, total GM volume correlated positively with performance on the Block Design test (r=0.36, p=0.02) and negatively with time to complete the Trail-making test A while total WM volume correlated positively with VCI score (r=0.38, p=0.01).

4. Discussion

The first major finding of this study is that GB/GF-exposed veterans had reduced total GM volume compared to their unexposed peers. This unexpected finding, which was contrary to our hypothesis, prompted us to use VBM to further explore regionally specific group difference in GM density. However, regional group difference survived correction for multiple comparisons, suggesting that the total GM reduction observed in GB/GF exposed veterans may be a global rather than a local phenomenon. Further studies with more subjects and larger statistical power might be required to detect subtle focal structural alterations.

Although we found group differences in total GM volume, there were no group differences in total WM or CFS volume. There was also no significant dose–response association between GB/GF exposure and total WM or CSF volumes as Heaton et al. (2008) had reported. While these results may initially appear to be at odds with each other, it could be argued that they are, in fact, complementary. Together these findings suggest that low-level GB/GF exposure has a deleterious effect on brain structure. This is in line with a recent report by Gullapalli et al., (2010) that a single exposure of 26.6 μg/kg, sc, 1.0×LD50 of the organophosphorous compound soman to guinea pigs causes significant and long-lasting damage to the structural integrity of the brain. Moreover, Yamasue et al. (2007) found regional reductions in both gray and white matter in victims of the 1995 Tokyo subway GB attack, providing critical evidence that exposure to GB affects both gray and white matter in humans. The fact that we found GM atrophy in GB/GF exposed veterans while Heaton et al. inferred a dose-dependent reduction in WM volume suggests that low-level exposure to GB/GF may have caused something pathological to take place at the boundary between gray and white matter. This, in turn, may have caused a shift in favor of one tissue class or another by the different segmentation algorithms used by our group and Heaton et al. Numerous factors may potentially cause tissue

misclassified along the transition from gray to white matter, including vascular changes, myelin changes, inflammation, and iron accumulation. Further studies with different types of imaging modality (e.g., diffusion tensor and susceptibility-weighted imaging) may reveal more about the potential source of pathology associated with low-level GB/GF exposure.

Despite the significant group difference in total GM volume, there were no significant correlations between individual unit-level dose-estimates and total GM volume or a significant dose-response association between GB/GF exposure level and total GM volume. A couple of different factors may have contributed to these negative findings. First, individual unit-level dose-estimates were not available for all of the GB/GF exposed veterans in our sample (we only had unit-level dose-estimates for 31 GB/GF exposed veterans). This is because the office of the Deputy Assistant Secretary of Defense for Force Health Protection and Readiness has gathered more information about individual soldiers who were in the hazard area, but for some reason were not identified in initial data collections for the modeling effort that determined the exposure estimates in 2002. Second, only 7 exposed veterans had unit-level dose estimates between 0.0129–0.072 mg min/m^3, which was classified as moderate exposure by Proctor et al. (2006). The remaining GB/GF exposed veterans had unit-level dose-estimates greater than 0.072 mg min/m^3, which was classified high exposure by Proctor et al. Thus, the unbalanced groups (7 with moderate and 24 with high exposure) may have affected the validity of the linear trend analysis. However, categorizing the exposed GB/GF veterans by tertiles of exposure estimates for the linear trend analysis also did not yield a significant dose–response association between GB/GF exposure and total GM volume.

The second major finding is that GB/GF exposed veterans had smaller hippocampal volumes than unexposed veterans. This finding is generally consistent with previous research that has shown that subchronic, low-level exposure to anticholinesterase compounds can result in serious neurotoxic consequences to the mammalian hippocampus (Veronesi et al. 1990) and the recent report that victims of the 1995 Tokyo subway GB attack have smaller than normal hippocampal volumes (Yamasue et al., 2007). However, it is noteworthy that hippocampal volume was positively correlated with the WAIS-III verbal comprehension index, a measure of crystallized verbal intelligence, in GB/GF exposed veterans. Other studies have linked hippocampal size to IQ (Gurvits et al., 1996) and genetic factors (Gilbertson et al., 2002). Therefore, it is possible that the smaller hippocampal volumes observed in these GB/GF exposed veterans may be a pre-existing trait rather than a consequence of low-level GB/GF exposure. The fact that we did not find any significant group differences in neuropsychological tests of memory or any significant correlations between hippocampal volume and memory in veterans with suspected GB/GF exposure further supports the conjecture that the smaller hippocampal volumes detected in the GB/GF exposed veterans may be a pre-existing trait.

