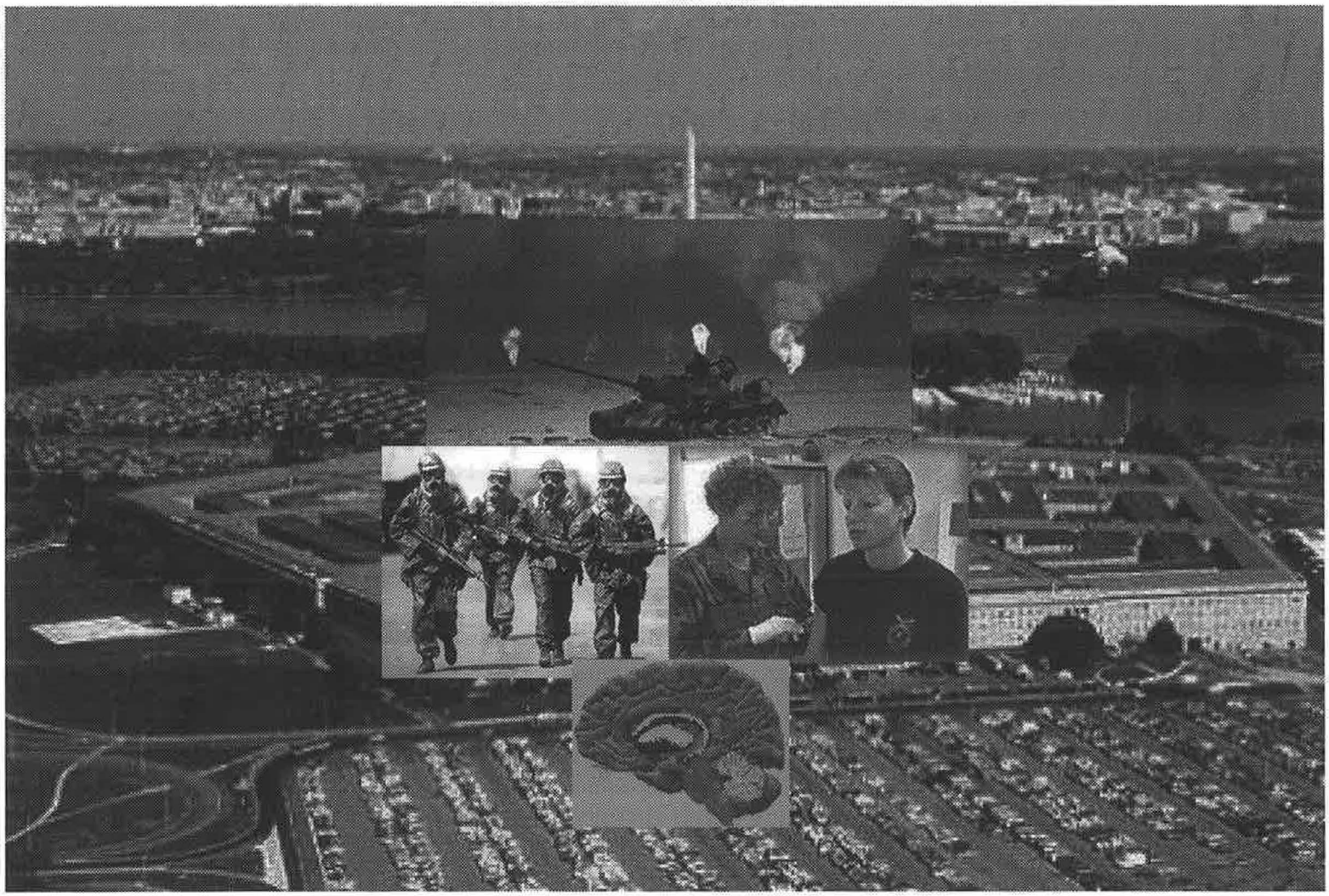
INTERNAL MEDICINE GRAND ROUNDS

December 2, 2004

Gulf War Syndrome: Clinical Science at the Interface with Politics



Robert W. Haley, M.D.

Department of Internal Medicine
University of Texas Southwestern Medical Center
Dallas, Texas

Robert W. Haley, M.D., is Professor and Chief of the Epidemiology Division in the Department of Internal Medicine, having attended on the medical services of the Dallas Veterans Affairs Medical Center and Parkland Memorial Hospital. During his ten years as a division director at the U.S. Centers for Disease Control and Prevention and his first ten years on the UT Southwestern faculty, he conducted research on the epidemiology and prevention of hospital-acquired infections, including national studies in random samples of U.S. hospitals. For the past 10 years he has devoted his research efforts to study of the Gulf War syndrome with support of the Perot Foundation and the Department of Defense.

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In April 1994 our research team at UT Southwestern undertook an epidemiologic and clinical study of the apparent epidemic of Gulf War syndrome. At an NIH consensus conference covering the Gulf War exposures and veterans' current symptoms,[1] we learned that three years after the war no one had performed the usual CDC-style epidemic investigation, used successfully to solve prior epidemics of Legionnaire's disease, toxic shock syndrome, HIV/AIDS, Four Corners (hantavirus) pneumonia and others.[2] No case definition had been formulated, and none was contemplated. Without a case definition, however, no meaningful epidemiologic study was possible.[2] At the conference, we formulated the hypothesis that Gulf War syndrome might represent a subtle brain injury from combinations of organophosphate pesticides and chemical nerve agents, pyridostigmine bromide, and DEET-containing insect repellants, and began designing an epidemiologic study to develop a case-definition and use it to test the hypothesis.[2]

Developing a Case Definition: The Seabees Survey

In December 1994 and January 1995 we performed a field survey of 249 members of the 24th Reserve Naval Mobile Construction Battalion (Seabees) in cities near their homes throughout the five southeastern states.[3,4] We administered a questionnaire on symptoms, one on war-zone exposures, and a psychological test, the Personality Assessment Inventory.[5] After computerizing the survey data, the first task was to derive a case definition from analysis of the survey data.

An epidemiologic case definition is a simple statement of the clinical features required to make a diagnosis of the epidemic disease.[2] In most epidemics, the case definition is obvious from examining a few typical cases, but this was not true for Gulf War syndrome.[1] Consequently, we used a mathematical technique called *factor analysis* to search for a syndrome-like structure in the symptom endorsements of the 249 seabees who participated in the survey.

We performed a hierarchical factor analysis involving two sequential factor analysis steps.[6,7] In the first step, we analyzed the anatomic distributions, clinical variations, and other important features of each of the 22 typical symptoms of Gulf War syndrome to subdivide each symptom into unambiguous symptom scales.[3] For example, we found that there are two distinct types of "chronic fatigue," one being excessive daytime sleepiness and the other, excessive muscle tiredness. This step yielded 52 unambiguous symptom scales that were normally distributed with mean zero and standard deviation of 1.

In the second step, we performed a factor analysis of 52 unambiguous symptom factor scales measured in the 249 subjects.[3] The results are displayed in a graph called a scree plot[6,7] (**Figure 1**). In the scree plot, the vertical axis measures the strength of clustering of the symptoms, and the horizontal axis indexes the

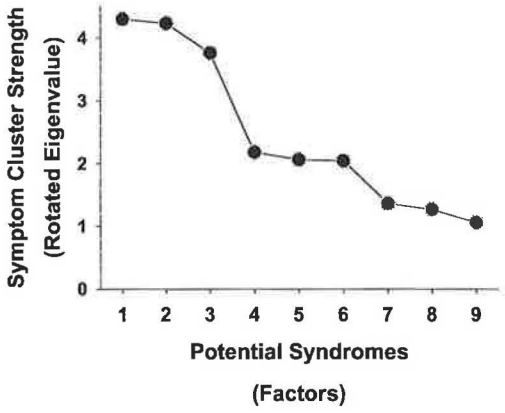


Figure 1. Scree plot from hierarchical principal factor analysis orthogonally rotated with varimax rotation. The 9-factor rotated model of the 52 symptom scales confirmed that 3 strong factors and 3 weak ones followed by a decreasing slope of trivial factors were evident after rotation was performed. Consequently, 6 rotated syndrome factors were extracted in the final model. The variance explained is knows as the eigenvalue. (From Haley et al. *JAMA* 1997; 277: 215-222.)

possible syndromes found in the data. In this case, the analysis identified three strong syndrome factors and three weak ones, the remaining points being too low on the strength scale to be meaningful. In further analysis we discovered that the weak syndrome factors 4-6 were strongly overlapping with syndrome factor 2, probably representing subgroupings of syndrome 2. As a result, we confined further analysis to syndrome factors 1-3.

Figure 2, top graph, shows the distributions of all 249 subjects on each of the six syndrome factor scales.

To evaluate the possibility that the six syndromes might have resulted from our over-fitting to random noise in the data, we subsequently repeated the epidemiologic survey in 336 North Texas-area Gulf War veterans identified through the Gulf War Veterans' Clinic at the Dallas VA Medical Center.[8]

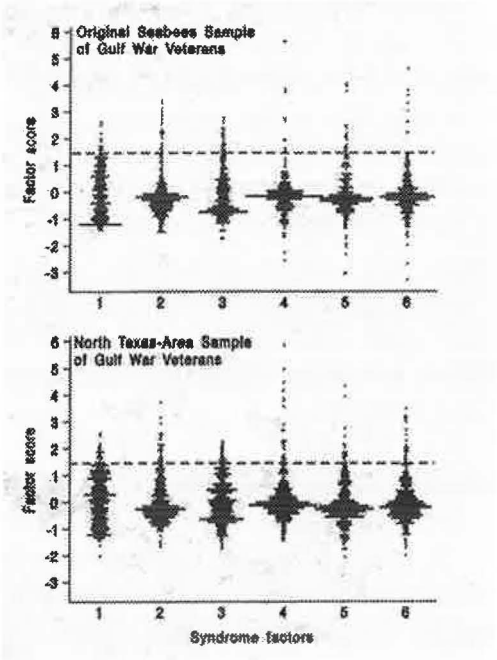


Figure 2. Distribution of the 249 veterans on each of the 6 syndrome factor scales. Visual inspection of the distributions indicated that dividing all of the distributions at 1.5 (horizontal line) would provide dichotomous syndrome variables distinguishing at least 9 extreme individuals from the bodies of the distributions. For further analysis, veterans falling above the line were considered to have a syndrome, and those falling below the line were considered not to have it. The top graph presents the findings from the survey of the Seabees battalion, and the bottom one presents the findings from the survey of North Texas Gulf War veterans at the Dallas VA Medical Center. (From Haley et al. *JAMA* 1997; 277: 215-222 and unpublished data.)

