Exp Brain Res (2017) 235:2777–2786
DOI 10.1007/s00221-017-5010-8

RESEARCH ARTICLE

Subcortical brain atrophy in Gulf War Illness

Peka Christova[1,2] · Lisa M. James[1,2,3] · Brian E. Engdahl[1,2,4] · Scott M. Lewis[1,5] · Adam F. Carpenter[1,5] · Apostolos P. Georgopoulos[1,2,3,5]

Received: 18 March 2017 / Accepted: 12 June 2017 / Published online: 20 June 2017

Abstract Gulf War Illness (GWI) is a multisystem disorder that has affected a substantial number of veterans who served in the 1990–1991 Gulf War. The brain is prominently affected, as manifested by the presence of neurological, cognitive and mood symptoms. Although brain dysfunction in GWI has been well documented (EBioMedicine 12:127–32, 2016), abnormalities in brain structure have been debated. Here we report a substantial (~10%) subcortical brain atrophy in GWI comprising mainly the brainstem, cerebellum and thalamus, and, to a lesser extent, basal ganglia, amygdala and diencephalon. The highest atrophy was observed in the brainstem, followed by left cerebellum and right thalamus, then by right cerebellum and left thalamus. These findings indicate graded atrophy of regions anatomically connected through the brainstem via the crossed superior cerebellar peduncle (left cerebellum → right thalamus, right cerebellum → left thalamus). This distribution of atrophy, together with the observed systematic reduction in volume of other subcortical areas (basal ganglia, amygdala and diencephalon), resemble the distribution of atrophy seen in toxic encephalopathy (Am J Neuroradiol 13:747–760, 1992) caused by a variety of substances, including organic solvents. Given the potential exposure of Gulf War veterans to "a wide range of biological and chemical agents including sand, smoke from oil-well fires, paints, solvents, insecticides, petroleum fuels and their combustion products, organophosphate nerve agents, pyridostigmine bromide, …" (Institute of Medicine National Research Council. Gulf War and Health: Volume 1. Depleted uranium, pyridostigmine bromide, sarin, and vaccines. National Academies Press, Washington DC, 2000), it is reasonable to suppose that such exposures, alone or in combination, could underlie the subcortical atrophy observed.

Keywords Gulf War Illness · Brain atrophy · Brainstem · Cerebellum · Thalamus · Toxic encephalopathy

Introduction

More than a quarter of the veterans who served in the 1990–1991 Persian Gulf War experience chronic unexplained health problems collectively referred to as Gulf War Illness (GWI; Research Advisory Committee on Gulf War Veterans' Illnesses 2014). GWI is characterized by multiple symptoms including fatigue, musculoskeletal pain, cognitive and neurological deficits, and mood disturbance, as well as gastrointestinal, respiratory, and skin problems (Fukuda et al. 1998; Steele 2000). Although the characteristic symptoms span multiple systems, several involve the brain including memory loss, word finding difficulty, concentration problems, headaches, dizziness, tremor, and mood changes. These symptoms and others, collectively referred to as the neurological/cognitive/mood

✉ Apostolos P. Georgopoulos
omega@umn.edu

1 Brain Sciences Center, Department of Veterans Affairs Health Care System, Minneapolis VAHCS, One Veterans Drive, Minneapolis, MN 55417, USA

2 Department of Neuroscience, University of Minnesota Medical School, Minneapolis, MN 55455, USA

3 Department of Psychiatry, University of Minnesota Medical School, Minneapolis, MN 55455, USA

4 Department of Psychology, University of Minnesota, Minneapolis, MN 55455, USA

5 Department of Neurology, University of Minnesota Medical School, Minneapolis, MN 55455, USA

symptoms, are among the most commonly reported symptoms in veterans who deployed during the Gulf War (Steele 2000), strongly suggesting brain involvement. Nonetheless, there are relatively few neuroimaging studies of GWI to date and findings of brain effects have been inconsistent (see Research Advisory Committee on Gulf War Veterans' Illnesses 2014; White et al. 2016, for review).

Although several early studies failed to find brain differences in GWI veterans compared to controls, more recent studies utilizing more sophisticated neuroimaging have identified a number of abnormalities. Using magnetoencephalography (MEG), we recently demonstrated GWI-related functional neural anomalies involving primarily the cerebellum and frontal cortical areas (Engdahl et al. 2016). A number of studies have demonstrated cortical abnormalities associated with GWI. For instance, reduced cortical volume (frontal, parietal, and occipital) (Chao et al. 2016) and total gray and/or white matter volume (Chao et al. 2011; Heaton et al. 2007) have been reported in Gulf War veterans likely exposed to sarin or cyclosarin. In addition, reduced brain activity in veterans with GWI has been demonstrated in several cortical areas using functional magnetic resonance imaging (fMRI) (Gopinath et al. 2012) and magnetic resonance spectroscopy (MRS) (Rayhan et al. 2013a). Far less research, however, has focused on cerebellar alterations despite evidence that cerebellar dysfunction results in broad-ranging neurobehavioral impairment akin to characteristic GWI symptoms; typical neurobehavioral changes associated with cerebellar dysfunction include impairments in executive functioning, spatial cognition, language, and emotion regulation (Schmahmann and Sherman 1998). To our knowledge, only one other study has reported cerebellar alterations associated with GWI. Specifically, Rayhan et al. (2013b) found evidence of volumetric reductions in the brainstem, the latter of which parallels previous research (Haley et al. 2000) demonstrating neural loss or dysfunction in the basal ganglia and brainstem using MRS (c.f. Weiner et al. 2011). Although several studies using functional neuroimaging have identified various subcortical impairments linked to GWI (see White et al. 2016 for review), there is limited evidence of additional structural anomalies.

We suspect that the limited evidence of cerebellar and other subcortical anomalies in GWI is not a reflection of "normality" but is instead attributable to a tendency in neuroimaging research to focus on particular regions of interest at the exclusion of other brain differences. That, along with other methodological issues including small samples, inconsistent GWI case definitions, and varied neuroimaging techniques and parameters has resulted in what the Research Advisory Committee on Gulf War Veterans' Illnesses (2008) refers to as "one-of-a-kind" findings. In the present study, we aim to further explore cerebellar and other subcortical abnormalities associated with GWI by comparing subcortical volumes in veterans with GWI to healthy veterans.