The third major finding of this study is that GB/GF exposure was not associated with deficits on neurobehavioral measures of attention, memory, visuospatial abilities, or manual dexterity as reported by others (Miyaki et al., 2005; Nishiwaki et al., 2001; White et al., 2001; Yokoyama et al., 1998). We also failed to find a dose-response association between GB/GF exposure and neurobehavioral function, as previously reported by Proctor et al. (2006). The absence of a GB/GF effect on neurobehavioral function could be a consequence of the long interval between exposure and testing in the current sample of GW veterans. However, reports of chronic cognitive impairments many years after the 1995 Tokyo subway sarin attack have been reported among victims who did not necessarily present with signs of acute toxicity at the time of the incident (Miyaki et al., 2005; Yokoyama et al., 1998). Similar long-lasting behavioral deficits have also been found in guinea pigs (Mamczarz et al., 2010), which are considered the best non-primate model of organophosphorous intoxication (Inns and Leadbeater 1983). Alternatively, it could be that we lacked the power to detect group differences in a neuropsychological test battery of this size with relatively small sample size of 80. We further

reduced our statistical power in our reanalysis of the neuropsychological data when we excluded the subjects who failed the TOMM. Nevertheless, there was some evidence that the reduced GM volume observed in GB/GF exposed veterans had a deleterious effect on cognitive function: Performance on several some neuropsychological test (i.e., the COWAT, Block Design, Trail-Making test, A, and Grooved pegboard) were positively correlated with total GM volume in GB/GF exposed veterans. Moreover, the finding that performance on these neuropsychological tests, which assessed executive function, visuospatial abilities, attention, and manual dexterity, correlated with total GM volume in GB/GF exposed veterans is in line with the regional pattern of GM density reduction observed in the VBM contrast comparing exposed and unexposed veterans (i.e., bilateral frontal, parietal, and occipital lobes).

We did not detect a dose–response association between GB/GF exposure and visuospatial abilities or WM volume as Proctor et al. (2006) and Heaton et al. (2007) had previously reported in the Devens cohort. However, we did find a significant, positive correlation between performance on the Block Design test (the same test of visuospatial abilities utilized by Proctor and colleagues) and total WM volume in GB/GF exposed subjects. Because this relationship between total WM volume and visuospatial abilities did not exist in unexposed veterans, one could speculate that low-level GB/GF exposure also had a deleterious affect on WM, which in turn, compromised cognitive function in GW veterans.

In 1994 the Centers for Disease Control and Prevention used factor analysis to define a symptom complex termed “chronic multisymptom illness” (CMI). CMI was defined as the presence, for 6 months or longer, of one or more symptoms from at least two of the following clusters: general fatigue, mood and cognitive abnormalities, and musculoskeletal pain (Fukuda et al. 1998). Shortly after the 1991 Gulf War, CMI was noted to be quite common in all veterans but more prevalent in deployed veterans than in nondeployed veterans (Steele 2000; Unwin et al. 1999; Wolfe et al. 2002). In the present study, there were no significant group differences in the prevalence of cases with CMI. Although CMI can be subclassified as severe in cases where the defining symptom(s) is/are rated as severe, the self-report questionnaire that we employed only asked about the presence or absence of these symptoms and not about severity. Therefore we cannot comment on the severity of CMI in the exposed versus non-exposed groups.