The distributions of the 336 North Texas veterans on the six syndrome factors was highly similar to those of the 249 seabees (**Figure 2**). To test definitively whether the same syndrome structure was present in the North Texas veterans' symptom data, we performed a confirmatory factor analysis by expressing the factor structure in the seabees' data as simultaneous structural equations and testing the goodness of fit of the stipulated factor model in the North Texas data.[9] We found that the original factor model provided an excellent fit to the new data, measured by Bentler's comparative fit index (CFI) of 0.95 and non-normed fit index (NNFI) of 0.93 (satisfactory values are >.90). Finding that he three syndrome factors were highly inter-correlated (r=0.71, 0.64 and 0.66), we posited an overall Gulf War syndrome with the three syndromes representing variants. We found that this model fit just as well as the model of three separate syndromes, supporting our original suggestion that the three primary syndromes are overlapping variants of a common illness. These findings reassured us that the same syndrome structure is present in both populations and it is not simply due to overfitting to random variation.

Since reporting these findings, two independent studies have further replicated the syndrome factor structure in different Gulf War veteran populations. Researchers at CDC performed a similar analysis in Air Force reservists who remained in the service through 1995 and found our syndrome factors 1 and 3, but they did not measure the symptoms that would have detected our syndrome 2.[10,11] More recently, researchers at the VA Central Office reported a symptom factor analysis in approximately 10,000 Gulf War veterans selected randomly from the full deployed population, and in approximately 9,500 selected randomly from the nondeployed Gulf War-era military population.[12] In their factor analysis, syndrome factors almost identical to our factors 1-3 were identified, appearing in the same order even. British researchers obtained

similar results in a factor analysis of symptoms in British Gulf War veterans,[13] and several other groups developed factor models with varying success related to limitations of their data. Taken together, these studies suggest that the syndrome structure we found reflects the distribution of a new disease process in the population of Gulf War veterans.

To obtain binary syndrome indicator variables for analyses to identify environmental risk factors associated with the syndromes, we dichotomized each of the syndrome factor distributions at the 1.5 standard deviation point (**Figure 2**).[3] The distribution of the seabees sample by these syndromes is given in **Table 1**. Of the 249 seabees surveyed, 70 remained well, 116 had had health problems since the war but did not fit into one of the new syndromes, 12 had syndrome 1 ("impaired cognition"), 21 had syndrome 2 ("confusion-ataxia"), and 22 had syndrome 3 ("arthro-myo-neuropathy," later renamed "central pain"). This classification, which constitutes our case definition of Gulf War syndrome, identified three primary subgroups of ill Gulf War veterans. This is important because we hypothesized that each group would have different risk factors and different distributions of brain dysfunction on objective tests. The symptoms that constituted each of the three primary syndrome variants are given in **Table 2**.

Group description	No. of veterans	% of battalion
No serious health problems	70	11.6
Serious health problem but no factor-derived syndrome	116	19.1
Any of the factor syndromes	63	10.4
Syndrome 1	12	2.0
Syndrome 2	21	3.5
Syndrome 3	33	3.6
Syndrome 4	11	1.8
Syncrome 5	16	2.6
Syndrome 6	9	1.5
Total survey participants	249	

Table 1. Classification of the 606 veterans of the 24th Reserve Naval Mobile Construction Battalion deployed to the Gulf War, according to reported health problems, factor analysis-derived syndromes, and participation in the UT Southwestern survey. (From Haley et al. *JAMA* 1997; 277: 215-222.)

To obtain more evidence on whether the syndromes reflect different clinical conditions, we analyzed the level of occupational disability and the psychological profiles of each.[3] The percentage who were unemployed at the time of the survey was low (2%) in the 70 veterans with no health problems, and not appreciably higher in the 116 with health problems but no Gulf War syndromes, or in syndromes 1 and 3. In syndrome 2, however, over 50% were unable to work, and those who were employed generally reported reduced job duties and problems carrying our their jobs due to severe fatigue, cognitive problems and emotional intolerance of the work environment.

Syndrome 1 (Impaired Cognition)
- Distractibility
- Memory problems
- Depression
- Middle/terminal insomnia
- Fatigue (daytime sleepness)
- Slurring of speech
- Confused thought
- Migraine-type headaches

Syndrome 2 (Confusion-Ataxia)
- Thinking/reasoning problems
- Getting confused or lost
- Getting disoriented
- Losing balance
- Stumbling often
- Feeling like the room is spinning
- Physician's diagnosis of PTSD/depression
- Sexual impotence

Syndrome 3 (Arthro-Myo-Neuropathy)
- Generalized joint and muscle pain
- Increased difficulty lifting heavy objects
- Fatigue (muscle weakness after exertion)
- Tingling/numbness of extremities

Table 2. Unambiguous symptoms that define each of the three primary syndromes derived by factor analysis.

The psychological profiles, measured by the Personality Assessment Inventory (PAI), were entirely within normal limits (within 2 SD, or 20 points, of the norm of 50) in the seabees with no health problems and in those with health problems but no syndromes, but in all six syndrome groups we found the same abnormal profile (**Figure 3**).[3] This profile was not compatible with PTSD or other psychological disorders but resembled the profile seen when the test is administered to patients with neurologic disease or injury. Although this does not prove the presence of neurologic disease, it weighs against any of the standard psychiatric diagnoses as explanations for the syndromes.

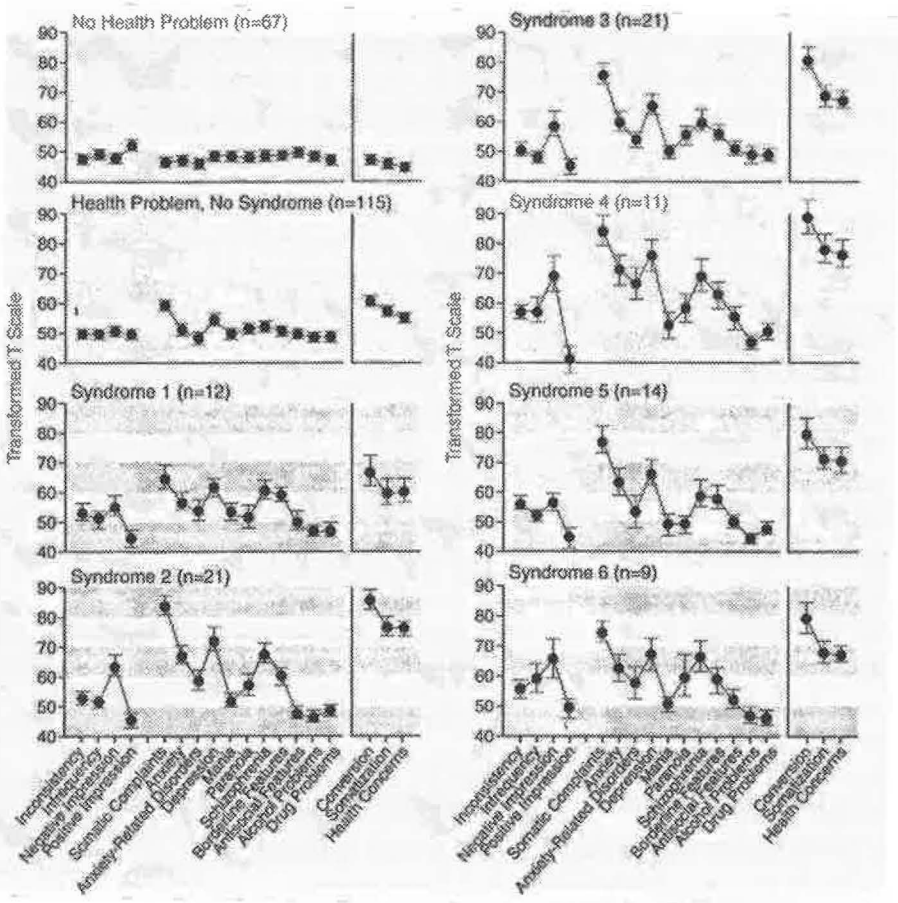


Figure 3. Psychological profiles of 243 Gulf War veterans in RNMCB-24 who completed the Personality Assessment Inventory, by presence of health problems or Gulf War syndromes. The vertical axis is measured on a transformed T scale, with the mean of a normal reference population at 50 with a SD of 10. Values outside 2 SDs (i.e., >70 or <30) are clinically significant. On the horizontal axis, the first 4 measures are the validity scales, and the horizontal axis on the right of each graph measures the 3 subscales for interpreting the Somatic Complaints scale. The points represent mean scale values, and the error bars represent 1 SEM. (From Haley et al. *JAMA* 1997; 277: 215-222.)

Preliminary Evaluation of Neurologic Function: First Nested Case-Control Study

From the above findings, we were still not convinced that the new syndromes were reflections of true physical illness. The possibility of over-fitting to random effects or of an unusually strong effect of communication among the veterans still seemed possible. To obtain evidence to confirm or refute these possibilities, we undertook a case-control study nested in our cross-sectional survey population of seabees.[14]

We selected 5 veterans with syndrome 1 (impaired cognition), 13 with syndrome 2 (confusion-ataxia), 5 with syndrome 3 (arthro-myo-neuropathy/central pain), and 1 each with syndromes 4-6. We oversampled syndrome 2 because it appeared to be the most severe, and we wanted to be more certain of findings on it. For comparison, we selected 20 well control veterans, all from the same seabees battalion, age-sex-education-matched to the veterans with syndrome 2. Ten of the controls had been deployed to the war but remained well (the deployed controls), and ten had not been deployed (the non-deployed controls). We brought them to Dallas in pairs and, with informed consent, performed a battery of neurophysiologic,

audiovestibular, and neuroradiologic tests to determine whether those with the syndromes had evidence of brain impairment compared with the controls. The faculty and staff who performed the testing were kept blinded to the subjects' case- or control-group status.

As expected, there were no significant differences between the cases and controls on the clinical neurological examination, a battery of blood tests, brain MRI, or clinical reading of regional cerebral bloodflow measured by single photon emission computed tomography (SPECT). However, there were statistically significant and important differences on more sensitive tests of neurologic function recommended for use in detecting neurotoxic brain damage (**Figure 4**).[14,15]

Slow sinusoidal harmonic acceleration showed greater inter-ocular asymmetry of gain in rotational nystagmus in ill veterans than in controls (**Figure 4, graph A**). This difference in asymmetry between cases and controls was most marked for syndrome 1 at rotational speeds of .01 and .02 Hz and for syndrome 2 at all rotational speeds. In a repeated measures analysis of variance of asymmetry values at .01, .02 and .04 Hz, the differences from controls were statistically significant for syndrome 1 (p=.015) and for syndrome 2 (p=.002) but not for syndrome 3 (p=.8). Moreover, in the controls the magnitude of asymmetry decreased monotonically in a normal fashion as rotational speed increased; however, this pattern was not present in any of the 3 syndrome groups (**Figure 4, graph A**).

