Parkland Memorial Hospital/UT Southwestern Medical Center

MEDICAL GRAND ROUNDS

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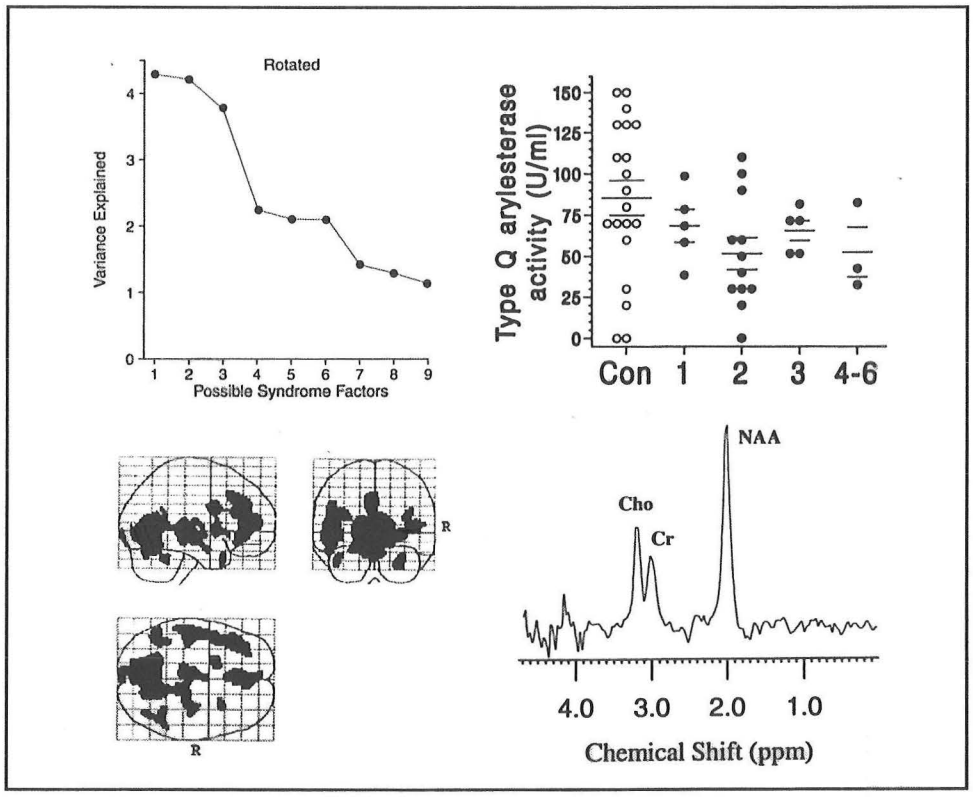
Gulf War Syndrome: Stress or Neurotoxic Brain Damage?



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BACKGROUND

In August 1990 Iraq invaded Kuwait. Between August and December the U.S. military's Operation Desert Shield involved the most complex movement of U.S. forces in history, second in size only to those around the Battle of the Bulge in World War II. Operation Desert Storm, launched on January 16, 1991, consisted of a 5 week bombing campaign and a 4 day ground war, February 24-28, ending in decisive victory with few U.S. casualties.[1] Punctuating two decades of negative public perception from the Vietnam War, Operation Desert Shield/Desert Storm, or the Gulf War, soon became known as "The Perfect War."

In all, approximately 697,000 U.S. military personnel, along with approximately 100,000 troops from Britain, France, Australia, Saudi Arabia, Kuwait, Syria and other countries, were deployed to the Kuwaiti Theater of Operations. In contrast to prior wars, the deployed U.S. force comprised 10% Reserves and 6% National Guard as well as 7% women, and the mean age was around 30 years.[1]

Despite its relative brevity, the Gulf War exceeded the Vietnam War in the variety of potentially toxic environmental exposures. These included low-level chemical warfare agents in fallout from our bombing, massive spraying of pesticides, uniforms impregnated with pesticides, unauthorized wearing of pesticide-containing flea collars, copious use of highly concentrated DEET insect repellant, near universal ingestion of pyridostigmine bromide (Mestinon 30 mg TID) tablets as a prophylactic nerve gas antidote, antimicrobial prophylaxis including ciprofloxacin for anthrax warfare and chloroquine for malaria, multiple immunizations including anthrax and botulinum toxoids, dense smoke from oil well fires, petroleum fumes, carbon monoxide from burning jet fuels inside tents, petroleum in drinking water, mildly radioactive dust from exploded depleted uranium munitions, CARC (chemical absorption-resistant coating) paint being sprayed on combat vehicles, and brief combat stress for the small numbers of actual combatants.[1-4]

As the ground war ended and troops were air-lifted home, large numbers began complaining of a unique constellation of symptoms consisting of various combinations of the following: unusual fatigue; difficulty concentrating; pain like a toothache in neck, shoulders, arms, lower back, and hips without signs of arthritis; watery diarrhea; hot flashes, night sweats, and a drop in usual body temperature; insomnia; skin rashes; emotional lability with difficulty controlling anger; sensory changes such as paresthesias or numbness of the extremities; and intolerance of common chemical odors.[1,2,4] Several surveys have confirmed that the prevalence of these and other symptoms is 2-3 times higher in Gulf War veterans than in military personnel who were not deployed to the war.[5-7]