Haley et al., (1997) previously described 6 syndromes in GW veterans. Syndrome 2 was called “confusion-ataxia” because it was characterized by problems with thinking, disorientation, balance disturbances, vertigo, and impotence. Haley and Kurt (1997) reported that veterans with Syndrome 2 had significantly more neuropsychological evidence of brain dysfunction on the Halstead Impairment Index, greater interside asymmetry of the wave I to wave III interpeak latency of brain stem auditory evoked potentials, greater interocular asymmetry of nystagmic velocity on rotational testing, increased asymmetry of saccadic velocity, more prolonged interpeak latency of the lumbar-to-cerebral peaks on posterior tibial somatosensory evoked potentials, and diminished nystagmic velocity after caloric stimulation bilaterally. Moreover, Haley and Kurt (1997) theorized that this syndrome may have resulted from sublethal exposures to chemical nerve agents. However, in the current study, we found no significant group difference in the factor analysis-derived syndromes.

Together these findings suggest that low-level exposure to GB/GF, independent of the symptoms associated with Gulf War Veteran's Illness, can have deleterious effects on brain structure and brain function more than decade later.

This study has some limitations that should be considered in its interpretation. First, we did not have information about the veteran's unit or whether the GW veterans were officers or enlisted personnel during the first Gulf War. We also did not obtain information about 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. It is possible that military personnel more likely to be in the higher GB/GF exposure areas are different from those in low exposure areas. However, we are not able to address this question in the current study. Second, we did not have cumulative estimated GB/GF exposure levels for all GW veterans whose units were deemed to be located within the modeled plume areas. Furthermore, only a minority of the exposed GW veterans had moderate (i.e., 0.013–0.072 mg min/m^3) cumulative estimated GB/GF exposure levels. Thus, our ability to examine dose–effect relationships between estimated GB/GF exposure levels and brain function and volumes was limited. Third, cumulative GB/GF exposure level estimates were calculated at the unit rather than individual level. Therefore, there is no definitive way of checking 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, thus would have limited influence on the present results. These limitations notwithstanding, the current findings indicate that this cohort of GW veterans with possible low-level exposure to GB/GF have reduced GM and hippocampal volumes compared to their unexposed peers. Furthermore, there was indirect evidence that low-level GB/GF exposure may have also had a detrimental effect on brain WM, which, together with GM atrophy, compromised cognitive function. Finally, there was no significant group difference in the factor analysis-derived syndromes described by Haley et al., (1997) or in the prevalence of cases with CMI. This suggests that the central nervous system (CNS) effects that we observed in GB/GF exposed veterans are not confounded by CMI or other factors associated with Gulf War Veterans Illness. Together with the findings of Heaton, Proctor and colleagues, these results provide a compelling argument to conduct a follow-up study with more subjects and more sophisticated imaging technology.