The ENG-measured velocity of nystagmus normally induced by caloric stimulation of the ear was significantly diminished in veterans with syndrome 3 compared to controls for all four irrigations (cool right, p=0.02; cool left, p=0.004; warm right, p=.009; warm left, p=.004) (**Figure 4, graph B**). Interaural asymmetry of caloric responses appeared greater in veterans with syndrome 2 than in controls (p=.07).

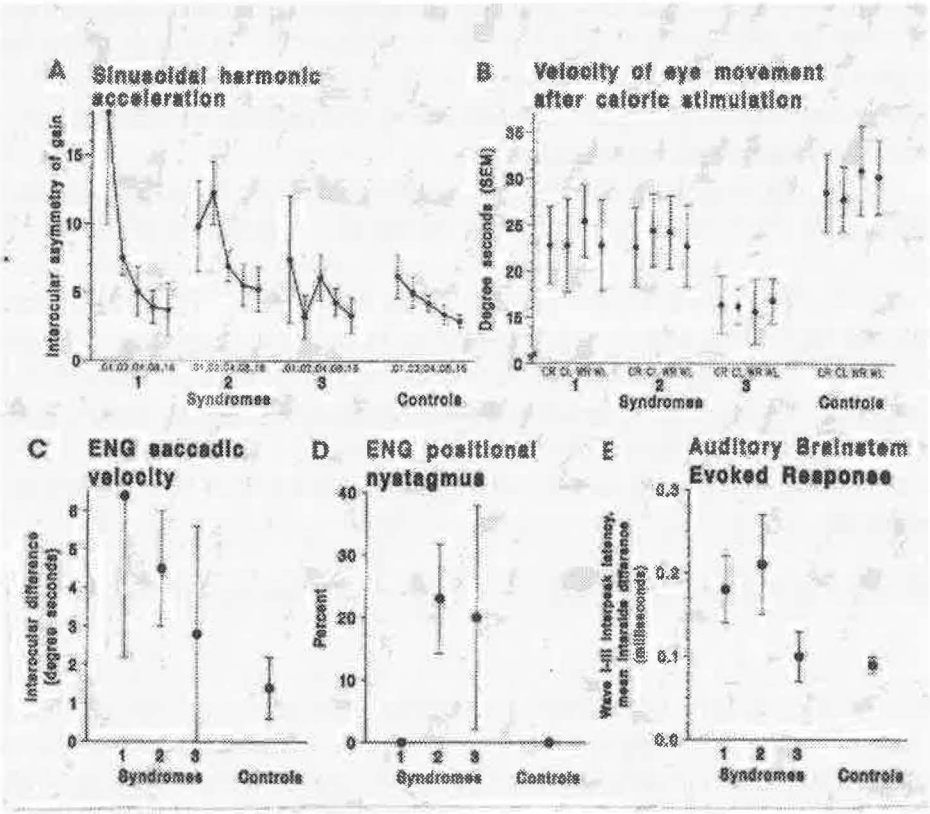


Figure 4. Comparison of mean values of selected audiovestibular tests in 23 veterans with syndromes 1, 2 or 3 and 20 age-sex-education-matched controls. On the horizontal axis of graph A, rotational speeds of .01, .02, .04, .08 and .16 are measured in Hz. On the horizontal axis of graph B, the conditions of the test stimuli are abbreviated as temperature (C=cool, W=warm) and side (R=right ear, L=left ear); for example, CR=cool stimulation of the right ear. On the horizontal axis of graph F, the response strength in each group is given for RF=right front and LF=left front of the test apparatus. (From Roland et al., *Otolaryngol Heal Neck Surg* 2000: 122:319-329.)

When saccadic eye movements were evaluated by ENG, veterans with syndrome 2 had either an abnormal saccadic accuracy or velocity (**Figure 4, graph C**). Asymmetry of saccadic velocity was significantly greater in syndrome 2 than in controls ($p<.05$). Pathological spontaneous nystagmus was demonstrated in various head positions in four ill veterans (3 with syndrome 2 and 1 with syndrome 3) but in none of the controls ($p=.09$, **Figure 4, graph D**).

Interaural asymmetry for ABR was manifested as wave I – wave III interpeak latency differences between ears that were greater in cases than in controls ($p=.02$, **Figure 4, graph E**). This was true of syndromes 1 ($p=.005$) and 2 ($p=.07$) but not syndrome 3.

An overall measure of organic brain dysfunction is the Halstead-Reitan neuropsychological impairment scale.[16] A summary score of results from 51 objective tests of diverse brain functions, it has been used to monitor the degree of brain damage in neurology and neurosurgery for decades and is not elevated by psychological conditions in patients in the absence of organic neurologic damage. The Impairment Index is measured on a scale from 0 (no impairment) to 1.0 (maximal impairment) with 0.4 as the upper limit of normal in males in the fifth decade of life. Compared with the 20 simultaneously tested controls (Impairment Index, 0.38 ± 0.05), our seabees with syndrome 2 (impaired cognition) had a slightly elevated mean ($\pm$SEM) score just above normal (0.43 ± 0.09, $p=.3$); whereas, those with syndromes 2 (impaired cognition) and 3 (arthro-myo-neuropathy/central pain) had evidence of substantial organic brain dysfunction (0.59 ± 0.06, $p=.006$ and 0.54 ± 0.10, $p=.09$, respectively).[14] Analysis of the individual neurologic measures indicated a pattern of generalized deficits on almost all tests but no single test indicated an extreme abnormality, a picture consistent with a generalized brain injury from exposure to low-levels of neurotoxic chemicals.[17]

The results of these clinical tests, performed under investigator blinding, demonstrate that veterans with Gulf War syndromes have statistically significant differences from controls on objective tests of vestibular function. This particular combination of vestibular abnormalities is best explained by a pathological process in the brainstem.[15] These differences, however, are subtle and difficult to detect, as has generally been found in patients with documented neurotoxic brain injury.[18] This degree of dysfunction is almost certain to be missed if an individual patient's performance is compared to published norms of audiovestibular tests, which are designed to detect more profound dysfunction from tumors, strokes, and traumatic injuries. This, we believe, has contributed importantly to why an organic basis for Gulf War syndrome was originally overlooked.

Evaluation of Neurologic Function: Study of a Monozygotic Twin Pair

We studied a 48 year old white male 27-year active duty colonel of U.S. Army Special Forces, who developed a debilitating neuropsychological condition shortly after the Gulf War, and his identical, non-military twin.[19] Qualified in Airborne, Ranger, Special Forces, underwater combat diving, and free fall parachuting, the officer served in 7 regions of the world including Operation Just Cause, speaks three languages, received service awards, and was fit on periodic Army HALO/SCUBA physical examinations through 1990. In the Gulf War he commanded a battalion, received the Legion of Merit and Valorous Unit Citation, was promoted in rank, and was anticipating increasing command opportunities.

Within a year of the war, he developed stuttering; slowed thinking; difficulty writing, pronouncing polysyllabic words, and learning new information; problems with balance descending stairs; apractic slowness in initiating actions such as stepping on the brake in his car;

middle and terminal insomnia; and fluctuating fatigue. For several months at a time, he experienced paroxysms of coughing, severe myalgias, hot flashes and night sweats, and worsening of fatigue, often triggered by exposure to fumes. Evaluation in the military's CCEP registry yielded diagnoses of mild PTSD and "adult-type ADD." Our evaluation comparing the officer with his twin confirmed the negative findings on routine medical tests including rheumatologic and pulmonary evaluations, clinical neurologic examination, nerve conduction testing, somatosensory evoked potentials, brain MRI and blood testing. However, psychiatric evaluation including structured clinical interview for DSM-IV (SCID) and the clinician-administered PTSD scale (CAPS) found no evidence of present or lifetime PTSD or ADD.

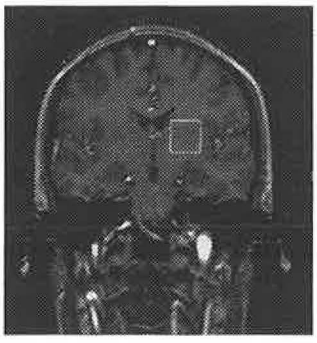
In contrast to his twin who was normal on the test, sleep studies revealed normal sleep latency and REM but multiple awakenings in the last 2/3 of sleep, central sleep apnea (>30 per hour), and loss of circadian rhythm of tympanic membrane temperature. Night sweats were accompanied by temperature spikes to 40°C. Radiologist's visual interpretation of high resolution 3-dimensional, full volume brain SPECT scans found reduced blood flow in the right putamen and left temporal cortex. Auditory brainstem response found asymmetrical delayed conduction in the upper brain stem and delay of the event-related potential (P300). Platform posturography revealed vestibular ataxia. Infrared oculography showed increased saccadic latency with decreased velocity and acceleration. Quantitative EEG showed excess beta activity similar to that described in symptomatic workers accidentally exposed to sarin.[20,21] Microneurography found sympathetic nerve hyperactivity. Three 24-hour urine analyses found excess norepinephrine excretion. Neuropsychological testing indicated cognitive impairment not typical of commonly diagnosed neurologic conditions.

Second Clinical Case-Control Study

From the differences found in this twin pair, we developed a battery of tests that would stand a good chance of identifying brain dysfunction in a clinical case-control study and performed the battery on the cases and controls studied previously. Of the original 46 study subjects, 22 case patients and 17 matched controls were brought to Dallas, hospitalized in the General Clinical Research Center at Parkland for a 7-day research protocol. The following major findings resulted.

Reduced NAA by Magnetic Resonance Spectroscopy

The cases and controls were identically scanned in a 1.5 Tesla clinical magnet with a proton MR spectroscopy (MRS) protocol, which acquired chemical spectra in a 2x2x4 cm voxel in each basal ganglia and a 2x2x2 cm voxel in the pons (**Figure 5**).[22]



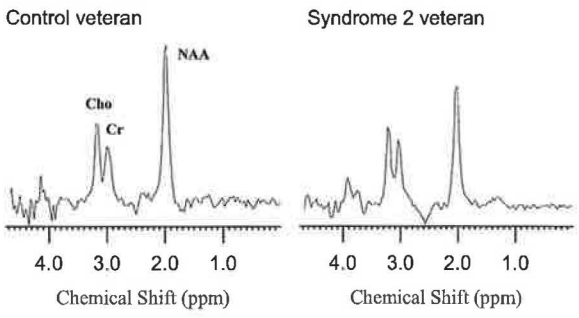


Figure 5. Chemical spectra from the right basal ganglia of a control and an ill veteran with syndrome 2.