Early investigations approached the problem as a possible epidemic of an occult infectious disease. Descriptive surveys of several affected military units confirmed high attack rates of the symptom constellation (Berg, S.W., unpublished reports). Clinical investigations of ill veterans at Walter Reed Army Medical Center identified 29 cases of leishmaniasis (17 cutaneous and 12 viscerotrophic) caused by *L. tropica*.[8,9] By mid-1993 planning for a CDC-style epidemic investigation began within the U.S. Department of Defense (DoD). Dr. Jay P. Sanford was commissioned to examine ill veterans and review medical records to develop a case definition of Gulf War syndrome for use in a large case-control study. In January 1994 the Sanford case definition was finalized, but growing opposition to an epidemic investigation ended the effort (personal communication from Dr. Sanford, 1994). Instead, the problem was referred to a series of blue ribbon committees that catalogued lists of symptoms and environmental exposures in official reports,[1,2,4] and DoD and the Department of Veterans Affairs' Central Office

(VACO) established a centralized board, the Persian Gulf Veterans Coordinating Board (PGVCB), to oversee further investigation and research.[10,11] The approach shifted from epidemic investigation by case-control studies of case-definitions to large-scale clinical examinations of ill volunteers and analysis of existing computerized population databases.[12-14] Disease registries were set up to list the complaining Gulf War veterans, and protocols prescribing a standardized clinical evaluation were established in both VA (Persian Gulf Veterans Protocol Examination)[15] and DoD (Comprehensive Clinical Evaluation Program, CCEP).[16,17]

From 1994 through 1997 several theories on the etiology of the Gulf War syndrome arose, some supported by empirical research and others not. Unsupported theories included infection by *Mycoplasma fermentans* (strain incognitus);[18-20] alleged use of vaccines containing the non-FDA approved adjuvant squalene;[21] and heavy metal poisoning from inhaled dust containing depleted uranium from exploded munitions.[1,2,4] The PGVCB recently initiated an $8 million collaborative study to test for *M. fermentans* in ill and well veterans and perform a clinical trial of empirical doxycycline therapy for the presumed chronic infection.

At present the prevailing view of the Departments of Defense and Veterans Affairs, however, is that the veterans' symptoms do not constitute a single new disease entity but instead a wide array of well known diseases and that the excess prevalence of symptoms in Gulf War veterans was caused by combat stress and the stress of deployment to a war theater (the Stress Theory).[17] The primary competing theory is that a sizeable subgroup of complaining veterans have a unique syndrome due to damage to deep brain structures caused by the delayed effects of exposures to combinations of neurotoxic chemicals (the Neurotoxicity Theory).[22-24] The evidence and arguments for and against these two theories are summarized below.

THE STRESS THEORY

The PTSD Argument

PRO. Following the Vietnam War, large numbers of U.S. servicemen who had experienced prolonged, intense combat developed a chronic psychiatric condition involving attacks of anxiety and physical symptoms that became known as post-traumatic stress disorder (PTSD).[25] In response Congress established a series of National Centers for PTSD to study the problem.

When the Gulf War deployment began, military planners anticipated intense ground combat with casualty rates as high as 20% of the combatant force.[1] To manage the expected large numbers of cases of PTSD, the national centers for PTSD planned large-scale surveys of returning troops for PTSD. When the conflict turned out to be brief with little close-hand combat and few casualties, the expectation of PTSD was greatly reduced. As the troops returned home, however, the planned PTSD surveys were carried out anyway. Between 1992 and 1996 nineteen papers were published in scientific journals reporting prevalence rates of PTSD averaging 9% (range 0% to 36%), and rates were higher in women veterans and in those who reported exposure to more war-zone stress.[26-44] These findings led to the conclusion that the physical symptoms comprising the Gulf War syndrome were due to PTSD or perhaps to lesser grades of "PTSD-related symptoms," or the Stress Theory. In late 1995 the Executive Branch formed the Presidential Advisory Committee on Gulf War Veterans Illnesses (PAC) which issued a report concluding that PTSD and the effects of general life stress from deployment to a war zone were important contributors to veterans' physical symptoms.[45,46]

CON. In late 1997 Haley published a technical commentary arguing that the apparent occurrence of PTSD was due entirely to errors of measurement.[47] The crux of this counter-

argument was that all prevalence rates of PTSD in Gulf War veteran populations were measured solely with self-administered screening questionnaires from which psychometric PTSD scales, such as the Mississippi PTSD Scale (M-PTSD)[48] and the Mississippi PTSD Scale–Modified for Desert Storm (M-PTSD-DS)*,[31] were calculated, but few followup structured interviews for PTSD were conducted by psychiatrists or psychologists to verify the PTSD diagnoses.[49] This was a strategic error because psychometric PTSD scales were developed for *screening* only to reduce the numbers of patients who need a full psychiatric evaluation, but they were never intended to make a diagnosis, which can only be done by psychiatric interview.[50,51] Cutpoints for screening scales are set to maximize sensitivity at the expense of low specificity, to avoid overlooking patients with PTSD but relying on followup psychiatric interviews to weed out the many false positive diagnoses.

The implications of the error can be estimated quantitatively from the published values of the sensitivity (U) and specificity (V) of the various psychometric screening tests used.[47] With Keane's original cutpoint of 107 (or 109) on the M-PTSD, U=0.83 to 0.93 and V=0.83 to 0.89. With Kulka's cutpoint of 89, U=0.77 to 0.94 and V=0.80 to 0.83. All other psychometric screening measures have far lower specificity. For example, for the Impact of Event Scale (IES), U=0.92 and V=0.66, and for the Keene-Fairbank subscale of the MMPI, U=0.90 and V=0.69.

The relative impacts of imperfect sensitivity and specificity on measured prevalence rates of a disease is given by

$$\hat{p} = pU + (1-p)(1-V) \qquad 1$$

where $\hat{p}$ is the observed prevalence rate of PTSD, p the true prevalence rate, U the sensitivity, and V the specificity, all measured on a scale of 0 to 1.[52] For most diseases and populations where prevalence rates are low (far less than 50 percent), imperfect specificity, which influences the far larger quantity 1-p, has a much greater biasing impact on the observed prevalence rate than the same value of sensitivity, which influences the far smaller quantity p.