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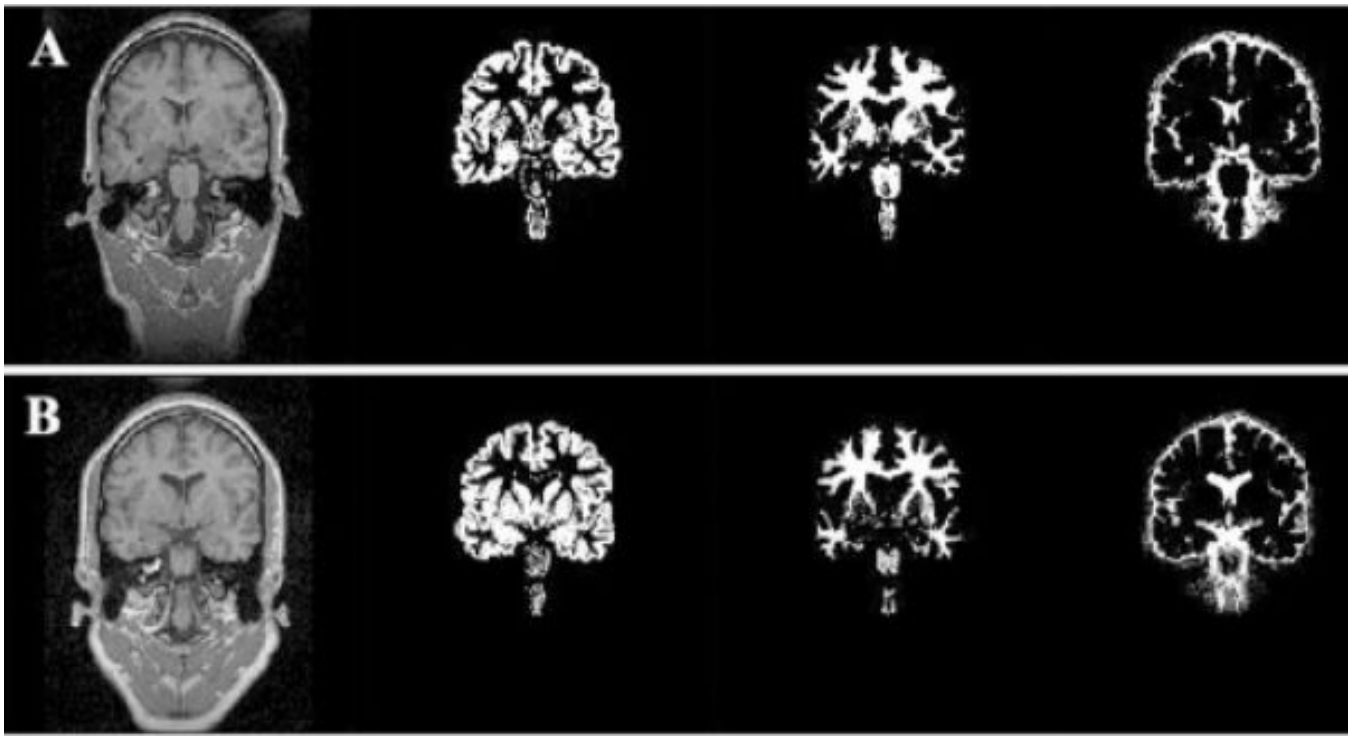

Figure. 1.
Examples of SPMP8 segmentation of high resolution T1-weighted MRI in a 40 year-old unexposed male GW veteran (A) and a 45 year-old GW veteran with suspected GB/GF exposure. The images at the far left show the coronal T1-weighted images. The images to the right show the SPM8 classification of GM, WM, and CSF.