Materials and methods

Participants

Nineteen veterans with GWI (17 men and 2 women; age 48.1 ± 4.9 years, mean ± SD) and 24 veterans as controls (23 men and 1 woman; age 62.4 ± 7.1 years) participated in the current study. All subjects participated in the study after providing informed consent, in adherence to the Declaration of Helsinki, and were financially compensated for their time. All study protocols were approved by the respective Institutional Review Boards. GWI status was determined using a self-report symptom checklist that permits classification as GWI case or control according to the Center for Disease Control (Fukuda et al. 1998) and the Kansas criteria (Steele 2000). All GWI veterans in the present study met both case definitions. Study participants completed diagnostic interviews including the Clinician-Administered PTSD Scale for *DSM-IV* (Blake et al. 1995) and the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (First et al. 2002) to evaluate mental health status. None of the participants in the present study met diagnostic criteria for any mental health condition. A neurological examination was conducted on GWI subjects (but not on control subjects) by a board-certified neurologist.

MRI acquisition

All data were acquired using a 3T MR scanner (Achieva, Philips Healthcare, Best, The Netherlands) with a phased array SENSitivity Encoding (SENSE) 8-channel head coil for reception. For each participant a high resolution T1-weighted image Turbo Field Echo (T1w TFE SENSE) was obtained (168 sagittal slices, TR = 8.1932 ms, TE = 3.752 ms, Acquisition matrix 256 × 256, Flip angle 8°, voxel size 0.9375 × 0.9375 × 1 mm). A T2-weighted image (T2w VISTA HR SENSE) was also obtained (180 slices, TR = 2500 ms, TE = 363.072 ms, acquisition matrix 256 × 256, voxel size = 0.7813 × 0.7813 × 1 mm). In advance of each acquisition a capsule of Vitamin E was taped to the participant's right temple to determine orientation in the imaged data.

MRI analysis

A 704-core High Performance Computing system (CentOS 6.5 Linux, Rocks 6.1.1) with Matlab R2012 (64 bit), Human Connectome Project (HCP humanconnectome.org)

pipeline with FreeSurfer (FS; http://surfer.nmr.mgh.harvard.edu) HCP version (freesurfer-hpc) was used for data processing. MRI data with high contrast between gray matter, white matter, and cerebrospinal fluid, as well as high spatial resolution are necessary for accurate results. We acquired T1w and T2w images with high spatial resolution ($\leq$1 mm^2) to achieve precise surface reconstruction. This is critical in regions with thin gyral blades of white matter and for thin cortex regions (Glasser et al. 2013). Standard FS software requires only T1w images as input. However, we used a modified version of FS, implemented in the structural HCP pipeline, which utilizes both T1w and T2w images to eliminate uncertainty due to the fact that dura and blood vessels are isointense to gray matter in the T1w image alone. In addition, T2w allows improved pial surface reconstruction (Glasser et al. 2013). Specifically, we used the first two structural HCP pipelines, namely *PreFreeSurfer* and *FreeSurfer*. One goal of the *PreFreesurfer* pipeline is to align the T1w and T2w images. *PreFreeSurfer* pipeline processing was followed by *FreeSurfer* pipeline processing which is based on FS version 5.2 with improvements. FS reconstructs the cortical surface (surface-based analysis) and subcortical segmentation (volume-based analysis). The surface-based analysis involves constructing models of the boundary between white matter and gray matter, as well as the pial surface and computing registration based on aligning the cortical folding patterns (Dale et al. 1999; Fischl et al. 1999a, b). In the volume-based stream, subcortical regions are automatically labeled (Fischl et al. 2002, 2004).

From the parcellation statistics output (Desikan-Killiany atlas, Desikan et al. 2006) we obtained volumes of the following bilateral cortical regions: banks of the superior temporal sulcus, caudal anterior-cingulate cortex, caudal middle frontal gyrus, cuneus, entorhinal cortex, fusiform gyrus, inferior parietal cortex, inferior temporal gyrus, isthmus–cingulate cortex, lateral occipital cortex, lateral orbitofrontal cortex, lingual gyrus, medial orbitofrontal cortex, middle temporal gyrus, parahippocampal gyrus, paracentral lobule, inferior frontal gyrus pars opercularis, inferior frontal gyrus pars orbitalis, inferior frontal gyrus pars triangularis, pericalcarine cortex, postcentral gyrus, posterior-cingulate cortex, precentral gyrus, precuneus, rostral anterior cingulate cortex, rostral middle frontal gyrus, superior frontal gyrus, superior parietal cortex, superior temporal gyrus, supramarginal gyrus, frontal pole, temporal pole, transverse temporal cortex, insula, and hippocampus. Total, left and right cortical volumes were estimated from the corresponding sums to volumes of the individual areas above. Finally, from the segmentation statistics output we obtained estimated total intracranial volume (eTIV), brainstem volume, and volumes of the following bilateral regions: cerebellar cortex (including, separately, gray matter and white matter volumes), thalamus, caudate, putamen, pallidum, amygdala, accumbens area, and ventral diencephalon. From these, total, left and right subcortical volumes were estimated from the corresponding sums of the lateralized (e.g., excluding brainstem) subcortical areas above.

Data analysis

Statistical analyses were carried out for total, left and right cortical and subcortical volumes, and for individual cortical and subcortical areas. Univariate analyses of covariance (ANCOVA) and multivariate analyses of covariance (MANCOVA) were performed with specific brain volumes as dependent variables (as detailed below), Group (GWI vs. control) as the fixed factor and eTIV, age and sex as covariates. The possible dependence of brain volumes on age was assessed by performing a multiple linear regression where the volume of an area was the dependent variable, age was the independent variable, and sex and eTIV were covariates. This analysis was performed separately for the control and GWI groups, and the partial regression coefficients for age were compared between groups using the bootstrap (Efron and Tibshirani 1993) by random resampling with replacement ($N = 1000$ bootstrap samples). An estimate of the average percent change in brain volume with age was obtained as the percentage of the partial coefficient for age with respect to the mean volume. Statistical analyses were performed using the IBM-SPSS statistical package (version 24) and ad hoc computer programs written in FORTRAN.

Results

General

The neurological examinations of GWI participants were generally unremarkable, other than the presence of tremor in 5/19 (26.3%) participants.

Cortical volumes

We adopted the following steps for comparing cortical volumes between GWI and control participants. First, we performed a MANCOVA where the volumes of the 70 left and right cortical areas listed above were the dependent variables, the Group was the fixed factor, and sex, age and eTIV were covariates. This analysis showed that the multivariate effect of the Cortical Areas was not statistically significant ($P = 0.741$ for all 4 multivariate tests provided by SPSS, namely Pilai's Trace, Wilk's Lambda, Hotelling's Trace and Roy's Largest Root). To account for a possible global effect, separate ANCOVAs were performed for Total, Left, and Right Cortical volumes; no statistically significant Group effect was found ($P = 0.677$,

0.529, and 0.908, respectively; *F* test; Fig. 1). Therefore, no further analyses were carried out on cortical volumes.