Rearranging equation 1 to estimate the true prevalence rate as a function of the observed prevalence rate and the sensitivity and specificity of the scale gives

$$\hat{p}_c = [\hat{p}_u - (1-V)] / (U + V - 1) \qquad 2$$

where $\hat{p}_c$ is the observed prevalence rate of PTSD corrected for known values of sensitivity and specificity, and $\hat{p}_u$ is the original observed value uncorrected for errors in measurement .[52]

In the seven studies reporting PTSD prevalence rates, 20 of the reported rates were calculated from validated psychometric PTSD scales.[47] After using equation 2 to correct for the published values of sensitivity and specificity of the tests,[47] the estimated true prevalence rates were 0 percent for 18 of the 20 rates (Table 1). This suggests that virtually all of the PTSD

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*In 1992 the National Center for PTSD published a new version of the M-PTSD scale modified for Desert Storm veterans (M-PTSD-DS).[31] The changes involved altering the wording of questions to refer to traumatic situations specific to Operation Desert Shield/Desert Storm, adding three new 5-point questions, and making the wording applicable to both genders.[31] The addition of 15 points from the new questions changed the range of the scale from 35-175 for the M-PTSD to 50-190 for the M-PTSD-DS; however, validation studies were not repeated, and cutpoints for defining PTSD were not uniformly moved up. Sutker et al. estimated that the change would cause a positive bias of eight points on the M-PTSD-DS scale, thus further reducing its specificity.[30,41]

reported in Gulf War veterans was due to false positive errors of measurement and that the true prevalence rates of PTSD were too low to be distinguished from zero.

There were two possible exceptions. Sutker et al. found a PTSD prevalence rate on the M-PTSD-DS of 19 percent (corrected rate 0 percent) in 215 veterans referred by unspecified criteria from five military units (containing possibly as many as 3,000 veterans) for study four to 10 months after returning from the war.[41] In a subgroup of 110 veterans exposed to high war-zone stress measured by a self-report combat questionnaire administered at the same time as the M-PTSD-DS, the PTSD rate was 36 percent (corrected rate 22 percent).

Perconte et al. identified five cases of traumatic stress-related illness in 20 members of the 14th Quartermaster unit present at the bombing of their barracks and tested within a month of the traumatic event.[42] Only one-third of the unit's members volunteered to be tested; the psychometric testing followed a week of educational seminars covering the causes and symptoms of PTSD; and the symptoms substantially diminished after a month of group psychotherapy.

Seven studies reported 16 mean PTSD scores measured by the M-PTSD or M-PTSD-DS.[47] Analysis of these scores suggested that the number of PTSD-related symptoms was higher in women and in veterans who reported exposure to more war-zone stress. The studies, however, differed on whether higher mean scale values were found in veterans deployed to the war zone than in non-deployed veterans and whether scores increased over time after return from the war. The two highest mean PTSD scores for Gulf War veterans (**Figure 1**) were measured with the M-PTSD-DS scale, on which scores may be inflated by as much as eight points.[30,41] Most importantly, the distribution of the 16 mean PTSD scores from studies of Gulf War veterans was entirely in the range found by Keane et al. to be typical of the nonspecific symptoms of well-adjusted Vietnam veterans (50 to 89) and were far below the range of scores (120 to 140) in Vietnam veterans with psychiatrically confirmed PTSD.

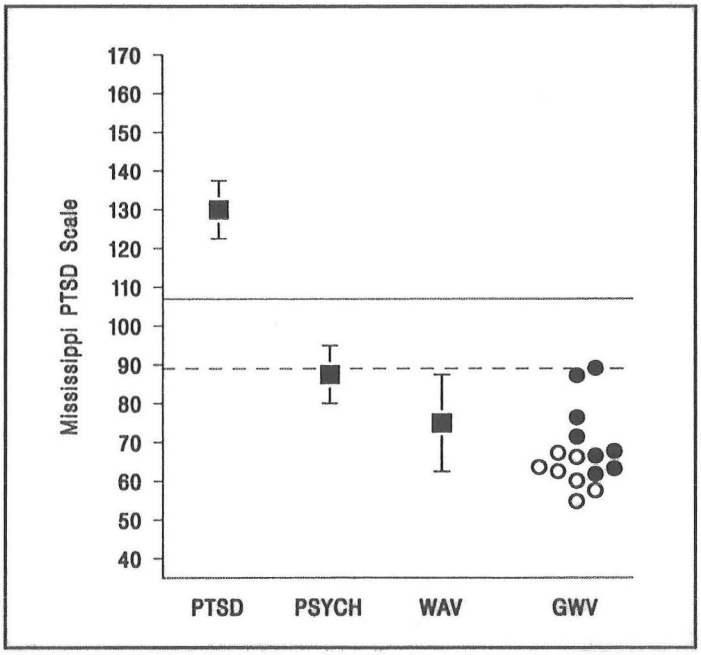

Figure 1. Mean scores (square markers) and standard deviations for the Mississippi PTSD Scale in Vietnam veterans with interview-confirmed PTSD ("PTSD"), Vietnam veterans with other psychiatric conditions ("PSYCH") and in well adjusted Vietnam veterans ("WAV"), compared with mean scores of Gulf War veterans with low (open circles) and high (solid circles) combat exposure. (From Haley, *Am. J. Epidemiol.* 1997; 146: 695-703)

Finally, two studies have performed direct analyses of the association between the intensity of combat exposure and chronic postwar physical symptoms (Gulf War syndrome).[6,53] Both found no statistical association, thus directly refuting the role of combat stress in the etiology of veterans' chronic physical symptoms.