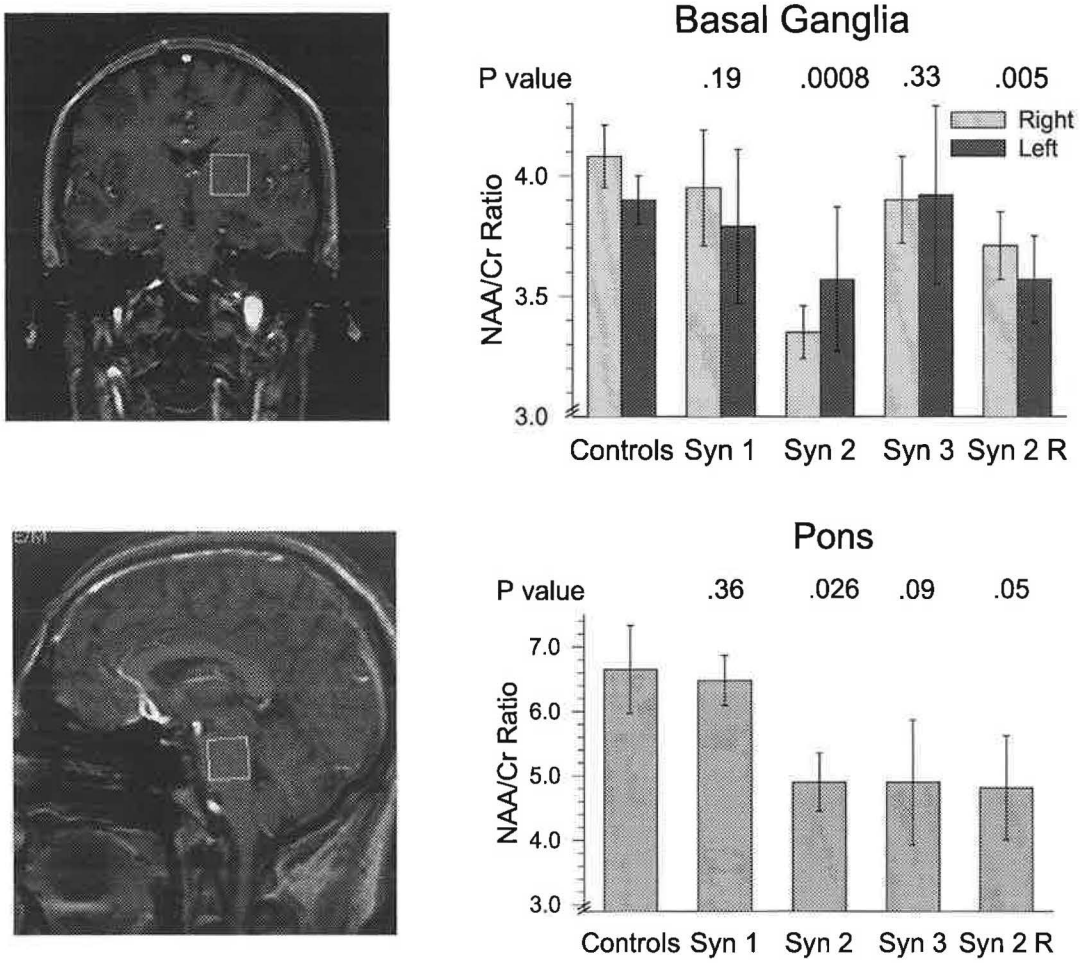


Figure 6. Comparison of mean NAA/Cr ratio among Seabees controls and syndrome 1-3 as well as a replication sample of syndrome 1 patients recruited at the Dallas VA Medical Center (Syn 2R). The top half shows the means for the left and right basal ganglia, and the bottom half shows those for the pons. The brain slices show the position of the voxels in which the spectra and metabolite ratios were derived.

Post-processing of the spectra on a SUN SPARC workstation estimated the concentrations of choline (Cho), creatine (Cr) and N-acetylaspartate (NAA). A statistical analysis compared the NAA/Cr ratio, an index of neuronal health, in the three syndrome groups and the control group. The NAA/Cr ratio was significantly lower in the syndrome 2 group compared with controls in both basal ganglia and the pons ($p = 0.009$, **Figure 6**). The NAA/Cr ratio was lower in the syndrome 1 group than controls in the basal ganglia only, but the difference was not significant ($p = 0.19$). It was lower in the syndrome 3 group than controls in the pons only ($p = 0.09$). These findings suggested that the three syndromes represent neuronal damage in different, though overlapping, anatomical distributions in deep brain structures.

Subsequently, Meyerhoff et al. of M. Weiner's research group at UCSF and the San Francisco VA Medical Center repeated the MR spectroscopy scanning protocol of the right basal ganglia in 11 Gulf War veterans resembling syndrome 2 and 11 civilian controls and found reduced NAA/Cr ratio in the syndrome 2 patients compared with controls ($p = 0.05$).[23] Recently, Menon et al. of the Jackson, Mississippi, VA Medical Center repeated the MRS protocol in 6 ill Gulf War veterans and 6 controls and found a similar difference in the hippocampus bilaterally[24]

and in the basal ganglia (personal communication, M.Menon, 2004). Weiner and colleagues are conducting an MR spectroscopy study of 400 ill and well Gulf War veterans.

Study of Brain Dopamine Turnover

While the subjects were hospitalized in the GCRC, they were provided a low stress environment and a high sodium, low tyrosine diet. On day 6 at 7:30 a.m., a venous blood sample was obtained, immediately cooled on ice, and sent for assay of homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenlyglycol (MHPG).[25] HVA is the major product of the metabolic degradation of brain dopamine. MHPG is a proportional co-metabolite of the breakdown of peripheral catecholamines to HVA, and the HVA/MHPG ratio has been shown to be a highly correlated index of brain dopamine turnover.

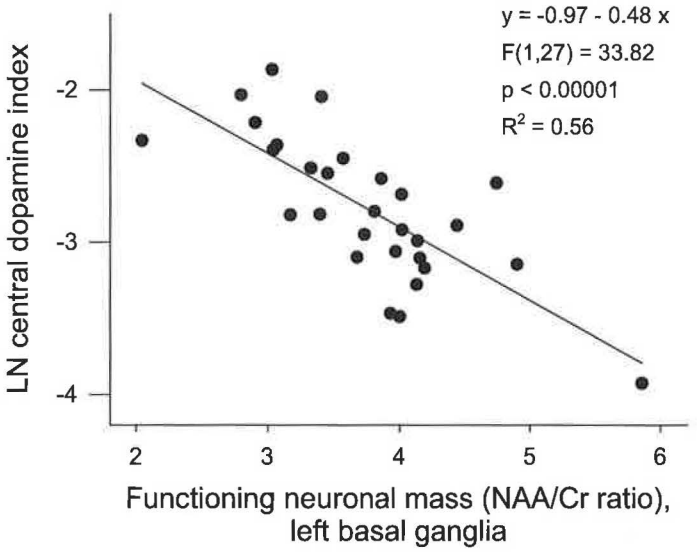


Figure 7. Excess central dopamine turnover in Gulf War syndrome 2 veterans and controls with reduced NAA/Cr ratio by MR spectroscopy.

In the syndrome 2 and control group together, the logarithm of the age-standardized HVA/MHPG ratio was inversely associated with functioning neuronal mass in the left basal ganglia ($R^2 = 0.56$, $F[1,27] = 33.82$, $p < 0.00001$) but not with that in the right ($R^2 = 0.04$, $F[1,26] = 1.09$, $p = 0.3$) (**Figure 7**). The association was not apparent in the syndrome 1 and 3 groups. Controlling for age, renal clearances of creatinine and weak organic anions, handedness, and smoking did not substantially alter the associations (**Table 3**).

Table 3. Multiple regression analysis predicting the central dopamine index* by functioning neuronal mass (NAA/Cr ratio)† in the left basal ganglia and potential confounding variables

Predictor variables	Adjusted significance F(1,22)	p
Functioning neuronal mass (NAA/Cr)†	25.95	<0.0001
Age (years)	5.67	0.03
Creatinine clearance (ml/min)	0.12	0.7
5-HIAA‡ concentration (μg/ml)	0.90	0.4
Left handed (1 = yes, 0 = no)	1.49	0.2
Smoker (1 = yes, 0 = no)	0.05	0.8

Model $R^2 = 0.63$, $F(6,22) = 6.18$, $p = 0.0006$
*Ratio of plasma homovanillic acid to 3-methoxy-4-hydroxyphenylglycol (HVA/MHPG ratio)
†Ratio of N-acetyl-aspartate to creatine (NAA/Cr ratio); determined by MR spectroscopy
‡5-hydroxy-3-indoleacetic acid

The degree of ill health of neurons in the left basal ganglia of these veterans with Gulf War syndrome 2 appears to have altered central dopamine production in a lateralized pattern, reflecting the lateralization of dopamine control found in ablation studies in rodent models. This

finding supports the theory that Gulf War Syndrome is a neurologic illness, in part related to injury to dopaminergic neurons in the basal ganglia.

Abnormality of Parasympathetic Control

Since many Gulf War veterans report chronic symptoms suggesting autonomic dysfunction, such as chronic diarrhea, dizziness, fatigue and sexual dysfunction, we performed a thorough investigation of the main functions of the autonomic nervous system. Twenty-two ill Gulf War veterans and 19 controls underwent measurement of circadian rhythm of heart rate variability by 24-hour Holter electrocardiography, ambulatory blood pressure recording, Valsalva ratio, sympathetic skin response, sweat imprint test, and polysomnography, under investigator blinding.

High frequency spectral power of heart rate variability increased normally 2.2-fold during sleep in the controls but only 1.2-fold in the ill veterans ($P < 0.0001$) (**Figure 8**). It was lower in the ill veterans than the controls at night ($P = 0.0006$), higher than the controls during the morning ($P = 0.009$), but no different the rest of the day ($P = 0.9$). Heart rate-corrected QT interval, which is influenced by parasympathetic tone, tended to be longer over the full 24 hours ($P = 0.07$), particularly at night ($P = 0.03$).