Subcortical volumes

Group differences

We adopted the same procedure as above for comparing subcortical volumes between GWI and control participants. Specifically, we first performed a MANCOVA where the volumes of the 17 subcortical areas (8 lateralized plus the brainstem) above were the dependent variables, Group was the fixed factor, and sex, age and eTIV were covariates. This analysis showed a statistically significant multivariate effect of Group ($P = 0.019$ for all 4 multivariate tests above). This result was complemented by a significant effect of Group on Total, Left, and Right subcortical volumes (Fig. 1; $P = 0.001$, 0.002, and 0.004, respectively; *F* test in ANCOVAs). This result dictated further testing of the Group on individual subcortical areas. Indeed, we found a significant Group effect on the volumes of brainstem, cerebellum and thalamus (Fig. 2; $P = 0.001$, 0.004, and 0.014, respectively; *F* test in ANCOVAs). A case of cerebellar atrophy in GWI, as compared to an age- and sex-matched control participant, is shown in Fig. 3. Finally, we further tested for a lateralized effect in the cerebellum and thalamus. We found a significant effect of Group on all (left and right cerebellum and thalamus) (Fig. 4). Interestingly, the effect size (and significance level) were higher for left cerebellum and right thalamus, as opposed to right cerebellum and left thalamus (Fig. 4). This grouping together of contralateral cerebellum and thalamus reflects the known dense anatomical projections from the cerebellum to the contralateral thalamus via the crossed superior cerebellar peduncle.

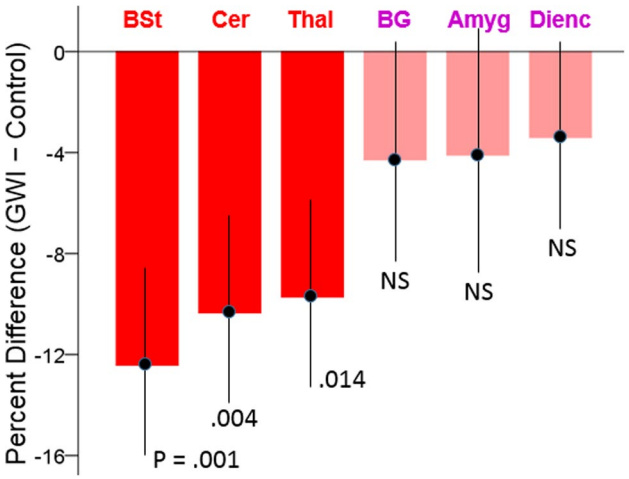

Fig. 2 Mean (±SEM, adjusted for age, sex and eTIV) percent differences in GWI vs. controls in the volumes indicated. Probability (*P*) values are from the *F* test in ANCOVA (see text for details). *NS* not statistically significant, *Bst* brainstem, *Cer* cerebellum, *Thal* thalamus, *BG* basal ganglia, *Amyg* amygdala, *Dienc* diencephalon

In terms of effect size, all subcortical areas showed a reduction in volume in GWI participants compared to controls (Fig. 2) but brainstem, cerebellum and thalamus showed highly significant reductions of −12.3, −10.4 and −9.7%, respectively (Fig. 2). Within that group, the highest reduction was found in (a) the brainstem (−12.3%), followed by (b) the left cerebellum (−12.1%) and right thalamus (−10.6%), and then (c) the right cerebellum (−8.6%) and left thalamus (−9%) (Fig. 4). Finally, we took advantage of the fact that FreeSurfer provides estimates of the volumes of both white and gray matter of the cerebellum and carried out the same analysis for these two cerebellar

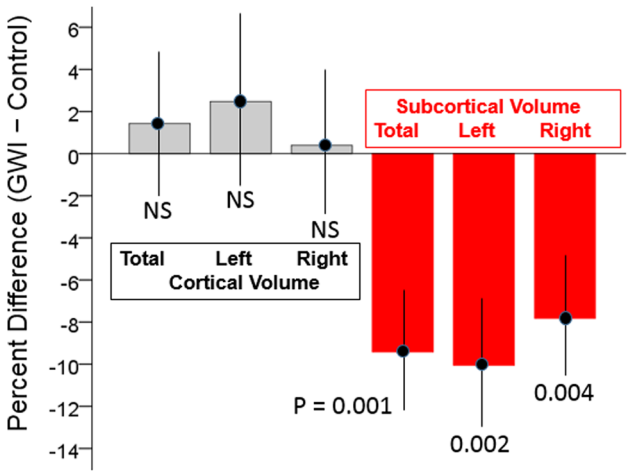

Fig. 1 Mean (±SEM, adjusted for age, sex and eTIV) percent difference in GWI vs. controls in the volumes indicated. Probability (*P*) values are from the *F* test in ANCOVA (see text for details). *NS* not statistically significant

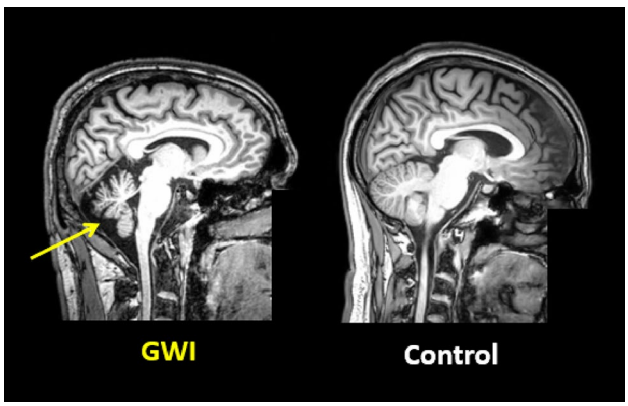

Fig. 3 Sagittal T1-weighted MRI images from a level just *left of midline*, from a participant with GWI and an age- and a sex-matched healthy control participant. Note the markedly decreased cerebellar volume (*arrow*) in the GWI brain

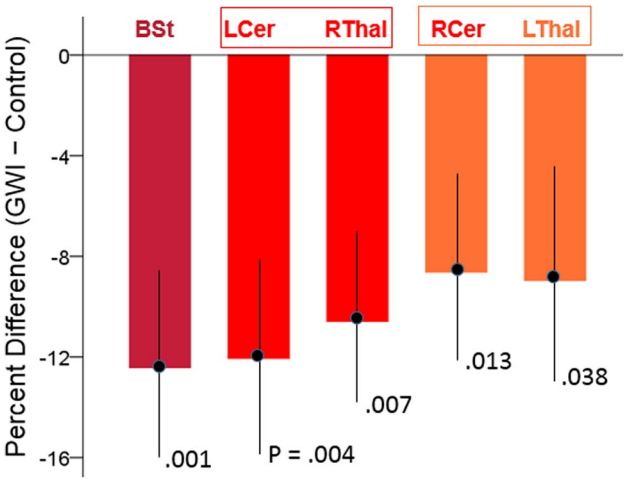

Fig. 4 Mean (±SEM, adjusted for age, sex and eTIV) percent differences in GWI vs. controls in the volumes indicated. Probability (*P*) values are from the *F* test in ANCOVA (see text for details). Abbreviations as in Fig. 2. L and R prefixes denote *left* and *right* hemisphere (for illustration purposes, the brainstem bar of Fig. 2 is shown again here)