The Argument from No Ill Effects

PRO. In 1996 and 1997 the *New England Journal of Medicine* published three articles by

U.S. government investigators examining the possibility of health effects of the Persian Gulf War.[12-14] The first (*the mortality study*)[12] compared the rates of postwar deaths, ascertained from Department of Veterans Affairs and Social Security Administration death claims, in all of the 695,516 active duty and reserve personnel deployed to the war zone and in a random sample of approximately half (746,291) of all nondeployed active duty and reserve personnel who were in the U.S. armed forces during the war period. The second (*the hospitalization study*)[13] compared rates of postwar hospitalization in all 579,931 active duty personnel deployed to the war zone and a similar random sample of approximately half (700,000) of the personnel on active duty on September 30, 1990, but not deployed to the war zone. The third *(the birth defects study)*[14] compared the rates of birth defects in offspring of veterans included in the two samples used in the second study. Whereas virtually all deaths were equally ascertained in both comparative populations for the first study, records of hospitalizations, births and birth defects for the second and third studies were obtained only from military hospitals serving personnel remaining on active duty; hospital records of personnel who separated from active duty during the follow-up period and were treated in nonmilitary hospitals were excluded.

From the results of the mortality study the authors concluded that deployed veterans had a higher rate of postwar death from motor vehicle accidents compared with the nondeployed sample, which they speculated was due to psychological adjustment problems, but no higher rate of death from natural causes, suicide or homicide. From the other two studies, they concluded that the deployed group of active duty veterans had no higher rates of hospitalization (**Figure 2**) and birth defects than the nondeployed sample. These conclusions have been cited as reassurance to veterans and the public that Gulf War veterans are suffering only reactions to psychological stress but not from physical illnesses contracted in the war.[45,46]

CON: To illustrate the problems with these arguments, consider a graph presented in the hospitalization paper depicting the odds ratio of the prevalence of hospitalization in the deployed military population compared with that in the nondeployed military population who were in the service on September 30, 1990 (Figure 2).[13] In this graph,

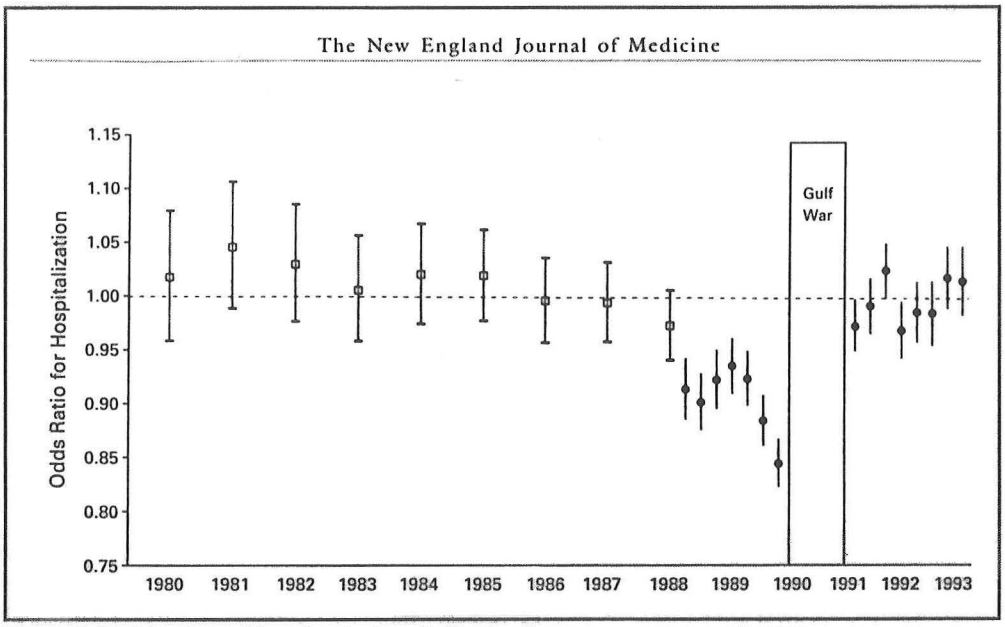

Figure 2. Multivariate odds ratios for hospitalization for any cause among Gulf War veterans as compared with other veterans before and after the war.
Data for the seven successive three-month periods from November 1988 through July 1990 and the eight three-month periods from August 1991 through July 1993 (solid circles) are for Army, Navy, Marine Corps, and Air Force personnel. Calendar-year data from 1980 through 1988 (open squares) are for Navy and Marine Corps personnel studied similarly. Odds ratios were adjusted for sex, age, race or ethnic group, branch of service, marital status, rank, length of service, salary, and occupation. Vertical lines indicate 95 percent confidence intervals (from Gray et al. NEJM 1996; 335:1505-1513).

values above 1.0 denote excess hospitalization rates in the deployed, and a decrease below 1.0, excess hospitalization rates in the nondeployed. They observed that the hospitalization odds ratio decreased below 1.0 (excess rate in the nondeployed) in the two years before the Gulf War, but it returned to around 1.0 in the three years after the war, similar to where it had been in the decade before 1989. From these data they concluded that deployed Gulf War veterans experienced no increase in conditions that caused increased post-war hospitalization.[13]

In Haley's commentary[54] he criticized the construction of the graph and the authors' interpretation as follows. First, the part of the graph from 1980 to 1988 portrays a biased picture of the hospitalization odds ratio in those remote years and must be excluded from consideration. This is due to the fact that soldiers hospitalized for chronic illnesses in the remote years 1980-1988 would have gradually been discharged from military service as unfit for duty and would not have been on active duty on September 30, 1990, the date that defined eligibility to be selected into the nondeployed group. Consequently, differences in chronic disease rates between Gulf War-deployed and -nondeployed populations cannot be measured for those early years from the available data and must be removed from consideration. In addition, these early year comparisons were calculated only for Navy and Marine personnel (only one-third of total personnel), and their lack of comparability is indicated by the discontinuity of the lines at the last quarter of 1988.