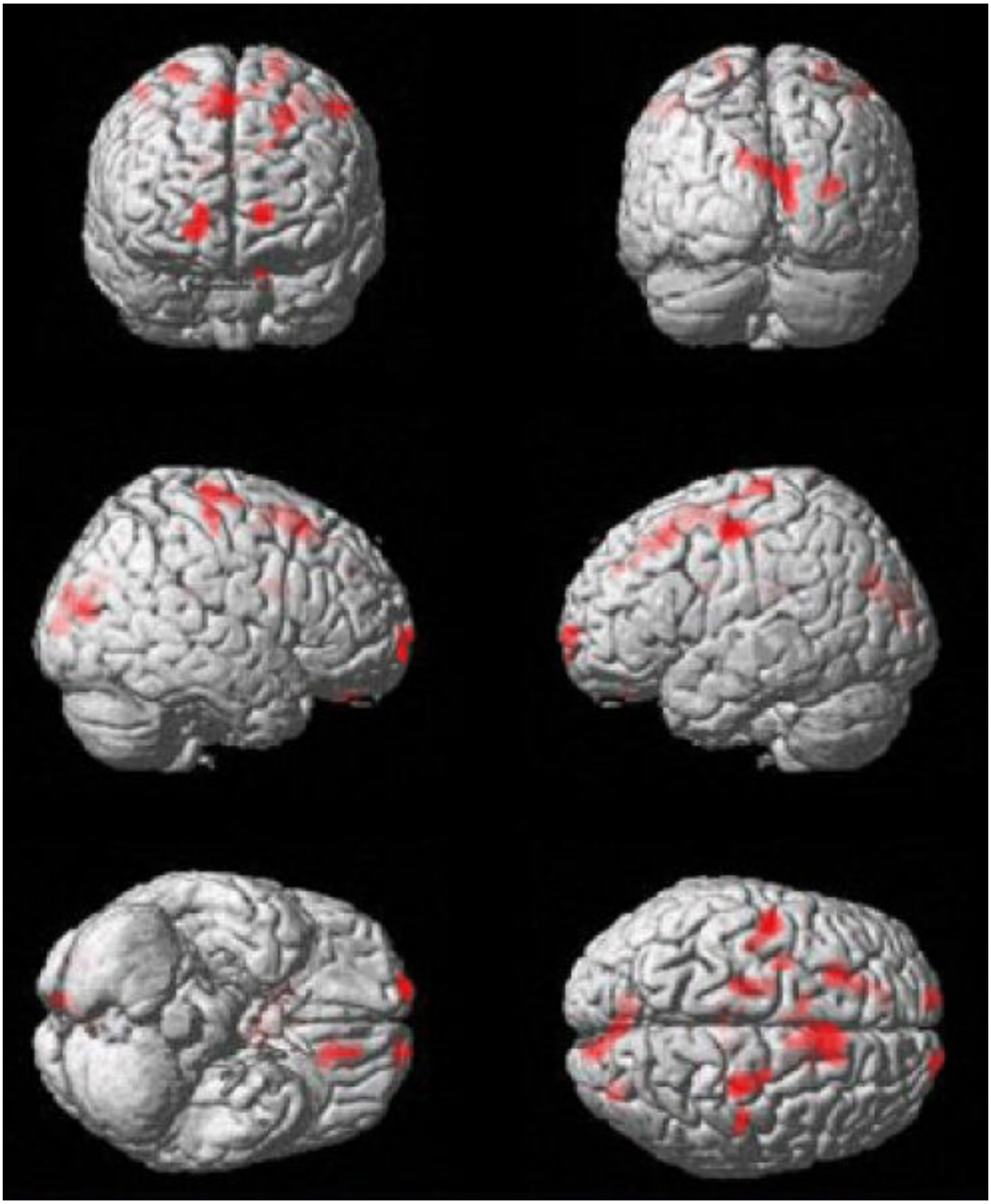

Figure 2.
Regional GM density reduction in GB/GF exposed GW veterans. Regions showing reduced gray matter (GM) density in veterans with suspected GB/GF exposure compared with unexposed veterans are rendered onto the image of a single unexposed GW veteran (voxel threshold: p= 0.001, uncorrected).

Table 1

Neuropsychological test battery

Functional domain	Neuropsychological test	Function assessed	Outcome measure(s) analyzed	Task included in prior study battery (Proctor et al. 2006)
Attention	Continuous Performance Test	Sustained attention	Mean response time of hits	Yes
	Trail Making Test, A	Spatial attention, simple visuospatial tracking	Time to completion	Yes
	WAIS-III Digit Span	Simple attention, concentration, tracking	Span score	Yes, WAIS-R version
Executive Function	Trail Making Test, B	Spatial attention, set-shifting	Time to complete	Yes
	Short Category Test	Cognitive flexibility, abstract reasoning	Number of correct test items	No
	Controlled Oral Word Association Test	Verbal fluency	Number of words generated	No
Psychomotor function	Grooved Pegboard	Performance speed in a fine motor task	Time to put in all pegs, dominant and non-dominant hands	Yes, Purdue pegboard version
	WAIS-III Digit Symbol, matching	Visual perception, psychomotor speed	Raw score	No
Visuospatial abilities	WAIS-III Block Design	Spatial perception, visual abstract processing, problem solving	Raw Score	Yes, WAIS-R version
Short-term memory	California Verbal Learning Test-II	Verbal episodic memory	Short and long delay free recall, raw scores	Yes
	WMS-III Logical Memory	Ability to recall orally presented information	Delayed recall, raw score	No
	Brief Visual Memory Test-R	Visuospatial memory	Total and delayed recall, raw score	No
Genuine Effort	Test of Memory Malingering	Tendency to purposefully perform poorly	Trails 1, 2, retention, raw score	Yes, but only Trial 1

WAIS-III, Wechsler Adult Intelligence Scale-III; WAIS-R, Revised Wechsler Adult Intelligence Scale; WMS III, Wechsler Memory Scale-III