Compared with controls, mean heart rate of the ill veterans, measured by Holter, declined less at night ($P = 0.0002$, **Figure 9**). The blunted heart rate dip in the ill veterans was confirmed by analysis of data from both 24-hour automatic ambulatory blood pressure monitoring ($P = 0.05$) and polysomnography over 3 nights ($P = 0.03$, **Figure 9**).

These differences remained significant after controlling for potential confounders. Cases and controls were similar on measures of sympathetic adrenergic and sudomotor function, sleep architecture, respiratory function, and circadian variation in blood pressure and body temperature.

These findings suggest that some symptoms of ill Gulf War veterans may be due to subtle dysfunction of the control of parasympathetic nervous system activity.

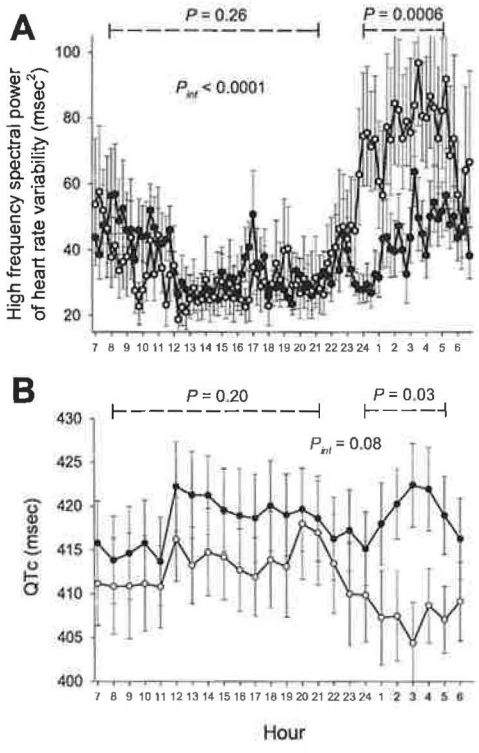


Figure 8. Circadian variation in parameters of autonomic regulation of the cardiovascular system, measured by 24-hour Holter monitoring. Shown are (A) high frequency spectral power of heart rate variability, and (B) heart rate-corrected QT interval (QTc) in 21 ill Gulf War veterans (solid circles) and 19 age-sex-education-matched control veterans (open circles). High frequency spectral power was measured in 5-minute epochs every 15 minutes over 24 hours, and QTc, in 5-minute epochs every hour. Error bars indicate one standard error of the mean. *P* values test the difference between ill veterans and controls during the day or at night in the repeated-measures mixed effects model, and the P_{int} values test the day-night by group interaction.

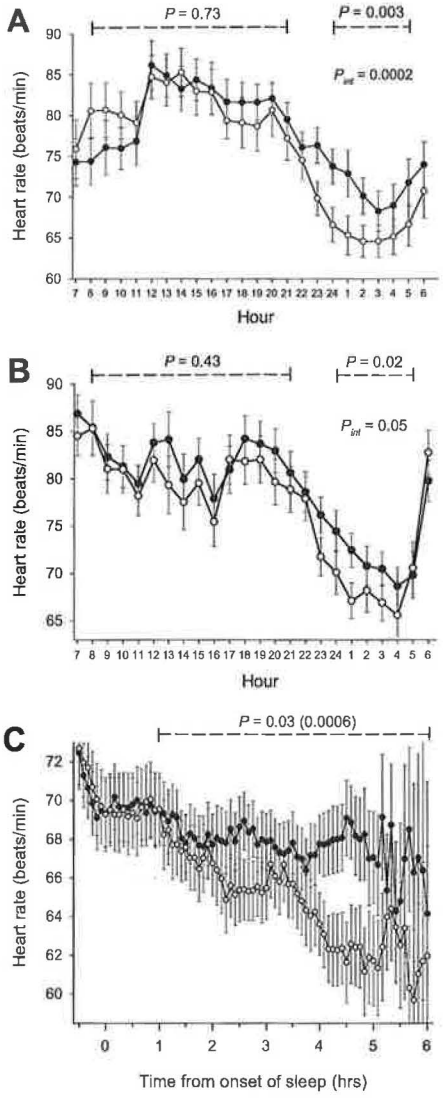


Figure 9. Circadian variation in mean heart rate measured (A) in 5-minute epochs every hour by Holter monitoring, (B) every 20 minutes during the day and every 60 minutes at night by 24-hour automated ambulatory blood pressure monitoring, and (C) in all 5-minute epochs from 30 minutes before to 6 hours after onset of sleep by polysomnographic recordings on three consecutive nights in a sleep study unit in 21 ill Gulf War veterans (solid circles) and 19 age-sex-education-matched control veterans (open circles). *P* values test the difference between ill veterans and controls during the day or at night in the repeated-measures mixed effects model, and the P_{int} values test the day-night by group interaction. The *P* value in parentheses is from the mixed effects model controlling for respiratory rate, $PSaO_2$, measures of sleep quality, and the other covariates. Transient increases in heart rate every 1-2 hours in the control group in (C) are compatible with effects of REM sleep reported in previous studies of normal subjects.

Excess Incidence of ALS in Young Gulf War Veterans

In the wake of publicity following the publication of our three papers in *JAMA* in January 1997, we became aware of cases of amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease) in Gulf War veterans in their 40s and younger. A brief networking and publicity campaign identified 21 Gulf War veterans with ALS, 17 below age 45—the flexion point of the age-specific incidence curve where ALS incidence starts increasing rapidly (**Figure 10**).[26]

Multiplying the age-specific incidence rate in each one-year age group of the U.S. population by the number of Gulf War veterans in that age group each year after the Gulf War yielded annual estimates of the number of Gulf War-associated ALS cases expected in the absence of any unusual clustering (line and confidence intervals in **Figure 11**). Superimposing this expected line on the number of Gulf War-associated cases observed in each post-war year suggested an increase above the expected.[26]

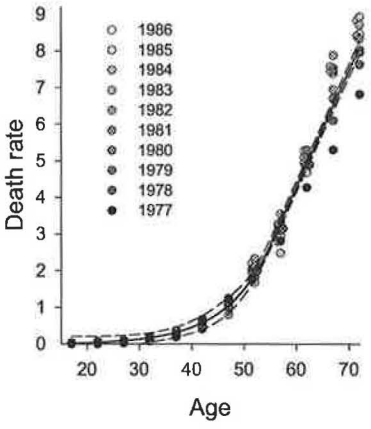


Figure 10. Age-specific incidence of ALS in the U.S. population from vital statistics.

Estimation of the standardized morbidity ratio (SMR) and its 95% confidence limits indicated that the increase in ALS cases in young Gulf War veterans above the expected was statistically significant in the period 1995-1998 (p =0.006) and in the single year 1998 (p = 0.02), and the increasing trend of the SMR from 1991 to 1998 was significant (p = 0.05, **Table 4**).[26]

Following this finding, the Department of Veterans Affairs undertook an independent study of the problem, which found a significantly higher rate of ALS in the Gulf War-deployed military population than in the non-deployed Gulf War-era military population.[27] Continuing surveillance found that the excess incidence has continued at least through 2003.

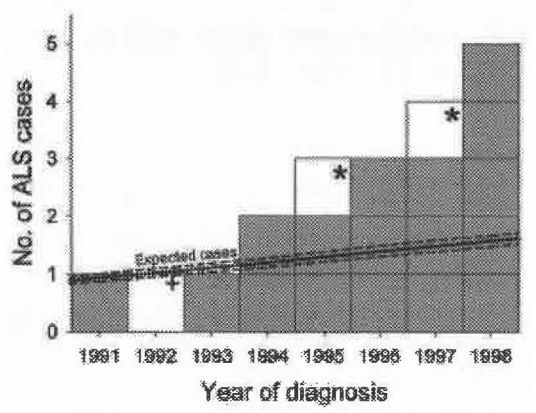


Figure 11. Number of definite ALS cases (shaded boxes) and possible cases (open boxes) and expected cases (solid line with 95% CI lines) in young (aged <45 years) Gulf War veterans.

Table 4. Standardized.morbidity ratio of numbers of new ALS cases observed compared with those expected in Gulf War veterans <45 years of age by year of diagnosis following the 1991 Gulf War (from Haley *Neurology* 2003;61:750-756).

Year of diagnosis	Observed ALS cases	Expected ALS cases		SMR (Ratio O:E)	p*
		Estimate	95% CI		
1991	1	0.93	0.87–1.00	1.07	0.6
1992	0	1.02	0.96–1.09	0.00	0.4
1993	1	1.10	1.03–1.18	0.91	0.7
1994	2	1.20	1.12–1.28	1.67	0.3
1995	2	1.29	1.21–1.38	1.55	0.4
1996	3	1.39	1.30–1.48	2.16	0.16
1997	3	1.48	1.38–1.58	2.03	0.19
1998	5	1.57	1.47–1.67	3.19	0.02
1991–1994	4	4.25	3.97–4.55	0.94	0.6
1995–1998	13	5.72	5.36–6.11	2.27	0.006

* Exact Poisson probability of finding the observed number of ALS cases or more, given the number expected.[31] The SMR increased monotonically from 1991 through 1998 (Poisson trend test, $\chi^2 = 3.715$, $df = 1$, $p = 0.05$).

SMR = standardized morbidity ratio; O = observed; E = expected.

Research on the Role of Low-Level Sarin in the Etiology of Gulf War Syndrome

Epidemiologic Study of Etiology

If our case definitions have identified real syndromes reflecting subtle brain injury, the next question would be what caused it. Now armed with a case definition,[3] we were prepared to analyze the questionnaire responses of the seabees sample on war-zone environmental exposures to see which, if any, were associated with the case definitions for any of our three syndromes. This is the standard approach to epidemic investigation that has solved the classic epidemics of the past half century.[2]

In analysis of cross-sectional survey data for risk factor associations, two potential biases of concern are type I sampling errors from multiple hypothesis tests (e.g., with 20 tests at the p=.05 level, one is expected to be significant purely by chance) and recall bias (e.g., sick people are more likely to recall exposures than well people).[28-31] To avoid these errors, we required a p value of ≤.005 to be considered statistically significant. To avoid recall bias, we hypothesized a priori that of the 19 risk factors tested, only those associated with organophosphate and related chemical exposures would be associated with the syndrome indicators, and the other risk factors,

which had been publicized equally in the press, would not be significantly associated.[4] Finding such a pattern would not be compatible with recall bias and would suggest etiologic associations. Moreover, as in all epidemiologic studies of etiology, the causal inference would be strengthened by finding large relative risks (usually greater than 3), monotonically increasing dose-response effects, and synergistic effects.[32]

Syndrome 1 ("impaired cognition") prevalence was 8 times more common in veterans who reported having worn pet flea-and-tick collars to repel insects during the war (**Table 5**).[4] Although the numbers of veterans in the subgroups were small, the risk increased with the likelihood that flea collars were worn in contact with the skin: 7 of 229 (3%) in those who never wore them, 3 of 17 (18%) in those who wore them but never next to skin, and 2 of 3 (67%) in those who sometimes wore them next to their skin (X^2 for trend, $p<.0001$).