Examination of the graph redrawn by Haley[54] without the early years and appropriately annotated (**Figure 3**) reveals another, more serious, selection bias. To understand this problem, think of what kind of soldiers we send to a war zone – only healthy soldiers! We do not send military personnel with chronic diseases, such as acquired immuno-deficiency syndrome (AIDS), cancer, diabetes, angina, etc. And yet, in so large a population as the U.S. military, such conditions obviously occur continuously, do not result in immediate dismissal from the service as long as personnel can go to work and perform a job, and yet they prevent soldiers from being deployed to a war zone. In an epidemiologic analysis, then, all military personnel with chronic diseases would be concentrated in the nondeployed population, giving it an excess risk of hospitalization both before the war (as seen in Figure 3) and after the war. Therefore, the expected hospitalization odds ratios in the three years after the war should be at the same level, or lower, than those in the two pre-war years (dashed line in Figure 3). Instead, the observed odds ratios in the post-war years were higher than that, around 1.0. The distance from the dashed line to the median post-war value (approximately 1.0) measures the excess in

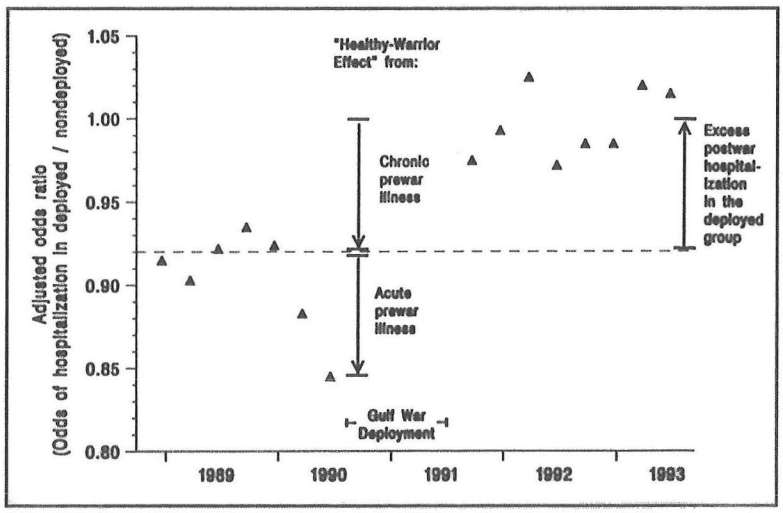

Figure 3. Haley's redrawn version of the Gray et al. graph (see Figure 2 above). Odds ratios >1.0 indicate that the rate of hospitalization in the deployed exceeded that in the nondeployed; odds ratios <1.0 indicate that the rate in the deployed was less than that in the nondeployed. Data from 1980 through 1988, shown in the original graph, were excluded because they would not validly estimate the selection bias from the "healthy-warrior effect" and were based on unrepresentative samples. Vertical arrows indicate the magnitude of biases due to the "healthy-warrior effect" from chronic and acute diseases and of excess postwar illness in the deployed group. (From Haley. *Am. J. Epidemiol.* 1998; 148: 315-323.)

hospitalization rates in the deployed population due to illnesses attributable to the war.

What is the explanation for the finding that the hospitalization odds ratio precipitously declined in the last two quarters before the wartime deployment below the plateau around 0.92 where it had remained constant for the prior 5 quarters (Figure 3)?[54] Whereas the drop in the hospitalization odd ratio to 0.92 was due to excess hospitalization from chronic illnesses in the nondeployed, the further drop from 0.92 to 0.85 was due to excess hospitalization for acute illness in the nondeployed (Figure 3). Acute illnesses requiring hospitalization would not prevent a soldier from being deployed to the war zone if the acute condition was cured by hospital care 7 to 21 months before the deployment; whereas, some of those occurring within 6 months would prevent deployment, and most occurring within 3 months would. Of the reasons for hospitalization 7-21 months before the war, only those related to chronic disease would have prevented deployment, and those would be expected to have caused excess postwar hospitalization. Consequently, the hospitalization odds ratio of 0.92 (the mean of odds ratios occurring between 7 and 21 months before deployment began) is the best estimate of the "healthy-warrior effect" due to prewar chronic illness and the best estimate of the expected postwar hospitalization odds ratio under the null hypothesis that the deployed group experienced no excess illness caused by the war (figure 3).

The fact that the expected post-war hospitalization odds ratio is measured by the pre-war odds ratio is a type of selection bias which Haley named the "healthy-warrior effect."[54] By analogy to the "healthy-worker effect" in occupational epidemiology,[55-57] the "healthy-warrior effect" designates the selection bias from systematic differences in the health of military personnel who are deployed to a war zone and those who are not deployed due to the selective withholding of chronically ill soldiers from deployment. Its presence was clearly demonstrated in the hospitalization paper[13] and corroborated in the mortality paper.[12]