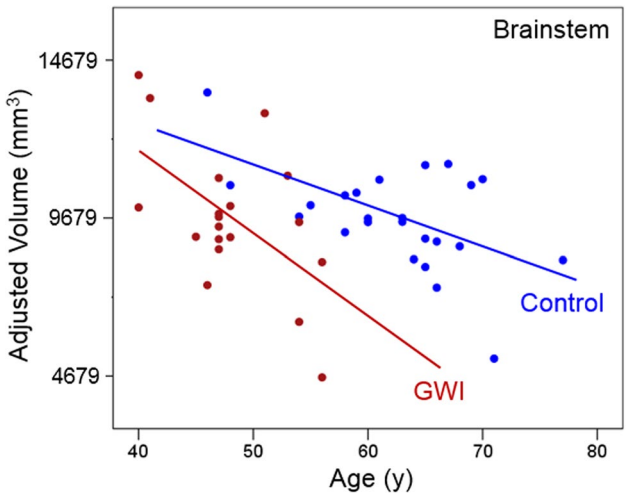

Fig. 5 Scatter plots of control and GWI brainstem volumes (adjusted for eTIV and sex) against age at MRI acquisition. Statistics for *fitted lines*: control, $P = 0.007$, $R^2 = 0.286$, $\hat{\beta} = -0.535$, $N = 24$; GWI, $P = 0.011$, $R^2 = 0.321$, $\hat{\beta} = -0.567$, $N = 19$

components. We found that both volumes were smaller in GWI than in control brains by −10.7 and 10.2% for the cerebellar white and gray matter, respectively. However, this volume reduction was statistically significant only for the gray matter ($P = 0.02$) but did not reach significance for the white matter ($P = 0.08$).

Effect of age

Given the significant decrease of brainstem, cerebellar and thalamic volumes in GWI participants (Figs. 2, 3), we tested the hypothesis that such a reduction may be associated with an accelerated atrophy in GWI, beyond that expected in controls. For that purpose, we carried out a multiple linear regression for each participant group, where the specific brain volume was the dependent variable and age, sex and eTIV were independent variables (covariates). Given the observed statistically significant Group effect in cerebellum, brainstem and thalamus, we tested the age effect on the volumes (summed over left and right) of those 3 areas. We found the following. First, the partial regression coefficient for age was negative for both groups and all areas, indicating a reduction of volume over time. Second, the age coefficients for thalamus did not differ significantly between the two groups. Third, the age coefficients for cerebellum and brainstem were appreciably higher in absolute value in the GWI group than the control group, as follows.

(a) Brainstem (Fig. 5): control = −127.4 mm^3/years, GWI = −276.7 mm^3/years. These rates of volume reduction with age, when expressed as percentages of the average volumes (adjusted for eTIV and sex), corresponded to −5.6%/decade for control and −12.4%/decade for GWI. We compared those percentages between groups by testing the corresponding distributions of 1000 bootstrap samples (Fig. 6); the larger negative percentage in GWI was highly statistically significant ($P < 0.001$, Mann–Whitney *U* test on the bootstrap distributions). (The same testing procedure was used for the comparisons below.) The ratio of GWI/control was −12.4/−5.6 = 2.21.

(b) Cerebellum (total; Fig. 7): control = −745.4 mm^3/years, GWI = −1253.6 mm^3/years. These rates of vol-

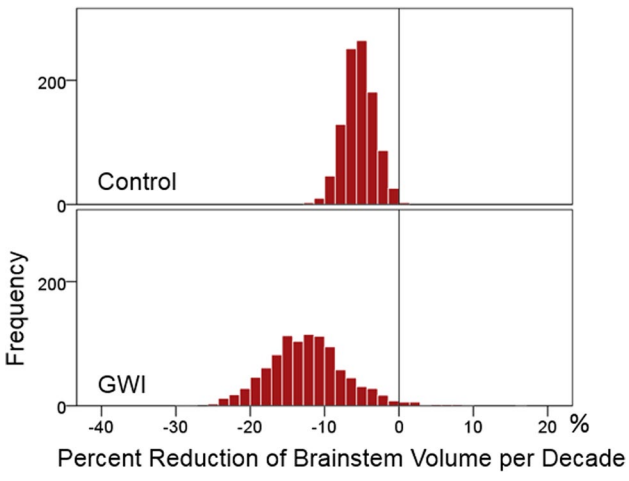

Fig. 6 Relative frequency histograms of 1000 bootstrap values of percent reduction of brainstem volume per decade (see text for details)

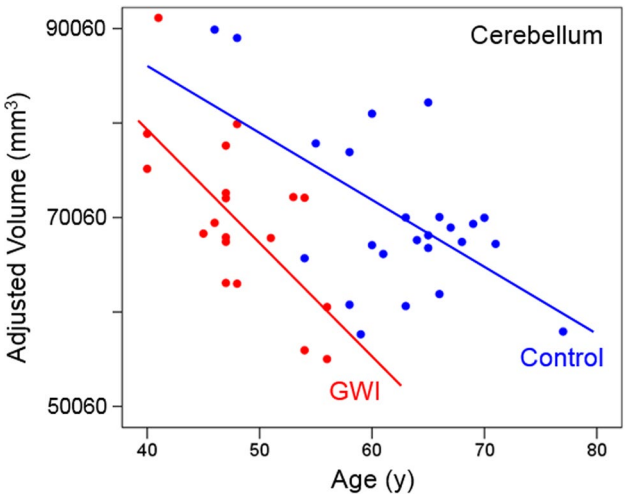

Fig. 7 Scatter plots of control and GWI total cerebellar volumes (adjusted for eTIV and sex) against age at MRI acquisition. Statistics for fitted lines: control, $P = 0.003$, $R^2 = 0.339$, $\hat{\beta} = -0.582$, $N = 24$; GWI, $P = 0.001$, $R^2 = 0.46$, $\hat{\beta} = -0.678$, $N = 19$

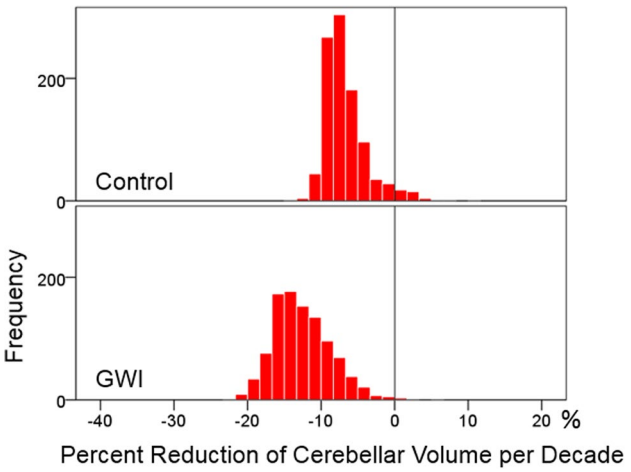

Fig. 8 Relative frequency histograms of 1000 bootstrap values of percent reduction of total cerebellar volume per decade (see text for details)

