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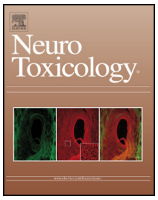

Effects of low-level sarin and cyclosarin exposure on hippocampal subfields in Gulf War Veterans

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ABSTRACT

Background: More than 100,000 US troops were potentially exposed to chemical warfare agents sarin (GB) and cyclosarin (GF) when an ammunition dump at Khamisiyah, Iraq was destroyed during the 1991 Gulf War (GW). We previously reported reduced hippocampal volume in GW veterans with suspected GB/GF exposure relative to matched, unexposed GW veterans estimated from 1.5 T magnetic resonance images (MRI). Here we investigate, in a different cohort of GW veterans, whether low-level GB/GF exposure is associated with structural alterations in specific hippocampal subfields, estimated from 4 T MRI.

Methods: The Automatic Segmentation of Hippocampal Subfields (ASHS) technique was used to quantify CA1, CA2, CA3 and dentate gyrus (DG), and subiculum (SUB) subfields volumes from high-resolution T2-weighted images acquired on a 4 T MR scanner in 56 GW veterans with suspected GB/GF exposure and 56 "matched" unexposed GW veterans (mean age 49 ± 7 years).

Results: GB/GF exposed veterans had smaller CA2 ($p = 0.003$) and CA3/DG ($p = 0.01$) subfield volumes compared to matched, unexposed GW veterans. There were no group difference in total hippocampal volume, quantified with FreeSurfer, and no dose–response relationship between estimated levels of GB/GF exposure and total hippocampal or subfield volume.

Conclusions: These findings extend our previous report of structural alterations in the hippocampi of GW veterans with suspected GB/GF exposure to volume changes in the CA2, CA3, and DG hippocampal subfields in a different cohort of GW veterans with suspected GB/GF exposure.

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1. Introduction

During the first Gulf War (GW), US combat engineers detonated a munitions storage pit at Khamisiyah, Iraq that was later found to contain stockpiles of sarin (GB; o-isopropyl methylphosphonoflouridate) and cyclosarin (GF; cyclohexyl methylphosphonoflouridate). The destruction of this bunker generated an airborne plume that potentially exposed as many as 100,000 troops in the surrounding area to low-levels of GB/GF. After the Gulf War ended, the Department of Defense (DoD) and the Central Intelligence Agency (CIA) tried to model possible GB/GF exposures over a 4-day period based on simulated meteorological conditions and analyses of likely chemical agent dispersal. Military personnel attached with units located in areas covered by the estimated zone of exposure were notified about their possible exposure (Directorate for Deployment Health Support of the Special Assistant to the Under Secretary of Defense (Personnel and Readiness) for Gulf War Illness, 1997). In 2002, the exposure plume model was refined and re-analyzed using additional meteorological modeling information, updated estimates of the total number of rockets destroyed, updated information about personnel and unit-level location, and updated exposure thresholds for GB and GF, the combined toxicity aspects of GB/GF, and consideration of agent removal mechanisms. This effort resulted in a second round of notification to service members that reversed some of the previous notifications and placed others into the possible zone of exposure (Assistant Secretary of Defense (Health Affairs) and Special Assistant to the Under Secretary of Defense (Personnel and Readiness) for Gulf War Illness Medical Readiness, and Military Deployments, 2002).

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Although two epidemiological studies have failed to find any health outcome differences between groups based on exposure predictions using the revised "plume" model data (Kang and Bullman, 1996; Enserink, 2001; Lindauer et al., 2004), we (Chao et al., 2010, 2011) and others (Proctor et al., 2006; Heaton et al., 2007) have reported neurobehavioral and structural brain changes in GW Veterans with suspected GB/GF exposure compared to unexposed GW veterans. One of the structural brain difference that we found was reduced hippocampal volume quantified from the 1.5 T magnetic resonance images (MRI) of 40 GW GB/GF exposed veterans relative to 40 matched, unexposed GW veterans (Chao et al., 2010). However, we were unable to replicate this hippocampal finding in a follow-up study of 64 different GW veterans with suspected GB/GF exposure and 64 matched controls scanned on a 4 T MR scanner (Chao et al., 2011).

One reason for our discrepant hippocampal results may be related to the different image processing software that we used to estimate hippocampal volume. We used a high dimensional brain mapping tool to estimate hippocampal volume in the 1.5 T dataset. Because of issues related to B1 inhomogeneity in the 4 T dataset, FreeSurfer (Martinos Center for Biomedical Imaging, Harvard-MIT, Boston, USA) (Fischl et al., 2002) was used to estimate hippocampal volume in the 4 T dataset. Compared to the gold standard of manual marking, the high dimensional brain mapping tool that we used on the 1.5 T dataset generates smaller hippocampal volumes because it excludes the alveus and fimbria (Hsu et al., 2002). In contrast, FreeSurfer generates larger hippocampal volumes than manual marking (Morey et al., 2009; Pardoe et al., 2009; Tae et al., 2008). This suggests that the FreeSurfer hippocampal mask includes neuroanatomical substrates beyond the hippocampus proper. Because animal studies suggest that organophosphate poisoning has selective effects on particular hippocampal subfields (Abdel-Rahman et al., 2002; Pazdernik et al., 2001), it is possible that FreeSurfer hippocampal volumetry is less sensitive to the effects of GB/GF exposure than the high dimensional brain mapping tool that we used to estimate hippocampal volume in the 1.5 T dataset.