When the "healthy-warrior effect" and the other biases pointed out in Haley's commentary are taken into account, the correct interpretation of the three *New England Journal of Medicine* papers is that Gulf War veterans suffered an excess risk of dying from motor vehicle accidents, and possibly from respiratory illnesses and suicides, in the first three years after the war; excess hospitalization from injuries, poisoning, and mental disorders also appears likely.[54] The authors' speculation that the excess mortality from motor vehicle accidents was due to psychological adjustment problems[12] was unsupported and has recently been questioned.[47] Neurological impairments from neurotoxic chemical exposures in the war is the only potential explanation for the excess deaths for which there is empirical support.[24] No conclusions can be drawn from the birth defects study regarding excess rates of birth defects after the war because of the severely biasing effects of the failure to obtain birth records from nonmilitary hospitals.[54]

Since the foregoing discussion recounts only a few highlights of a complex, intricate series of arguments that are central to understanding the debate over Gulf War syndrome, the interested reader is referred to the "Point," "Counterpoint," and "Countercountpoint" commentaries that appeared together in the *American Journal of Epidemiology*.[54,58-61]

Effects of Stress on the Blood-Brain Barrier

PRO. In 1996 the Stress Theory received a dramatic boost with the publication in *Nature Medicine* of an article by Friedman et al.[62] They demonstrated that in FVB/N mice two 4-minute periods of forced swim stress apparently caused the blood-brain barrier to open wide and allow a massive influx of pyridostigmine into the brain. Compared with a 20% inhibition of acetylcholinesterase (AChE) in the brains of the control mice by the dose of pyridostigmine,

those subjected to swim stress had 100% inhibition of AChE. The authors speculated that this phenomenon could explain the frequency of acute CNS symptoms during the war and chronic symptoms after the war.

Pyridostigmine bromide (Mestinon), a long-time treatment for myasthenia gravis, was administered to U.S. and coalition troops in a dose of 30 mg three times a day during several weeks of Operation Desert Storm as a pre-treatment antidote to the fatal effects of the chemical nerve agent soman.[63-65] Organophosphate pesticides and nerve agents produce clinically evident poisoning by binding to the active site of AChE and thereby causing a cholinergic crisis. Pyridostigmine binds to the same receptors, though less tenaciously. Its military use is to increase the efficacy of post-exposure atropine/pralidoxime therapy after soman exposure.[63] It does this by occupying the active site of AChE so that soman cannot attach to at least some portion of the body's AChE. The protective action of pyridostigmine is of use only with the nerve agent soman (possibly also with tabun), because soman's binding to AChE becomes irreversible too rapidly (within 5 minutes) for post-exposure pralidoxime therapy to work.[65]

Pyridostigmine was chosen for military use because it does not readily cross the blood-brain barrier and therefore generally produces few performance-altering side effects. Whereas approximately half of troops experienced mild muscarinic side effects from the standard pyridostigmine dose, approximately 10% experienced more severe nicotinic and CNS side effects as well.[66] Epidemiologic studies have linked the more severe side effects with chronic Gulf War syndrome.[24]

CON. The Friedman et al. finding was surprising because all prior literature indicated that stress does not affect the permeability of the blood-brain barrier in adults of diverse species, although the effect may be seen in immature animals.[67] Recently Lallement et al. raised further question about the Friedman et al. findings with experiments demonstrating that prolonged heat stress (up to 43 degrees C for two hours), an even more potent stimulus to the physiologic stress reaction, does not increase the entry of pyridostigmine into the brain in guinea pigs.[68] To date, four additional laboratories have attempted to replicate the finding in the same species and in different species, all unsuccessfully, although none of these has yet been published.

The Argument from Clinical Experience

PRO. It is widely perceived, though not well documented, that physicians who care for Gulf War veterans in the military and VA systems attribute the veterans' physical symptoms to psychological processes.[17,69,70] This perception appears to have arisen soon after the Gulf War as physicians were confronted by physical symptoms far out of proportion to objective findings on physical examination and laboratory and radiological tests, and it appears to have persisted. In the first three or four years after the war, common diagnoses used to explain the excessive symptoms were PTSD, depression, somatization disorder (hysterical neurosis), and "adult-onset attention deficit disorder" [sic]. In more recent years, as these diagnoses were challenged on nosological grounds, the term "undiagnosed illness" has come into uniform use. In some instances veterans have received service-connected disability on the basis of this diagnosis.

To date approximately 100,000 of the 697,000 Gulf War veterans have registered with either the DoD or VA Gulf War illness registries. The majority of these have gone through the standard protocol examinations which consist generally of a thorough history and physical examination, basic blood chemistries and serologies, and specialized laboratory work indicated by clinical findings, but no tests used by toxicologists to detect neurotoxic brain dysfunction. All diagnoses are recorded in the ICD-9 coding system.[16,17]

This huge clinical effort has failed to identify a new or unusual syndrome. Instead, it has found that the majority of ill volunteers have at least one medical condition to which an ICD-9 code can be attached and the distribution of these conditions is similar to what would be expected in any age-sex-matched U.S. population.[16,17] Consequently, an official conclusion has been reached that there is no Gulf War syndrome but only the usual spectrum of illness expected in an aging military population.[17]

Finally, a widely quoted essay by military and VA physicians has attributed the veterans' physical symptoms to the chronic effects of combat stress by drawing a parallel with unexplained symptoms that occurred in groups of soldiers after previous wars.[71] The authors reasoned that, since the prior post-war syndromes have been attributed to combat stress by some, this supports a stress etiology for the unexplained symptoms of Gulf War veterans.

CON. Since there presently is no widely agreed upon definition of the Gulf War illness and no consensus on etiology, the current position of the DoD and VA medical departments to regard veterans' unexplained physical symptoms as "undiagnosed illness" is a reasonable one. However, certain frequently repeated assumptions about etiology are not warranted. For example, the perception that the illness is a psychological condition in the sense of learned, conditioned or reactive behavior is unsupported empirically. Likewise, the conclusion that veterans are suffering only from a collection of traditional medical conditions captured by ICD-9 codes[17] fails to account for the remarkable similarity of the constellation of symptoms reported by veterans from different services, ranks and geographical locations.[6,22,72,73] The analogy with prior post-war syndromes is not a cogent argument because it is not clear whether the condition affecting Gulf War veterans is the same as those reported after prior wars, and the etiology of prior post-war syndromes was never determined.