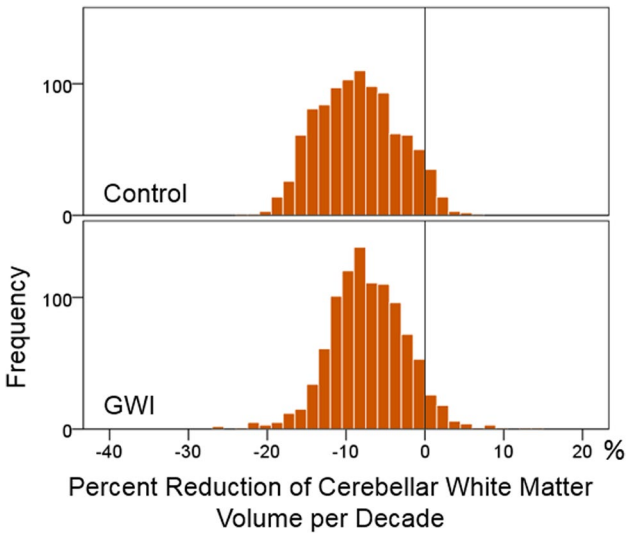

Fig. 9 Relative frequency histograms of 1000 bootstrap values of percent reduction of cerebellar white matter volume per decade (see text for details)

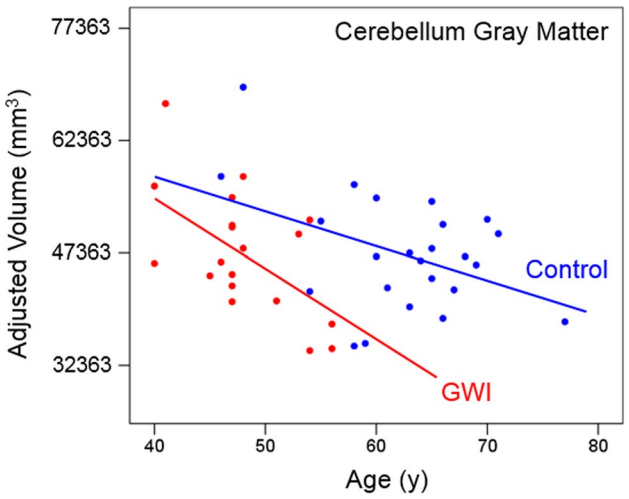

Fig. 10 Scatter plots of control and GWI volumes of cerebellar gray matter (adjusted for eTIV and sex) against age at MRI acquisition. Statistics for fitted lines: control, $P = 0.019$, $R^2 = 0.224$, $\hat{\beta}$ (standardized regression coefficient) $= -0.474$, N = 24; GWI, $P = 0.173$, $R^2 = 0.106$, $\hat{\beta} = -0.326$, $N = 19$

ume reduction with age, when expressed as percentages of the average volumes (adjusted for eTIV and sex), correspond to −7.5%/decade for control and −12.5%/decade of GWI cerebellum; the larger negative percentage in GWI was highly statistically significant (Fig. 8; $P < 0.001$). The ratio of GWI/control was $-12.5/-7.5 = 1.67$.

(c) Cerebellar white matter: control = −244.8 mm^3/years, GWI = −223.4 mm^3/years. These rates of volume reduction with age, when expressed as percentages of the average volumes (adjusted for eTIV and sex), correspond to −9.0%/decade for control and −8.3%/decade for GWI. These rates did not differ significantly (Fig. 9).

(d) Cerebellar gray matter (Fig. 10): control = −500.6 mm^3/years, GWI = −1030.1 mm^3/years. These rates of volume reduction with age, when expressed as percentages of the average volumes (adjusted for eTIV and sex), correspond to −6.9%/decade for control and −14.0%/decade for GWI; the larger negative percentage in GWI was highly statistically significant (Fig. 11, $P < 0.001$). The ratio of GWI/control was $-14.0/-6.9 = 2.03$.

Estimation of onset of subcortical atrophy in GWI

Given the steeper slopes of age on cerebellar and brainstem volumes in GWI, as compared to controls, we hypothesized that the faster atrophy in GWI might reflect the impact of adverse exposures during the Gulf War (GW). For that purpose, we estimated the age at which the adverse GW effect would have occurred by finding the age at which the control and GWI fitted lines would cross. The results are shown in Figs. 12, 13 and 14 for total cerebellar volume, volume of cerebellar gray matter, and brainstem volume, respectively (all volumes adjusted for eTIV and sex). The ages thus estimated, at which the hypothesized GW adverse effect would have occurred, were 27.7 years (total cerebellar volume, Fig. 12), 22.7 years (volume of cerebellar gray matter, Fig. 13), and 35.9 years (brainstem volume, Fig. 14). We took as an approximation of the age at military activation (i.e., called for military service) the participant's age at 1/1/1991 (GW mobilization lasted from August 1990 until April 1991). The estimated ages of GW insult for cerebellum are well within the range of age at activation (mean ± SD, 27.8 ± 4.1 years; range 18.3–32.3 years). Finally, the estimated age of insult for brainstem (35.9 years, Fig. 13), was later and outside the range of age at activation.

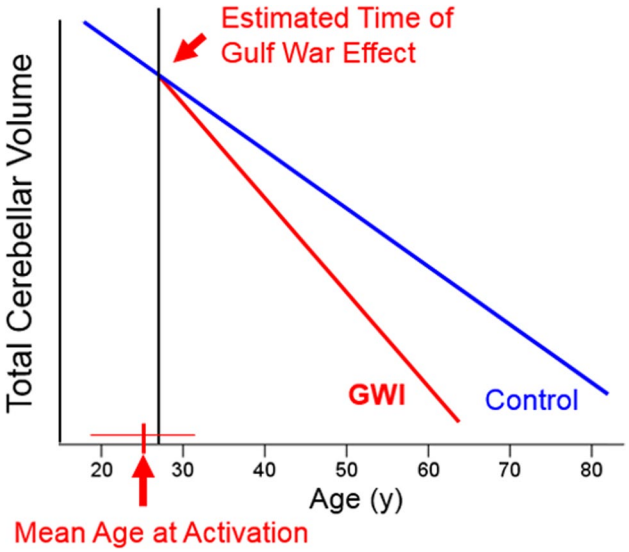

Fig. 12 Time of GW effect on total cerebellum volume estimated as the age at which the control and GWI lines intersect. The ordinate scale is the same as in Fig. 7 (see text for details)

Discussion

In this study, we document a systematic and significant reduction in subcortical brain volumes in GWI with the greatest differences observed in the brainstem, cerebellum, and thalamus. These findings complement and extend prior research on brain abnormalities associated with GWI and highlight dramatic and widespread brain effects of Gulf War Illness.