The hippocampus consists of two convoluted formations: the dentate gyrus (DG) and the Cornu Ammonis (CA; Duvernoy, 2005), which can be further separated into four subdivisions (CA1–4). In recent years, several groups have tried to measure hippocampal subfields at the macroscopic level. We (Mueller et al., 2007, 2008; Mueller and Weiner, 2009; Mueller et al., 2010) and others (La Joie et al., 2010; Malykhin et al., 2010; Thammaroj et al., 2005; Zeineh et al., 2000, 2003) have utilized a high-resolution T2-weighted MRI sequence that yields sufficient gray matter contrast to visualize the dark band of stratum moleculare and stratum lacunosum to serve as a key landmark for defining the boundary between the CA4/DG region and the other CA subfields and the subiculum (Amaral and Lavenex, 2007; Eriksson et al., 2008). In this study, we used an automatic hippocampal subfield segmentation (ASHS) technique that has shown good accuracy relative to manual segmentation (Yushkevich et al., 2010) to investigate the effect of GB/GF exposure. Based on the rodent studies cited above (Abdel-Rahman et al., 2002; Pazdernik et al., 2001), we hypothesize that GW veterans with suspected GB/GF exposure have smaller CA1 and CA3/DG hippocampal subfields than unexposed GW veterans.

2. Methods

2.1. Participants

All participants were GW veterans who took part in a 4 T imaging study on the effects of Gulf War Illness on the brain, which was conducted at the San Francisco Veterans Affairs Medical Center between 2005 and 2010. A detailed description of predicted exposure and exposure dosage estimates has been described previously (Chao et al., 2010, 2011). The current analysis focused on 56 GB/GF exposed GW veterans who had high-resolution T2-weighted MRIs. All 56 GB/GF exposed veterans in the current analysis were part of the sample described in Chao et al. (2011). Furthermore, there is no overlap with the sample of GW veterans in whom we previously reported reduced hippocampal volume from 1.5 T MRI (Chao et al., 2010). Fifty-six unexposed GW veterans were selected from a group of 127 GW veterans with high-resolution T2-weighted images to match the GB/GF-exposed veterans for age, sex, level of education, and diagnoses of chronic multisymptom illness (CMI, Fukuda et al., 1998), current PTSD according to the Clinician-Administered PTSD Scale (CAPS, Blake et al., 1995), and current major depressive disorder, according to the Structured Clinical Interview for DSM-IV (First et al., 1995). Thirty-four these 56 unexposed GW veterans were part of the sample described in (Chao et al., 2011).

The Institutional Review Boards of the University of California, San Francisco, the San Francisco Veterans Affairs Medical Center (VAMC), 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 San Francisco VAMC on a Bruker MedSpec 4 T MRI system equipped with a USA instruments eight-channel array head coil. The MRI scan protocol included a high-resolution T2-weighted fast spin echo sequence (repetition time, 3500 ms; echo time, 19 ms) with a train of 15 spin-echoes per k-space segment, 160° refocusing pulses, and 100% oversampling in the phase-encoding direction to avoid aliasing, yielding a nominal in-plane resolution of 0.4 mm × 0.4 mm. Twenty-four contiguous slices, each 2-mm thick, were acquired in interleaved fashion. The coronal oblique slices were angulated perpendicular to the long axis of the hippocampal formation to achieve consistent images of hippocampal subfields from subject to subject (Mueller et al., 2007). A volumetric T1-weighted magnetization prepared gradient echo (MPRAGE) sequence (repetition time, 2300 ms; time following inversion pulse, 950 ms; echo time, 4 ms; 7° excitation pulses; 1 mm × 1 mm × 1 mm resolution) was also acquired.

2.3. Determination of total hippocampal volume

An automated, non-biased atlas-based Bayesian segmentation procedure, applied in Freesurfer v.4.5 (http://surfer.nmr.mgh.harvard.edu/), was used to derive quantitative estimates of total hippocampal volume from the volumetric T1-weighted MPRAGE (Dale et al., 1999; Desikan et al., 2006; Fischl et al., 1999).

2.4. Automatic Segmentation of Hippocampal Subfields

Segmentations of the hippocampal formation were generated using the Automatic Segmentation of Hippocampal Subfields (ASHS; Yushkevich et al., 2010). ASHS uses a combination of multi-atlas segmentation, similarity-weighted voting, and a learning-based bias correction technique to segment hippocampal subfields. Briefly, each subject's T2-weighted image was registered to a set of atlases (i.e., T2-weighted images of 32 subjects (mean age: 64.8 ± 11.8 years) with manual segmentations) and the candidate segmentations provided by the different registered atlases are combined into a single consensus segmentation based on a voting scheme that is weighted locally by the image intensity similarity. The segmentation of each voxel was corrected by a classifier trained to