The risk of syndrome 1 was also 6 times more common in veterans whose main job during the Gulf War involved security (**Table 5**). Security guards often had night guard duties that would have exposed them to ambient risks occurring at night.

Whereas 95% of the veterans in the survey reported having taken PB during the war, these associations were not modified by the number of PB tablets taken or by having experienced side effects from PB. Veterans who reported having entered an enemy bunker also had a significantly elevated risk of syndrome 1 (RR 5.4; 95% CI 1.8-16.0), but this effect did not remain significant after controlling for the flea collar and security job variables (analysis not shown).

Syndrome 2 ("confusion-ataxia") prevalence was 8 times more common in veterans who reported having experienced a likely chemical weapons attack and 4 times more common in those who were located in extreme northeastern Saudi Arabia (near Khafji) on 20 January 1991, the fourth day of the air war (**Table 5**). There was no evidence of increased risk associated with any geographical location around the second week in March 1991 that would implicate the CW ammunition demolition incident at Khamisiyah. Whereas the prevalence of syndrome 2 did not increase with the number of tablets of PB taken (X^2 for trend $p=.97$), it increased in a monotonic dose-related pattern with the scale of advanced side effects from PB (X^2 for trend $p<.0001$; **Table 5**).

Syndrome 2 was also significantly more common in those who reported an Iraqi artillery shell exploding within 5 km of their position (RR 4.9; 95% CI, 2.2-10.9) and in those who reported seeing the explosion of a suspected chemical land mine (RR, 5.6; 95% CI, 2.3-13.6), but these did not remain significant in an adjusted logistic regression analysis after controlling for perceived chemical weapons attack and presence in sector 7 on 20 January (analysis not shown).

There was a statistically significant synergistic interaction between self-reported perception of exposure to a likely chemical weapons attack and the scale of advanced side effects from PB, dichotomized at ≥5 (**Table 6**). The risk in those exposed to both risk factors was approximately 5 times greater than that expected if their effects were additive (Rothman's S=5.3; 95% CI, 1.04-26.7; Hogan's T=.31; 95% CI, .04-.57). The relative risk of exposure to both risk factors ($RR_{11} = 42.9$) was approximately 3 times greater than that expected if their effects were multiplicative ($RR_{10} \cdot RR_{01} = 14.6$; **Table 6**). Although the odds ratio (OR) of the interaction term in a saturated logistic regression analysis was not statistically significant (OR, 4.4; 95% CI, 0.18-107.3), the goodness of fit increased sequentially from the main effects model without interaction (goodness-of-fit $X^2=11.1$, df=8, p=.20), to the saturated interaction model (goodness-of-fit $X^2=6.5$, df=8, p=.59), and to the model with only the interaction term (goodness-of-fit $X^2=4.7$, df=8, p=.79).

Table 5. Associations of the 3 primary factor analysis-derived syndromes with self-reported exposures in the Kuwaiti Theater of Operations during the Gulf War (from Haley et al. *JAMA* 1997; 277: 231-237)

Syndrome, Abbreviated Description	No. of Veterans Affected by/At Risk for Syndrome	Prevalence Rate, %	Relative Risk (95% CI)*	P†
Syndrome 1 ("Impaired cognition")				
Wore pet flea-and-tick collars				
No	7/229	3	1.0	.001
Yes	5/20	25	8.2 (2.9-23.5)	
Main job involved security				
No	8/231	3	1.0	.007‡
Yes	4/18	22	6.4 (2.1-19.3)	
Syndrome 2 ("Confusion-ataxia")				
Experienced a likely chemical weapons attack				
No	3/141	2	1.0	<.0001
Yes	18/108	17	7.8 (2.3-25.9)	
Was located in sector 7 in northeastern Saudi Arabia on January 20, 1991§				
No	15/228	7	1.0	.004
Yes	6/21	29	4.3 (1.9-10.0)	
Scale of advanced adverse effects from pyridostigmine bromide				
0	0/27	0	0.0	<.0001‖
1-4	2/151	1	1.0	
5	4/36	11	8.4 (1.6-44.0)	
6	15/35	43	32.4 (7.8-135.0)	
Syndrome 3 ("Arthro-myo-neuropathy")				
Index of the amount of insect repellent typically applied to skin¶				
0-1	4/93	4	1.0	<.0001‖
2	6/87	7	1.6 (0.5-5.5)	
3	3/32	9	2.2 (0.5-9.2)	
4	3/19	16	3.7 (0.9-15.1)	
5	6/18	33	7.8 (2.4-24.7)	
Factor scale of advanced adverse effects from pyridostigmine bromide				
0	0/27	0	0.0	<.0001‖
1-3	5/115	4	1.0	
4	3/36	8	1.9 (0.5-7.6)	
5	8/36	22	5.1 (1.8-14.6)	
6	6/35	17	3.9 (1.3-12.1)	

*CI indicates confidence interval.
†*P* values were calculated for presentation in this table with the Fisher exact test or, where indicated, with the χ^2 test for trend using the risk factor variable's a priori categorization before categories were combined for presentation.
‡This association met our criterion for statistical significance ($P<.005$ in the unadjusted logistic regression analysis), and in the adjusted logistic regression analysis both flea collar and security job were significant ($P\leq.001$).
§Sector 7 was bounded on the north by the Kuwaiti border, on the east by the Persian Gulf coastline, on the south by the 48th parallel, and on the west by the 28th meridian, and contains the port city of Ra's al-Khafji. The relative risk was significantly elevated for those present in sector 7 between January 18 and January 23, 1991, but was greatest for those present there on January 20, 1991.
‖The χ^2 test for trend was used to calculate these *P* values.
¶Measured by the interaction of questions estimating the number of times per day repellent was typically applied and the amount typically applied each time (Table 2).

Syndrome 3 ("arthro-myo-neuropathy," late renamed "central pain") prevalence increased with the index of the amount of insect repellent veterans typically applied to their skin

(X^2 for trend p<.0001) and with the scale of advanced side effects from PB (X^2 for trend p<.0001; **Table 5**). In a multiple logistic regression analysis, the association of syndrome 3 with the 6-point index of the amount of repellent used held true for those who used government-issued repellent (adjusted OR, 1.54; 95% CI, 1.17-2.03; p=.002) but not for those who reported using *Off!*® (adjusted OR, 1.08; 95% CI, 0.79-1.46; p=.64) or Avon *Skin-So-Soft*® (adjusted OR, 0.87; 95% CI, 0.64-1.18; p=.37). Synergy between the effects of repellent use and PB side effects could not be assessed because there were too few participants in the off-diagonal cells for a powerful test.

Together the associations of these neurotoxicity risk factors, and the absence of other risk factor associations, suggest that each of our three syndromes is due to neurotoxic injury but with each syndrome caused by different combinations of chemical exposures. This conclusion ties the three different symptom constellations with different profiles of abnormalities on vestibular tests and with different chemical risk factors.

Table 6. Synergy between the effects of perceived exposure to chemical weapons attack and scale of advanced adverse effects from pyridostigmine bromide in predicting syndrome 2, the "confusion-ataxia" syndrome (from Haley et al. *JAMA* 1997; 277: 231-237)

Experienced a Likely Chemical Weapons Attack	Scale of Advanced Adverse Effects <5	Scale of Advanced Adverse Effects ≥5
No		
No. of veterans with syndrome 2/No. of veterans with the indicated combination of risk factors	1/114	2/27
Prevalence rate, %	0.9	7.4
Yes		
No. of veterans with syndrome 2/No. of veterans with the indicated combination of risk factors	1/64	17/44
Prevalence rate, %	1.6	38.6

*Synergy measured by a Rothman S of 5.2 (95% confidence interval [CI], 1.04-26.70) and a Hogan T of 0.31 (95% CI, 0.04-0.57).

Evidence of Chronic Effects of Low-Level Sarin Exposure in Humans

In the mid-1950s, Spiegelberg reported that personnel of the German Wehrmacht who had handled chemical nerve agents during World War II suffered from lowered vitality, reduced drive, and defective autonomic regulation 5 to 10 years after their last possible exposures.[33,34]

In 1974 Sidell reported persisting psychiatric symptoms in 2 workers of the Edgewood Arsenal accidentally overcome by sarin and soman, respectively.[35]

In 1979 Duffy et al. reported abnormal electroencephalographic (EEG) patterns in workers of a sarin manufacturing plant 1-5 years after they suffered accidental exposures compared with matched plant workers without exposures.[21] Burchfiel and Duffy experimentally produced the same EEG abnormalities by experimental administration of low-level sarin to primates.[20]

These findings are consistent with a scientific literature documenting the chronic neurologic and behavioral sequelae of exposure to certain organophosphate pesticides.[36-41]

Clinical research testing of subjects exposed to sarin in the 1995 terrorist attacks in the Tokyo subway and the Matsumoto housing complex has revealed the development of objective abnormalities of central, peipheral and autonomic nervous system function with severity in proportion to the acute sarin exposure (i.e., the degree of initial reduction in serum and red cell cholinesterase).[42] A man who died 15 months after the acute sarin exposure was found by neuropathologic examination to have evidence of distal sensory axonopathy in several peripheral nerves, indicating that sarin produces OPIDP at a much lower dose than suspected from prior studies.[43-45] Morever, although acutely exposed individuals were initially reported to be

neurologically asymptomatic 6-8 months after exposure, evidence of the delayed development of vestibulo-cerebellar ataxia (measured by platform posturography), autonomic dysfunction (by diminished heart rate variability), and other brain abnormalities (prolonged P300 wave on evoked potential measurements) were documented in comparison with matched control subjects.[42,46-50] These findings, uncorrelated with psychological sequelae such as PTSD, appear similar to those of the UT Southwestern seabees studies.[14,22,51]

Tests of Biological Plausibility in Animal Experiments

In 1993, Husain et al. reported that unprotected mice given 10 daily inhalation exposures to sarin at a low concentration not causing immediate cholinergic signs developed typical OPIDP with ataxia from histologically proven axonal degeneration of the spinal cord beginning 14 days after the first exposure.[52] Subsequently, they duplicated the finding in hens with 10 daily subcutaneous injections of 0.1 LD_{50} of sarin.[53]

To test the biological plausibility of the UT Southwestern epidemiologic findings in Gulf War veterans, Dr. Thomas Kurt of the UT Southwestern research team designed a series of laboratory studies that were carried out in collaboration with veterinary toxicologists at Kansas State University, Duke University and the U.S. Environmental Protection Agency.[54,55] The implicated chemicals, pyridostigmine bromide, chlorpyrifos (Dursban), permethrin, and diethyl toluamide (DEET), were administered daily for two months in doses approximating human exposure levels during the Gulf War to groups of hens alone and in all two-chemical and three-chemical combinations. The studies were designed to test the hypothesis that the chemicals would produce little or no long-term neurologic damage when administered alone but would act synergistically to produce long-term neurologic damage when given in combinations. Hens have long been the EPA-recommended animal in testing for the chronic effects of pesticides.