Table 2

Demographic, military, and clinical information for study population

	Exposed (N=40)	Unexposed (N=40)
Age, mean (S.D.)	44.0 (10.2)	42.7 (9.3)
No. left-handed or ambidextrous (%)	7 (18%)	5 (13%)
No. female (%)	7 (18%)	7 (18%)
No. White (%)	26 (65%)	19 (48%)
No. Married (%)	19 (48%)	25 (63%)
Years of education	14.9 (3.7)	14.5 (2.0)
No. with less than college education	5 (13%)	6 (15%)
Military status during Gulf War		
No. activity duty (%)	30 (75%)	31 (78%)
No. National Guard (%)	5 (13%)	2 (5%)
No. Reserves (%)	5 (13%)	7 (18%)
Years in the military	14.6 (9.2)	14.1 (7.8)
No. with service-connected disability (%)	18 (45%)	22 (55%)
Avg. percent VA disability (range)	38.6% (10–100%)	40.3% (5–100%)
No. currently employed (%)	26 (65%)	28 (70%)
No. with history of alcohol problem (%)	19 (48%)	22 (55%)
Avg. no. of alcoholic drinks/week during past yr.	3.7 (5.6)	2.3 (3.1)
None	21	18
1–3	8	13
≥ 4	11	9
No. CMI cases (%)	21 (54%)	23 (59%)
No. current PTSD diagnosis (%)	5 (13%)	5 (13%)
Current CAPS	14.8 (25.1)	15.5 (21.8)
No. current MDD diagnosis (%)	2 (5%)	3 (7%)
Estimated exposure range (mg min/m^3)	0.047–0.889	--
No. with no exposure estimate	9 (23%)	--
No. with moderate exposure (0.0469-0.072 mg min/m^3)	7 (18%)	--
No. with high exposure (>0.072–0.999 mg min/m^3)	24 (60%)	--

CMI, chronic multisymptom illness (as defined by Fukuda et al., 1998); PTSD, post-traumatic stress disorder; CAPS, Clinician Administered PTSD scale; MDD, major depression

Table 3

Relationship between GB/GF exposure and factor analysis-derived syndromes

Syndrome	Exposed	Unexposed	χ^2	*p*-value
	No affected (%)	No affected (%)		
1 “impaired cognition”	3/40 (8)	3/40 (8)	0.00	1.00
2 “confusion-ataxia”	5/40 (13)	4/40 (10)	0.12	0.73
3 “arthro-myo-neuropathy”	4/40 (10)	5/40 (13)	0.12	0.73
4 “phobia-apraxia”	5/40 (13)	4/40 (10)	0.12	0.73
5 “fever-adenopathy”	2/40 (5)	2/40 (5)	0.00	1.00
6 “weakness-incontinence”	1/40 (3)	2/40 (5)	0.34	0.56

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Table 4

Relationship between GB/GF exposure and neuropsychological performance in all subjects and in a subset of subjects with complete neuropsychological data