THE NEUROTOXICITY THEORY

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,[2] 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.[74] No case definition had been formulated, and none was contemplated. Without a case definition, no meaningful epidemiologic study was even possible.[74] At the conference, our research team 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.[74]

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.[22,24] We administered a questionnaire on symptoms, one on war-zone exposures, and a psychological test, the Personality Assessment Inventory.[75] 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.[74] In most epidemics, the case definition is obvious from examining a few typical cases, but this was not true for Gulf War syndrome.[2] 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. Despite excessive distrust of the technique among biomedical scientists, factor analysis has proved valuable over the century in identifying and classifying diseases (e.g., the psychiatric diseases).[76]

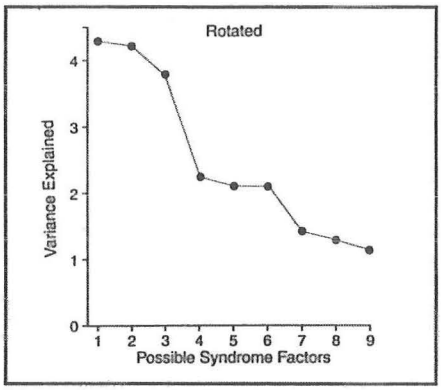

Figure 4. Scree plot from hierarchical principal factor analysis orthogonally rotated with varimax rotation. The 9-factor rotated model of the 52 symptom scales confirmed that 3 strong factors and 3 weak ones followed by a decreasing slope of trivial factors were evident after rotation was performed. 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.)

We performed a hierarchical factor analysis involving two sequential factor analysis steps.[77,78] 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.[22] 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 the 52 unambiguous symptom factor scales measured in the 249 subjects.[22] The results are displayed in a graph called a scree plot[77,78] (**Figure 4**). In the scree plot, the vertical axis measures the strength of clustering of the symptoms, and the horizontal axis indexes the 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 5 shows the distributions of all 249 subjects on each of the six syndrome factor scales in the seabees sample (top) and in a replication sample of North Texas Gulf War veterans (bottom). 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 Gulf War veterans identified through the Gulf War Veterans' Clinic at the Dallas VA Medical Center.[79] The distributions of the 336 North Texas veterans on the six syndrome factors was highly similar to those of the 249 seabees (Figure 5). 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.[79] We found that 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). The three syndrome factors were highly inter-correlated (r=0.71, 0.64 and 0.66), but the fit of the model was far superior to models with stipulated inter-factor correlations of zero (CFI=0.72; X^2 difference test, $p<.0001$) and unity

(CFI=0.82, X^2 difference test, $p<.0001$), 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.[6,72] 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.[73] In their factor analysis, syndrome factors almost identical to our factors 1-3 were identified, appearing in the same order even. British researchers obtained very different results in a factor analysis of symptoms in British Gulf War veterans, but their pool of symptom measurements was too dissimilar from any of the American studies to be compared.[7,80] 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 5).[22]

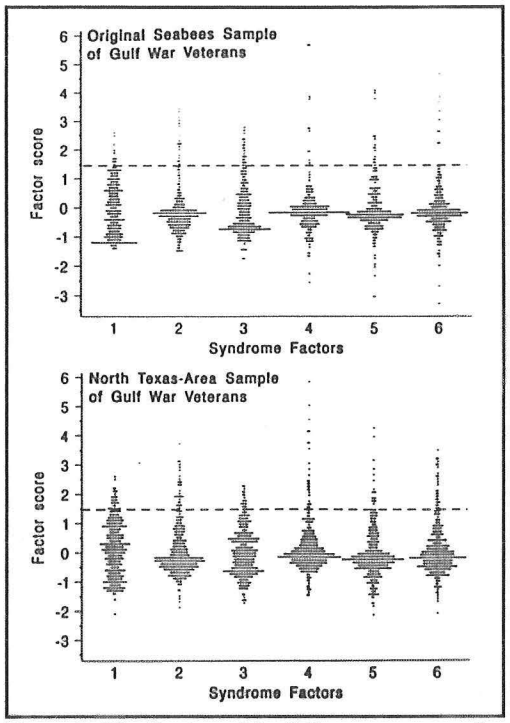

Figure 5. Distribution of the 249 veterans on each of the 6 syndrome factor scales. Visual inspection of the distributions indicated that dividing all of the distributions at 1.5 (horizontal line) would provide dichotomous syndrome variables distinguishing at least 9 extreme individuals from the bodies 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 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-

Group Description	No. of Veterans	% of All 606 Veterans†
No serious health problems and no syndromes	70‡	11.6
Serious health problems but no syndromes	116§	19.1
Any of the 6 syndromes	63‖	10.4
Syndrome 1 (impaired cognition)	12	2.0
Syndrome 2 (confusion-ataxia)	21	3.5
Syndrome 3 (arthro-myo-neuropathy)	22	3.6
Syndrome 4 (phobia-apraxia)	11	1.8
Syndrome 5 (fever-adenopathy)	16	2.6
Syndrome 6 (weakness-incontinence)	9	1.5
Total survey participants	249	41.1
Total survey nonparticipants	357	58.9
Total RNMCB-24 members deployed	606	100.0

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.)

neuropathy"). This classification, which constitutes our new case definition of Gulf War syndrome, identified three important subgroups of ill Gulf War veterans, each containing veterans with highly similar symptom profiles. 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 syndromes are given in **Table 2**.

