

VERIFICATION OF CHRONIC HIPPOCAMPUS PERFUSION ABNORMALITIES IN ILL GULF WAR VETERANS FROM A REPRESENTATIVE NATIONAL SAMPLE

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Introduction: Gulf War illness affects about 25% of the 700,000 veterans of the 1991 Persian Gulf War and causes chronic fatigue, memory loss, poor emotion control, attention problems, personality changes, and other problems (1,2). A case definition of Gulf War illness, derived by principal components factor analysis of symptoms (2,3) and validated in a national sample of Gulf War veterans (4), classified three primary Gulf War illness variants: syndrome 1 (impaired cognition), syndrome 2 (confusion-ataxia), and syndrome 3 (central neuropathic pain). Gulf War illness has been associated with the exposure to neurotoxic acetylcholinesterase inhibitors, including organophosphate (OP) pesticides, pyridostigmine bromide (PB) given to protect against nerve gas, and low-level sarin/cyclosarin nerve gas (2,3,5). Animal studies have shown that acetylcholinesterase inhibitors acutely affect the hippocampus (6, 7) and chronically impair hippocampal synaptic transmission (8). Hippocampal blood flow at baseline and after infusion of the reversible short-acting cholinesterase inhibitor physostigmine was found to be abnormal in ill Gulf War veterans of a Naval Reserve Mobile Construction Battalion (Seabees) studied by SPECT in 1998 (9) and ASL in 2008-2009 (10). The purpose of this 2009-2010 ASL study was to validate these results in a representative national sample of U.S. Gulf War veterans.

Materials and Methods: Subjects were selected as a nested case-control study from a national survey sample of 8,020 Gulf War-era veterans statistically representative of all in the U.S. Armed Forces during the 1991 Gulf War (4). Cases were those meeting the case definition of Gulf War Syndromes 1 (Syn 1), 2 (Syn 2), 3 (Syn 3), and controls were from the deployed and non-deployed healthy veterans (NC). Demographic data of veterans studied are provided in Table 1. All subjects were screened for contraindications and gave written informed consent according to a study protocol approved by the local Institutional Review Board.

Infusion and MR parameters were optimized in a preliminary experiment (11). For each subject, a two-session perfusion study, double-blinded by group and single-blinded by infusate, was performed with the infusion of saline during the first session and physostigmine (~0.6 mg) during the second session, each at 130 ml/hour for 30 minutes prior to imaging. To combat nausea, glycopyrrolate (0.3 mg) was injected prior to physostigmine infusion; a placebo saline injection was given prior to the saline infusion in the first session (Figure 1).

Oblique coronal imaging slices were used to measure ASL perfusion with a customized FAIR technique implemented on a 3T Siemens TIM Trio. Details of the imaging system, ASL sequence and parameters, and quantitative rCBF analysis have been described previously (10,12,13).

A linear statistical model was used to assess the effects of syndrome classification, cholinergic challenge, cerebral hemisphere and all interactions involving these factors on hippocampal CBF measures. Primary interest centered on differential cholinergic effects among the ill groups compared to the healthy controls.

Results: One healthy veteran's perfusion-weighted imaging maps from the saline session are shown in Figure 2. The national sample study results showed significant differences of the cholinergic effects across the four veteran groups ($p < 0.0001$). Physostigmine decreased hippocampus perfusion in healthy and Syn 1 veterans, but increased hippocampus perfusion in Syn 2 and Syn 3 veterans (Figure 3). The planned comparisons between control and ill veteran groups showed no significant difference between NC and Syn 1 groups, but significant differences between NC and Syn 2 and Syn 3 groups, the same pattern observed in the 1998 SPECT and 2008-9 ASL studies of the Seabees battalion (Figure 3). For Syn 2 and Syn 3, progression of rCBF abnormality with time is indicated in both hippocampi.

Discussion: This 2009-2010 ASL study of a national sample representative of U.S. veterans of the 1991 Persian Gulf War verifies our previous 1998 SPECT (9) and 2008-9 ASL (10) perfusion imaging studies of Gulf War veterans from a Seabees Navy battalion. It corroborates that hippocampal perfusion dysfunction in ill Gulf War veterans is chronic, persisting and perhaps progressing in the 20 years since the war, and occurs in a national sample representative of all Gulf War veterans as well as in the Seabees battalion in which the perfusion abnormalities were initially observed. This physostigmine challenge ASL experiment supports our pre-stated hypothesis that the abnormalities arise from neurotoxic exposures to cholinesterase inhibitors such as sarin/cyclosarin, pyridostigmine bromide (PB), and OP pesticides (2, 3, 5, 14). The test protocol can conveniently be administered as a 2-3 hour diagnostic test in a clinical setting (10).

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Table 1 Demographic information for veterans of national survey study*

Veteran Group	Male		Female		Both Gender	
	Number	Age	Number	Age	Number	Age
NC**	25	49 ± 8	6	53 ± 9	31	49 ± 8
Syn 1	13	51 ± 9	5	47 ± 9	18	50 ± 9
Syn 2	15	52 ± 9	4	49 ± 2	19	51 ± 8
Syn 3	14	51 ± 9	5	43 ± 1	19	48 ± 9

* No significant age differences were found across veteran groups for either gender or both. Subject ages are expressed in mean ± STD years.

** NC group included both deployed and non-deployed healthy veterans.

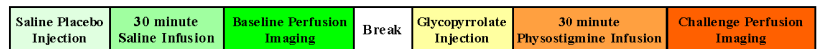


Figure 1. Diagram for Physostigmine challenge ASL perfusion study schedule.

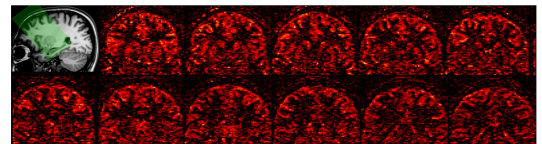


Figure 2. Oblique coronal imaging slab position for ASL perfusion imaging (top left) and perfusion-weighted imaging maps of one healthy veteran from saline session.

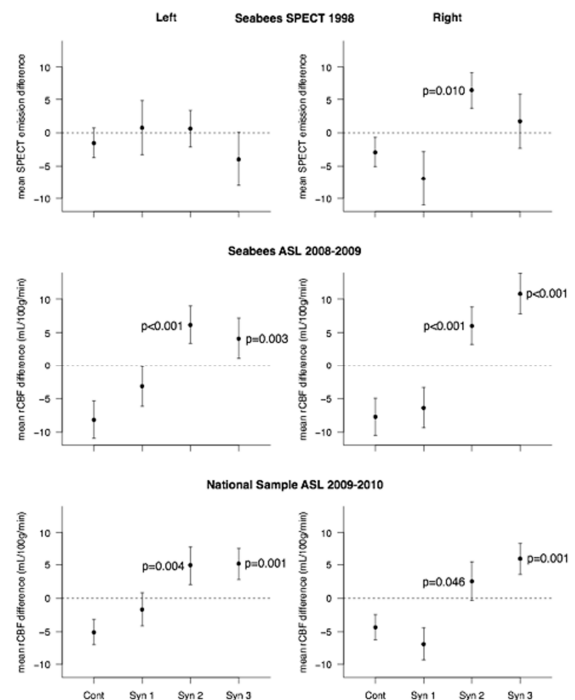


Figure 3. Hippocampus rCBF changes upon physostigmine treatment from three studies: SPECT (top) and ASL (middle) with veterans from a Seabees Navy Battalion, and ASL with veterans from a national sample (bottom). P values indicate significant differences of syndromes compared to controls.