The results of the experiments confirmed the synergistic effects of the compounds (**Figure 12**). When administered alone, they produced no signs of chronic locomotor disturbance and no to minimal neuropathologic evidence of neuronal degeneration in the spinal cord and sciatic nerve (**Figure 13**). All two-chemical combinations, however, produced definite signs of chronic locomotor disturbance and moderate neuropathologic evidence of neuronal degeneration in the spinal cord and sciatic nerve, and the three-chemical combinations produced severe chronic effects. Since these experiments were reported, additional evidence of neurotoxicity and environmental persistence of chlorpyrifos (Dursban) has led to phasing out of this, the most widely used domestic pesticide, from most consumer uses.

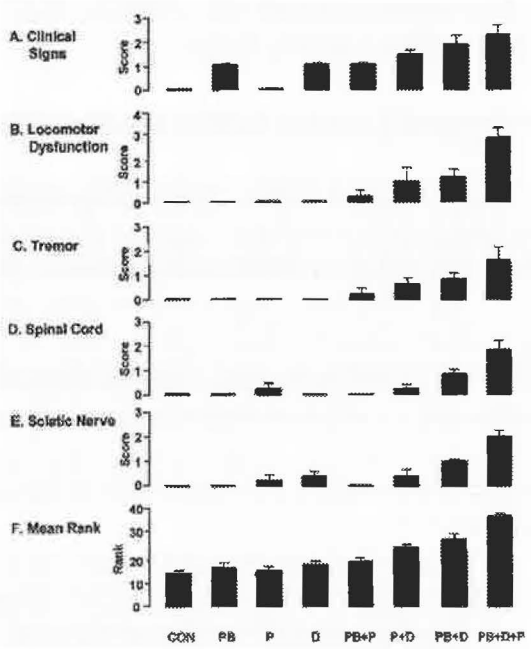


Figure 12. Severity of clinical signs, locomotor dysfunction, histopathological changes in spinal cord and sciatic nerve and mean rank following daily administration of pyridostigmine bromide (PB), DEET (D), and permethrin pesticide (P), either alone or in combination, to hens. CON is the control group. (From Abou-Donia et al. *Fundament Appl Toxicol* 1996; 34: 201-222.)

There is also considerable evidence that exposure to low-levels of the chemical nerve agent sarin can result in chronic neurologic injury. Although survivors of incapacitating, near fatal exposures to chemical nerve agents often suffer permanent brain and muscle damage from the effects of seizures, hypoxia and sustained cholinergic stimulation,[56] a basic question underlying our hypothesis is, can exposures to non-incapacitating concentrations of organophosphate chemical nerve agents cause permanent neurologic sequelae? At the time of the Gulf War, the body of scientific literature indicated that they do not.[43-45] Even though each of the main nerve agents--tabun (GA), sarin (GB), soman (GD) and VX--is known to bind to and inactivate neurotoxic esterase (NTE) and undergo "aging," Gordon et al.[43] and Willems et al.[44] found in pharmacologically protected hens that a single exposure does not inactivate a high enough proportion of NTE (>70%) to produce organophosphate-induced delayed polyneuropathy (OPIDP) unless the dose is far above the level that would prove lethal from the immediate effects of AChE inhibition (30-60 x LD_{50} for sarin, 100-150 x LD_{50} for soman, at unknown higher levels for tabun, and negligibly for VX). Despite a warning by Gordon et al. that pharmacologic protection of soldiers increases the likelihood that OPIDP from nerve agents will occur,[43] these findings formed the basis for the supposition that OPIDP could not have resulted from chemical nerve agents in the Gulf War in the absence of casualties from immediate cholinergic effects.

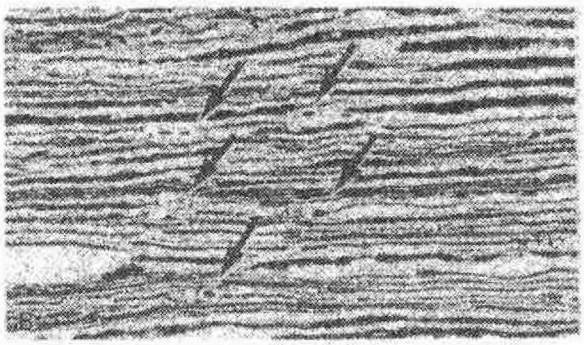


Figure 13. Photomicrographs of longitudinal sections through the lateral columns of the spinal cord (x100) from hens treated with pyridostigmine bromide, DEET and permethrin daily for six weeks. There are axonal varicosities and fragmented axons typical of organophosphate induced delayed neurotoxicity (from Abou-Donia et al. *Toxicol Environ Health* 1996; 48: 35-56)

In all, **16** animal studies have addressed the chronic effects following exposure to cholinesterase-inhibiting organophosphate chemicals (**Table 7**). This body of literature supports the biologic plausibility of the chronic neurologic damage resulting from low-level exposure to synergistic combinations of organophosphates and related chemicals. They further suggest that exposure to low levels of the nerve agent sarin may produce such a neurologic injury even without the synergistic effects of other chemicals.

The 2002 papers by Henderson et al.[63] and Kalra et al.[66] from the Lovelace Respiratory Research Laboratory at the University of New Mexico, funded by the U.S. Army Medical Research and Materiel Command, have proved particularly influential on the question of chronic effects of low-level sarin exposures. They definitively demonstrated the delayed onset of alteration in cholinesterase receptors in olfactory bulb, basal ganglia and other deep brain structures of rats exposed daily by inhalation for 5 or 10 days to low concentrations of sarin below the dose causing immediate pharmacologic effects. Rats sacrificed immediately after the completion of the sarin exposure period had no evidence of chronic brain changes, but those sacrificed 30 days later showed alteration in muscarinic M1 and M3 receptors as well as altered T cell function mediated by autonomic nervous system dysfunction (effects blocked by prior administration of a ganglionic blocker).

Table 7. Studies of chronic effects of low-dose sarin exposure in animal experiments

Study	Year	Animal model	Major findings
Burchfiel[20]	1976	monkey	Persistent effects on quantitative EEG
Husain[52]	1993	mouse	Delayed development of peripheral neuropathy and spinal cord lesions
Husain[53]	1995	hen	Delayed development of peripheral neuropathy
Jones[57]	2000	rat	Chronic reduction in nicotinic acetylcholine receptor binding in cerebral cortex.
Kassa[58]	2000	rat	Chronic alteration in immune function (lymphocyte proliferation, bactericidal activity of macrophages)
Kassa[59]	2000	rat	Persistent changes in DNA and protein metabolism in liver tissue
Kassa[60]	2001	rat	Subtle chronic signs of neurotoxicity and immunototicity with repeated exposures
Kassa[61]	2001	rat	Impaired spatial memory
Conn[62]	2002	rat	No persistent effects on reported indices of temperature regulation and motor activity
Henderson[63]	2002	rat	Delayed, persistent changes in cholinergic receptors in brain areas associated with memory loss and cognitive changes
Hulet[64]	2002	guinea pig	Persistent failure to habituate on functional test battery
Scremin[65]	2002	rat	Persistent increase in regional cerebral blood flow
Kalra[66]	2002	rat	Suppression of immune response (antibody-forming cells and T cell responses) mediated by the autonomic nervous system
Roberson[67]	2002	guinea pig	Chronic depression of AChE activity, persistent behavioral changes (disordered activity, increased rearing behavior)
Husain[68]	2003	mouse	Persistent reductions in respiratory exchange, blood AChE activity and BChE activity, NET activity in various tissues
Scremin[69]	2003	rat	Down-regulation of muscarinic receptors in hippocampus, decreased habituation
Kassa[70-72]	2003 2004 2004	mouse	Chronic alteration in immune function (increase in CD19 cells, CD4 cells, and mitogen-induced lymphoprolif-eration, increased NK cell activity)

From *2004 Report and Recommendations* of the VA Research Advisory Committee on Gulf War Veterans' Illnesses, found at www.va.gov/rac-gwvi.

Genetic Predisposition to Gulf War Syndrome

One of the most interesting questions about Gulf War syndrome has been why one person got sick when the person serving next to him did not. That is one of the major puzzles that encouraged attribution of the veterans' chronic symptoms to stress.

From our epidemiologic and laboratory findings, we hypothesized that environmental risk factors might be interacting with a genetically determined trait that put a subset of military

personnel at higher risk of developing chronic brain damage. Mammalian species are protected from organophosphate anticholinesterase poisons by at least two mechanisms[73-75]: first, butyrylcholinesterase(i.e., BChE, serum cholinesterase, pseudocholinesterase) binds and sequesters these poisons from neural tissue but does not destroy them; second, paraoxonase/arylesterase (PON1) destroys them by hydrolysis to harmless products that are excreted. BChE is inactivated in the process, but paraoxonase/arylesterase is not. Certain genetic variants of BChE (e.g., atypical [AA] or silent [SS]) result in abnormally low blood levels of the enzyme or forms of the enzyme that are less effective in binding organophosphates than the usual BChE (UU).[76] Of two common polymorphisms of the human PON1 gene, Arg or Gln at amino acid position 192, and Leu or Met at amino acid position 55, the former determines three genotypes (Q, QR and R) that explain the catalytic properties of two allozymes which hydrolyze organophosphates at different rates.[77,78]

The type Q allozyme, present in homozygous Q and heterozygous QR individuals, has higher hydrolytic activity against chemical nerve agents including sarin, soman and diazinon but lower activity against paraoxon, the metabolite of parathion.[78,79] In contrast, the type R allozyme in homozygous R and heterozygous QR individuals has the opposite hydrolytic affinities. The two allozymes, Q and R, have about equal activity as arylesterases with such other substrates as phenylacetate and chlorpyrifos-oxon, the metabolite of chlorpyrifos (Dursban). Within each of the PON1 Q/R genotypes, paraoxonase/ arylesterase activity varies many fold among different individuals.[73,80] Quantitative differences may predict susceptibility to acute toxicity in animals,[74,75,80-82] and dose-response curves for organophosphate toxicity are very steep,[81] suggesting that small differences in hydrolytic rates below a critical threshold could account for large differences in toxicity.[79] That the PON1 genotype might predispose to chronic neurodegenerative disease was recently supported by the finding of a higher prevalence rate of Parkinson's disease (odds ratio, 1.6) in people

Table 8. Association of threshold values of plasma allozyme activity and phenotypes of paraoxonase/arylesterase 1 (PON1) and butyrylcholinesterase (BChE) with chronic neurologic illness in 25 ill Gulf War veterans, including 12 with the more disabling syndrome 2, compared with 20 age-sex-education-matched well veteran controls (from Haley et al. *Toxicol Appl Pharmacol* 1999; 157: 227-233).