Brainstem alterations in GWI

We found the most substantial and most statistically significant reduction in brain volume for GWI veterans in the brainstem. These findings complement that of Haley

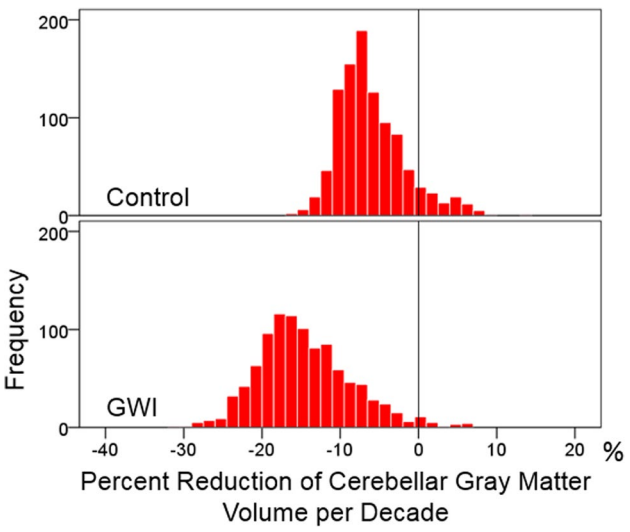

Fig. 11 Relative frequency histograms of 1000 bootstrap values of percent reduction of brainstem volume per decade (see text for details)

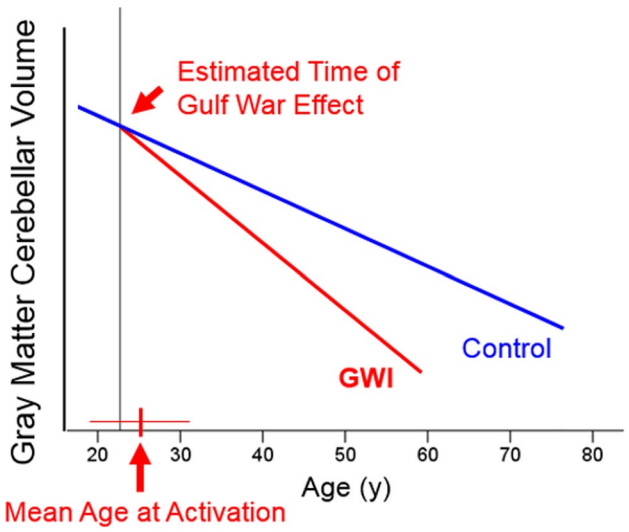

Fig. 13 Time of GW effect on cerebellar gray matter volume estimated as the age at which the control and *GWI lines intersect*. The ordinate scale is the same as in Fig. 10 (see text for details)

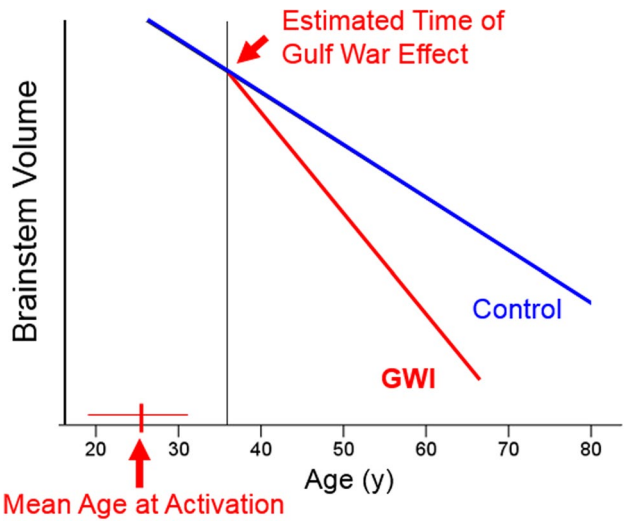

Fig. 14 Time of GW effect on brainstem volume estimated as the age at which the control and GWI lines intersect. The ordinate scale is the same as in Fig. 5 (see text for details)

and colleagues who have reported evidence of impaired brainstem auditory evoked potentials (Haley et al. 1997) and reduced "functional neural mass" in the basal ganglia and brainstem of GW veterans relative to controls using MRS (Haley et al. 2000). That study found particularly robust differences in a subset of GW veterans characterized by confusion-ataxia; however, a study aimed at replicating those findings failed to find evidence of brainstem and basal ganglia dysfunction in GWI veterans (Weiner et al. 2011). Using the CDC case definition of GWI, one other group reported brainstem atrophy in a subgroup of GWI veterans characterized by exertional tachycardia but not in GWI veterans characterized by exercise-induced hyperalgesia (Rayhan et al. 2013b). Taken together, most of the studies investigating GWI-related effects on the brainstem have found some evidence of brainstem dysfunction.

Thalamic alterations in GWI

Consistent with our findings of reduced thalamic volume in veterans with GWI, other studies have demonstrated thalamic alterations associated with GWI. An fMRI study found that compared to controls, veterans with GWI exhibited significantly different thalamic signal change which corresponded with deficits in semantic memory performance (Calley et al. 2010). Others have reported abnormal responses to physostigmine challenge in veterans with GWI relative to controls, with the thalamus being one of the most affected areas (Haley et al. 2009; Liu et al. 2011). Finally, evidence of thalamic cell death has been observed in a rat-model of GWI (Abdel-Rahman et al. 2002).

Cerebellar alterations in GWI

To our knowledge, only one other study described cerebellar volume alterations in veterans with GWI (Rayhan et al. 2013b). This study reported lower cerebellar gray and white matter volumes in GWI subjects who developed orthostatic tachycardia in response to exercise challenge, compared to a different group of GWI who developed hyperalgesia after exercise. Animal models of GWI have also suggested cerebellar dysfunction. Specifically, animals exposed to stress and low doses of chemicals that veterans were exposed to in the Persian Gulf War (e.g., diethyltoluamide (DEET), permethrin, and pyridostigmine bromide), but not either condition independently, showed evidence of loss of Purkinje cells and cytoskeletal abnormalities in the cerebellum (Abdel-Rahman et al. 2004).