	All Exposed		All Unexposed		All Exposed vs. All Unexposed		32 Exposed vs. 26 Unexposed	
	N	Mean (SD)	N	Mean (SD)	χ^2	*p*-value	χ^2	*p*-value
General Verbal Intelligence								
WAIS-III VCI	39	102.3 (12.1)	40	107.1 (13.7)	1.90	0.17	1.40	0.24
WRAT-III reading	40	47.8 (4.9)	40	48.4 (4.5)	0.17	0.68	0.21	0.65
Attention								
Continuous Performance Test[a]	28	408.5 (77.2)	32	382.0 (48.2)	1.44	0.23	1.64	0.20
TMT A, time to complete[a]	40	30.1 (10.1)	40	30.0 (11.3)	0.12	0.73	0.21	0.65
WAIS-III Digit Spans	39	16.5 (4.4)	40	16.5 (4.6)	0.08	0.78	0.02	0.88
Executive Function								
TMT B, time to complete[a]	40	66.1 (29.7)	40	67.3 (31.2)	0.00	0.99	0.01	0.91
Short Categoy Test[a]	38	28.0 (17.1)	40	25.2 (12.9)	0.33	0.57	0.40	0.53
COWAT, FAS total correct	40	40.7 (10.8)	40	38.2 (9.1)	0.96	0.33	1.53	0.22
Psychomotor Function								
WAIS-III Digit Symbol, coding	40	66.7 (18.9)	40	71.4 (14.2)	1.43	0.23	0.24	0.63
Grooved Pegboard, dominant[a]	40	70.6 (15.6)	40	70.6 (12.0)	0.00	0.99	0.49	0.49
Grooved Pegboard, non-dominant[a]	40	78.9 (24.5)	40	73.6 (12.1)	0.95	0.33	0.24	0.62
Visuospatial abilities								
WAIS-III Block Design	40	41.3 (12.9)	40	45.2 (11.4)	1.87	0.17	0.43	0.51
Memory								
CVLT-II, short delay free recall	38	10.8 (3.7)	38	11.7 (3.2)	1.19	0.27	2.03	0.15
CVLT-II, long delay free recall	38	10.9 (3.6)	38	12.0 (2.8)	1.31	0.25	4.33[b]	0.04[b]
WMS-III Logical Memory, delayed Recall	40	24.4 (9.3)	40	26.0 (8.3)	0.48	0.49	0.93	0.34
BVMT-R, total recall	40	22.8 (6.1)	40	25.4 (4.5)	3.30	0.07	2.10	0.15
BVMT-R, delayed recall	40	9.2 (1.8)	40	9.4 (1.9)	0.44	0.51	0.02	0.89
Test of Memory Malingering (TOMM)								
Trial 1	38	47.0 (5.0)	40	48.4 (2.4)	0.15	0.69	0.58	0.45
Trial 2	38	48.8 (3.2)	40	50.0 (0.2)	6.69	0.01	5.46	0.02

	All Exposed		All Unexposed		All Exposed vs. All Unexposed		32 Exposed vs. 26 Unexposed	
	N	Mean (SD)	N	Mean (SD)	χ^2	*p*-value	χ^2	*p*-value
Retention	38	48.8 (3.6)	40	49.9 (0.3)	2.67	0.10	1.44	0.23

VCI: Verbal Comprehension Index; TMT: Trail Making Test; COWAT: Controlled Oral Word Association Test; CVLT-II: California Verbal Learning Test-II, BVMT-R: Brief Visual Memory Test-R

[a] lower scores indicate better performance

[b] group difference no longer significant after the TOMM failures are excluded from analysis (χ^2=2.48, p=0.12)

Table 5

Volumetric measurements (in cc) of brain tissue classified by SPM8 in GB/GF exposed and unexposed veterans

	Exposed	Unexposed	F-value	p-value
Total GM[a]	654.14 (72.92)	675.89 (67.43)	7.68	0.007
Total WM[a]	539.28 (62.59)	521.53 (71.89)	0.70	0.95
Total CSF[a]	386.72 (131.31)	332.58 (100.48)	2.85	0.10
Hippocampus[b]	5.09 (0.67)	5.42 (0.69)	6.41	0.01
ICV[a]	1571.85 (178.37)	1530.01 (175.31)	1.39	0.24

[a] data available for 36 exposed and 40 unexposed subjects

[b] data available for 35 exposed and 40 unexposed subjects

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Table 6

Spearman's correlation coefficient between measures of brain volume and neuropsychological test performance

	Hippocampal volume		Total GM volume		Total WM volume	
	Unexposed (N=40)	**Exposed (N=32)**	**Unexposed (N=40)**	**Exposed (N=32)**	**Unexposed (N=40)**	**Exposed (N=32)**
WAIS-III VCI	0.28	0.48**	0.13	0.27	0.38*	0.22
TMT A[a]	−0.18	0.12	−0.38*	−0.35*	−0.10	−0.21
COWAT, FAS	−0.17	0.18	0.02	0.61**	0.20	0.43**
WAIS-III Block Design	0.14	0.29	0.36*	0.42**	−0.08	0.34*
Grooved Pegboard, non-dominant hand[a]	−0.02	−0.25	−0.14	−0.36*	0.21	−0.12

** $p<0.01$

* $p<0.05$

[a] lower scores indicate better performance