	Symptom complex 2 vs controls		All ill veterans vs controls	
Independent variable	Odds ratio (95% CI)[a]	*p*	Odds ratio (95% CI)	*p*
Quartiles of PON1 Type Q arylesterase activity				
Top three quarters	1.0		1.0	
Lowest quarter	9.00 (1.72–46.99)	0.009	4.5 (1.24–16.35)	0.02
Quartiles of BChE activity				
Top three quarters	1.0		1.0	
Lowest quarter	2.83 (0.51–15.77)	0.23	2.67 (0.60–11.80)	0.20
PON1 polymorphism at amino acid position 192				
Has no R allele[b]	1.0		1.0	
Has an R allele[c]	3.27 (0.73–14.55)	0.12	3.50 (1.01–12.18)	0.05
PON1 polymorphism at amino acid position 55				
Has no M allele[d]	1.0		1.0	
Has an M allele[e]	0.67 (0.16–2.82)	0.58	0.85 (0.26–2.80)	0.79
BChE phenotype				
UU	1.0		1.0	
AU	3.80 (0.31–47.21)	0.31	1.65 (0.14–19.65)	0.69

[a] Odds ratio and its 95% confidence interval were estimated by logistic regression analysis.
[b] Homozygous Q.
[c] Either heterozygous QR or homozygous R.
[d] Homozygous L.
[e] Either heterozygous LM or homozygous M.

who have the R allele (homozygous R or heterozygous QR) of the PON1 gene than in those who do not (homozygous Q).[83]

We therefore collected blood samples from the seabees who were participating in our clinical case-control study and performed tests to determine the BChE and PON1 genotypes and measured the enzymatic activity levels of the type Q and type R allozymes. Assays were performed by Dr. Bert La Du at the University of Michigan Medical School.

We found that the veterans' health status (case or control group) was significantly associated with their PON1 polymorphism at amino acid position 192: ill veterans were more likely than well controls to have the R allele (QR heterozygotes or R homozygotes) (**Table 8**). Since only 9% of Caucasian populations have the homozygous R genotype,[83] we had too few in this sample to evaluate it separately.

Within a given Q/R phenotypic group, ill veterans tended to have lower arylesterase activity than well controls (**Figure 14, graph a**), suggesting that enzyme activity levels are important over and above the genotype.

To pursue this possibility further, we compared total paraoxonase activity, total arylesterase activity, type Q arylesterase activity, type R arylesterase activity, and BChE activity in cases versus controls. Whereas total arylesterase activity tended to be lower in the ill veterans

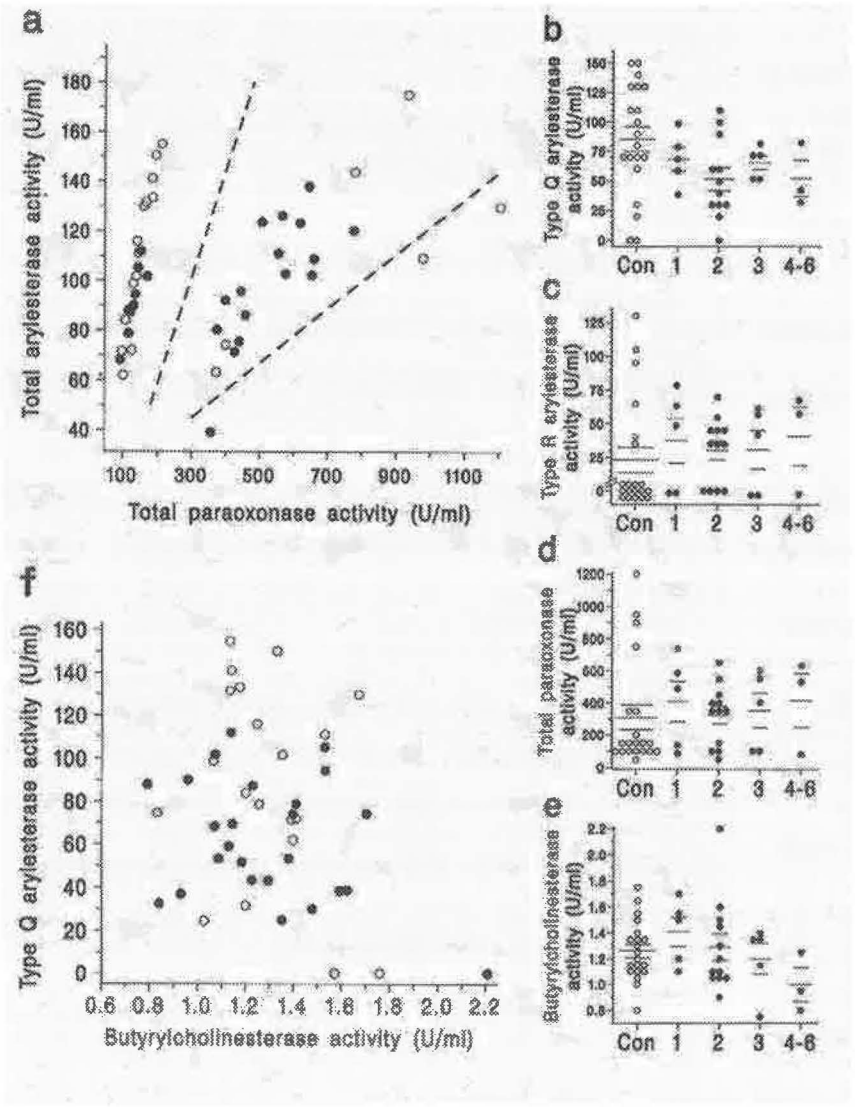


Figure 14. Distribution of ill veterans (solid circles) and well controls (open circles) by measures of paraoxonase/ arylesterase 1 (PON1) and butyrylcholinesterase (BChE) genotypes and plasma allozyme activity. In **a**, plotting cases and controls by total paraoxonase activity and total arylesterase activity identifies the three PON1 phenotypes separated by dashed lines: homozygous Q (leftmost), heterozygous QR (middle), and homozygous R (rightmost). In **b-e**, Con indicates the control group, followed by numbers indicating the Haley et al. Gulf War-associated neurologic syndromes 1-6. In **f**, a dot in the plot symbol marks one of the 10 controls who were not deployed to the Kuwaiti Theater of Operations during the Gulf War and thus were not exposed to the same environmental conditions. (From Haley et al. *Toxicol Appl Pharmacol* 1999; 157: 227-233)

(97±4) than in the well controls (113±7, p=.08 by t test), total paraoxonase activity tended paradoxically to be higher in the ill veterans (mean 384±34) than in the controls (mean 336±76, p=.6, **Figure 14, graph d**), and mean BChE activity did not differ significantly between ill veterans (1.29±0.06) and controls (1.29±0.05, **Figure 14, graph e**).

The type Q arylesterase activity, however, was significantly lower in the ill veterans (mean 62±6) than in the well controls (mean 88±11, **Figure 14, graph b**), and this difference was most pronounced for our syndromes 2 (mean 56±10) and 4-6 (mean 55±18), the most severely impaired groups clinically, than for syndromes 1 (mean 72±11) and 3 (mean 69±7). In contrast, type R arylesterase activity tended paradoxically to be higher in the ill veterans (mean 35±6) than in the well controls (mean 24±10, p=.34, **Figure 14, graph c**).

Plotting all subjects by their levels of PON1 type Q arylesterase activity and BChE activity demonstrated a strong association between illness and having a low plasma level of type Q arylesterase activity (**Figure 14, graph f**). There was a possible contribution from low levels of BChE activity as well (**Figure 14, graph f**). Dichotomizing the distributions at the lowest quartile of the control group to model a threshold effect, we found that being in the lowest quarter of expected type Q arylesterase activity was the strongest predictor of illness (**Table 8**). This association was strongest with syndrome 2 (**Table 8**), the condition previously demonstrated to have the highest rate of occupational disability,[3] the most severe neurologic impairment,[14] and the strongest epidemiologic associations with risk factors of wartime environmental chemical exposure.[4] Being in the lowest quarter of BChE activity also predicted illness, but the difference was not statistically significant (**Table 8**).

The PON1 Q192R polymorphism provides a potentially important clue to the pathogenesis and etiology of the chronic neurologic damage that appears to underlie the Gulf War syndrome. Not only does it help explain why certain personnel became ill while others did not, but it also links the illness to a certain set of chemicals, namely, those for which the PON-Q allozyme has high hydrolytic activity.

Future Directions of Research

To date, most of the findings pointing to brain cell damage as the basis for the symptoms of Gulf War syndrome variants have been obtained in small clinical case-control studies. Whereas the replication of the findings at different research institutions increase confidence in the findings, replication in larger population samples is now required. Weiner's group at UCSF is in the middle of a 5-year replication in 400 healthcare-seeking veterans at the San Francisco VA, and our group has DoD funding and all the necessary approvals to begin a replication study in national random samples of the deployed and nondeployed Gulf War-era military populations.

Our national replication survey is designed to estimate the relative prevalence of our case-definition of Gulf War syndrome variants and then to bring representative subsamples of ill veterans meeting the case definition and matched controls to our GCRC for study of objective biomarkers, including advanced brain imaging, neurophysiologic tests, and paraoxonase assays. In parallel, we have established an advanced brain imaging center with a Siemens 3 Tesla clinical magnet to develop and validate new high field MR spectroscopy, functional MRI, diffusion-weighted tractography routines for probing deep brain structures of concern in Gulf War syndrome variants. Clinical neuroscientists from universities throughout the Dallas area are collaborating in this methodologic development and validation project. In addition, animal models are needed to understand mechanisms upon which to base eventual treatment.

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