Age-related atrophy

We found a higher rate of cerebellar and brainstem atrophy in GWI, as compared to controls. The rate of volume reduction in the controls ranged from 5 to 7% per decade in the controls, whereas in GWI it ranged from 12 to 14% per decade. Interestingly, no significant difference was found for cerebellar white matter; in contrast, the rate of atrophy in GWI was more than 2× that of controls for cerebellar gray matter and brainstem. The different rates of atrophy between controls and GWI participants allowed us to estimate an approximate age at which a postulated GW insult affecting the brain would have occurred. For total cerebellar volume and cerebellar gray matter, this age (27.7 and 22.7 years, respectively) was well within the age of GWI participants on 1/1/1991 (18.3–32 years). The good correspondence between the estimated dates of GW effect and the time of activation supports the hypothesis that cerebellar gray atrophy began at the time of the GW conflict. In contrast, the brainstem atrophy began later, at 35.9 years. Since the brainstem is intimately connected to the cerebellum, and includes the three cerebellar peduncles (superior, middle and inferior), it is reasonable to suppose that brainstem atrophy was secondary to cerebellar atrophy. Finally, the fact that the rate of thalamic atrophy did not differ significantly between control and GWI participants could be explained by the fact that the thalamic nuclei receiving cerebellar input are only a fraction of the whole thalamus (the volume that was assessed in this study).

Subcortical brain atrophy in GWI: sequelae of toxic encephalopathy?

Given the widespread reduction in brain volume observed here and in other studies (Chao et al. 2011, 2016; Heaton

et al. 2007), it is not surprising that neurological/cognitive/mood symptoms are among the most common symptoms reported among veterans of the Persian Gulf War (Steele 2000). Evidence across studies suggests that multiple brain areas are impaired in veterans with GWI. Healthy brain functioning relies on the complex interactions among various interconnected regions; conversely, if one region is impaired, it follows that dysfunction may affect associated areas. The substantial (~10%) subcortical brain atrophy found in GWI comprised mainly the brainstem, cerebellum and thalamus, and, to a lesser extent, basal ganglia, amygdala and diencephalon. The highest atrophy was observed in the brainstem, followed by left cerebellum and right thalamus, then by right cerebellum and left thalamus. These findings indicate graded atrophy of regions anatomically connected through the brainstem via the crossed superior cerebellar peduncle (left cerebellum → right thalamus, right cerebellum → left thalamus). This distribution of atrophy, together with the observed systematic reduction in volume of other subcortical areas (basal ganglia, amygdala and diencephalon), resemble the distribution of atrophy seen in toxic encephalopathy (Valk and Knaap 1992) caused by a variety of substances, including organic solvents. Given the potential exposure of Gulf War veterans to "a wide range of biological and chemical agents including sand, smoke from oil-well fires, paints, solvents, insecticides, petroleum fuels and their combustion products, organophosphate nerve agents, pyridostigmine bromide, …" (Institute of Medicine 2000), it is reasonable to suppose that such exposures, alone or in combination, may underlie, in part, the subcortical atrophy observed.

Immune-related mechanisms

In addition to these possible directly toxic effects, additional brain damage is likely to have been inflicted by the lack of immunogenetic protection (Georgopoulos et al. 2016; James et al. 2016) to a multitude of vaccines administered to GW veterans (Institute of Medicine 2000). Cerebellar atrophy has been observed in autoimmune disorders, including multiple sclerosis (Bermel and Bakshi 2006; De Stefano et al. 2016), Hashimoto's autoimmune thyroiditis (Selim and Drachman 2001) and various other immune-related disorders (Mitoma et al. 2015, 2016). It seems that the cerebellum is a frequent target organ of such disorders as well as of toxic insults, as discussed above. This preferential vulnerability of the cerebellum to various circulating immune- and nonimmune-related factors may be due to the higher blood brain barrier (BBB) permeability of the cerebellum and brainstem, compared to the cerebrum (Lee et al. 1989). The loss of BBB integrity in inflammatory or immune-related responses of the central nervous system triggered by infection, autoimmunity and/or stress (Esposito et al. 2001) has been shown to affect the cerebellum extensively (Esposito et al. 2001; Fabis et al. 2008; Michalak et al. 2010; Muller et al. 2005; Phares et al. 2006) and preferentially, compared to cerebral cortex (Esposito et al. 2001; Phares et al. 2006).

The structural cerebellar abnormalities observed in the present study dovetails with the cerebellar focus of functional brain anomalies we previously detected in GWI brains using magnetoencephalography (Engdahl et al. 2016, James et al. 2016) and, together with the cortical abnormalities observed previously (Engdahl et al. 2016), they provide a compelling argument for the hypothesis that brain damage in GWI is the result of heterogeneous insults, as proposed earlier (see Fig. 1 in James et al. 2016).

Limitations of the study

The main limitation of the study is the relatively small sample size.

Acknowledgements We thank Dr. Effie-Photini Tsilibari for bringing to our attention the potentially crucial role of the blood brain barrier in cerebellar dysfunction. This study was supported in part by the US Department of Veterans Affairs and the University of Minnesota. The contents of this paper do not represent the views of the US Department of Veterans Affairs or the United States Government.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

Abdel-Rahman A, Shetty AK, Abou-Donia MB (2002) Disruption of the blood–brain barrier and neuronal cell death in cingulate cortex, dentate gyrus, thalamus, and hypothalamus in a rat model of Gulf-War syndrome. Neurobiol Dis 10:306–326

Abdel-Rahman A, Abou-Donia SM, El-Masry EM, Shetty AK, Abou-Donia MB (2004) Stress and combined exposure to low doses of pyridostigmine bromide, deet, and permethrin produce neurochemical and neuropathological alterations in cerebral cortex, hippocampus, and cerebellum. J Toxicol Environ Health A 67:163–192

Bermel RA, Bakshi R (2006) The measurement and clinical relevance of brain atrophy in multiple sclerosis. Lancet Neurol 5:158–170

Blake DD, Weathers FW, Nagy LM et al (1995) The development of a clinician-administered PTSD scale. J Trauma Stress 8:75–90

Calley CS, Kraut MA, Spence JS et al (2010) The neuroanatomic correlates of semantic memory deficits in patients with Gulf War Illnesses: a pilot study. Brain Imaging Behav 4:248–255

Chao LL, Abadjian L, Hlavin J, Meyerhoff DJ, Weiner MW (2011) Effects of low-level sarin and cyclosarin exposure and Gulf War Illness on brain structure and function: a study at 4T. Neurotoxicology 32:814–822

Chao LL, Reeb R, Esparza IL, Abadjian LR (2016) Associations between the self-reported frequency of hearing chemical alarms in theater and regional brain volume in Gulf War Veterans. Neurotoxicology 53:246–256

Dale AM, Fischl B, Sereno MI (1999) Cortical surface-based analysis. I. Segmentation and surface reconstruction. NeuroImage 9:179–194

De Stefano N, Stromillo ML, Giorgio A et al (2016) Establishing pathological cut-offs of brain atrophy rates in multiple sclerosis. J Neurol Neurosurg Psychiatry 87:93–99

Desikan RS, Segonne F, Fischl B et al (2006) An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. NeuroImage 31:968–980

Efron B, Tibshirani R (1993) An introduction to the bootstrap. Chapman & Hall, New York

Engdahl BE, James LM, Miller RD et al (2016) A magnetoencephalographic (MEG) study of Gulf War Illness (GWI). EBioMedicine 12:127–132

Esposito P, Gheorghe D, Kandere K, Pang X, Connolly R, Jacobson S, Theoharides TC (2001) Acute stress increases permeability of the blood–brain-barrier through activation of brain mast cells. Brain Res 888:117–127

Fabis MJ, Phares TW, Kean RB, Koprowski H, Hooper DC (2008) Blood–brain barrier changes and cell invasion differ between therapeutic immune clearance of neurotrophic virus and CNS autoimmunity. Proc Natl Acad Sci USA 105:15511–15516

First MB, Spitzer RL, Gibbon M, Williams JBW (2002) Structured clinical interview for DSM-IV-TR Axis I disorders, research version, non-patient edition (SCID-I/NP). Biometrics Research New York State Psychiatric Institute, New York

Fischl B, Sereno MI, Dale A (1999a) Cortical surface-based analysis II: inflation, flattening, and surface-based coordinate system. NeuroImage 9:195–207

Fischl B, Sereno MI, Tootell RBH, Dale A (1999b) High-resolution inter-subject averaging and a coordinate system for the cortical surface. Hum Brain Mapp 8:272–284

Fischl B, Salat DH, Busa E et al (2002) Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33:341–355

Fischl B, van der Kouwe A, Destrieux C et al (2004) Automatically parcellating the human cerebral cortex. Cereb Cortex 14:11–22

Fukuda K, Nisenbaum R, Stewart G et al (1998) Chronic multisymptom illness affecting air force veterans of the Gulf War. JAMA 280:981–988

Georgopoulos AP, James LM, Mahan MY et al (2016) Reduced human leukocyte antigen (HLA) protection in Gulf War Illness (GWI). EBioMedicine 3:79–85

Glasser MF, Sotiropoulos SN, Wilson JA et al (2013) The minimal preprocessing pipelines for the human connectome project. NeuroImage 80:105–124

Gopinath K, Gandhi P, Goyal A et al (2012) FMRI reveals abnormal central processing of sensory and pain stimuli in ill Gulf War veterans. Neurotoxicology 33:261–271

Haley RW, Hom J, Roland PS et al (1997) Evaluation of neurologic function in Gulf War veterans: a blinded case–control study. JAMA 277:223–230

Haley RW, Marshall WW, McDonald GG et al (2000) Brain abnormalities in Gulf War syndrome: evaluation with 1H MR spectroscopy 1. Radiology 215:807–817

Haley RW, Spence JS, Carmack PS et al (2009) Abnormal brain response to cholinergic challenge in chronic encephalopathy from the 1991 Gulf War. Psychiatry Res Neuroimaging 171:207–220

Heaton KJ, Palumbo CL, Proctor SP et al (2007) Quantitative magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. Neurotoxicology 28:761–769

Institute of Medicine National Research Council (2000) Gulf War and Health, vol 1. Depleted uranium, pyridostigmine bromide, sarin, and vaccines. National Academies Press, Washington DC

James LM, Engdahl BE, Leuthold AC, Georgopoulos AP (2016) Brain correlates of human leukocyte antigen (HLA) protection in Gulf War Illness (GWI). EBioMedicine. 13:72–79. doi:10.1016/j.ebiom.2016.10.019

Lee C, William O, Stonestreet BS, Cashore WJ (1989) Permeability of the blood brain barrier for 125I-albumin-bound bilirubin in newborn piglets. Pediatr Res 25:452–456

Liu P, Aslan S, Li X, Buhner DM et al (2011) Perfusion deficit to cholinergic challenge in veterans with Gulf War Illness. Neurotoxicology 32:242–246

Michalak S, Wender M, Michalowska-Wender G, Kozubski W (2010) Blood–brain barrier breakdown and cerebellar degeneration in the course of experimental neoplastic disease. Are circulating cytokine-induced neutrophil chemoattractant-1 (CINC-1) and -2alpha(CINC-2alpha) the involved mediators? Folia Neuropathol 48:93–103

Mitoma H, Hadjivassiliou M, Honnorat J (2015) Guidelines for treatment of immune-mediated cerebellar ataxias. Cereb Ataxias 2:14. doi:10.1186/s40673-015-0034-y

Mitoma H, Adhikari K, Aeschlimann D et al (2016) Consensus paper: neuroimmune mechanisms of cerebellar ataxias. Cerebellum 15:213–232

Muller DM, Pender MP, Greer JM (2005) Blood–brain barrier disruption and lesion localisation in experimental autoimmune encephalomyelitis with predominant cerebellar and brainstem involvement. J Neuroimmunol 160:162–169

Phares TW, Kean RB, Mikheeva T, Hooper DC (2006) Regional differences in blood–brain barrier permeability changes and inflammation in the apathogenic clearance of virus from the central nervous system. J Immunol 176:7666–7675

Rayhan RU, Raksit MP, Timbol CR et al (2013a) Prefrontal lactate predicts exercise-induced cognitive dysfunction in Gulf War Illness. Am J Transl Res. 5:212–223

Rayhan RU, Stevens BW, Raksit MP et al (2013b) Exercise challenge in Gulf War Illness reveals two subgroups with altered brain structure and function. PLoS ONE 8:e63903

Research Advisory Committee on Gulf War veterans' illnesses (2014) Gulf War illness and the health of Gulf War veterans: research update and recommendations, 2009–2013. Government Printing Office, Washington DC

Schmahmann JD, Sherman JC (1998) The cerebellar cognitive affective syndrome. Brain 121:561–579

Selim M, Drachman DA (2001) Ataxia associated with Hashimoto's disease: progressive non-familial adult onset cerebellar degeneration with autoimmune thyroiditis. J Neurol Neurosurg Psychiatry 71:81–87

Steele L (2000) Prevalence and patterns of Gulf War Illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. Am J Epidemiol 152:992–1002

Valk J, van der Knaap MS (1992) Toxic encephalopathy. Am J Neuroradiol 13:747–760

Weiner MW, Meyerhoff DJ, Neylan TC et al (2011) The relationship between Gulf War Illness, brain *N*-acetylaspartate, and post-traumatic stress disorder. Mil Med 176:896–902

White RF, Steele L, O'Callaghan JP et al (2016) Recent research on Gulf War Illness and other health problems in veterans of the 1991 Gulf War: effects of toxicant exposures during deployment. Cortex 74:449–475