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 syndrome group.[22] 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.

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 6**).[22] 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 strongly against any of the standard psychiatric diagnoses as explanations for the syndromes.

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.

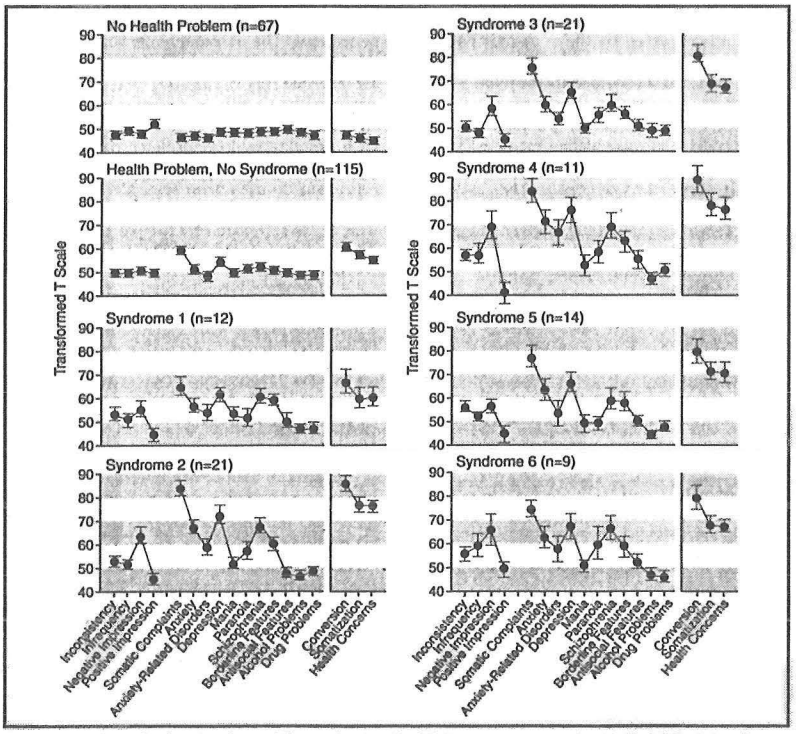

Figure 6. Psychological profiles of 243 Gulf War veterans in RNMCB-24 who completed the Personality Assessment Inventory, by presence of health problems or Gulf War syndromes. The vertical axis is measured on a transformed T scale, with the mean of a normal reference population at 50 with a SD of 10. 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.)

Evaluation of Neurologic Function: A 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 clinical case-control study nested in our cross-sectional survey population of seabees.[23]

We selected 5 veterans with syndrome 1 (impaired cognition), 13 with syndrome 2 (confusion-ataxia), 5 with syndrome 3 (arthro-myo-neuropathy), 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 gross examination 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 7**).[23,81]

Slow sinusoidal harmonic acceleration showed greater interocular asymmetry of gain in rotational nystagmus in ill veterans than in controls (Figure 7, 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

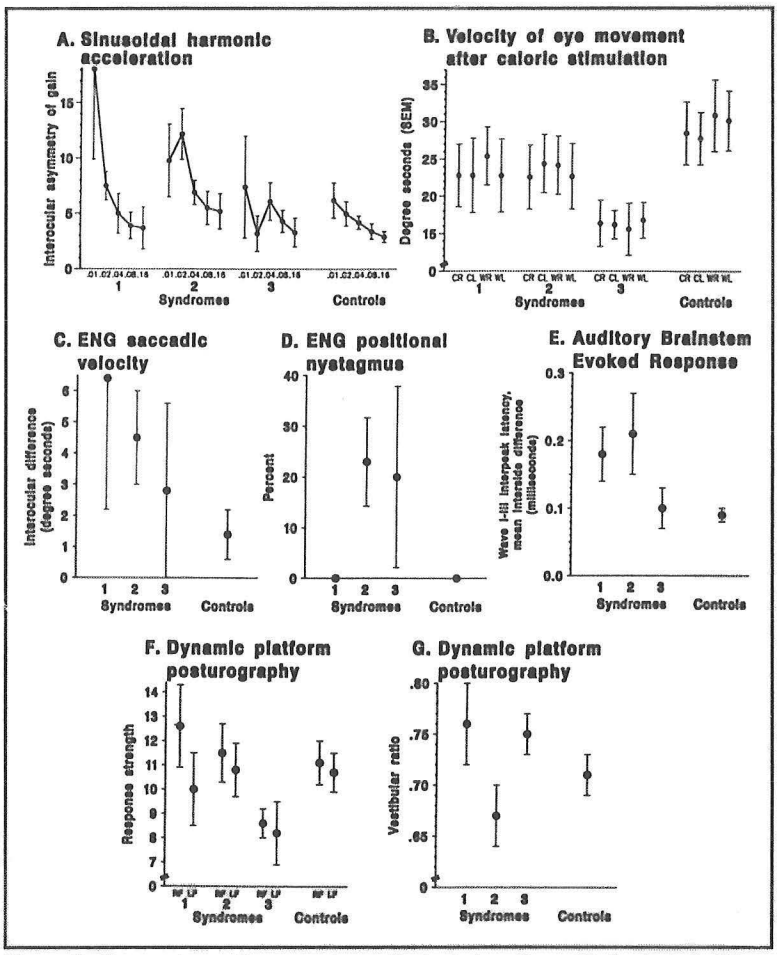

Figure 7. Comparison of mean values of selected audiovestibular tests in 23 veterans with syndromes 1, 2 or 3 and 20 age-sex-education-matched controls. On the horizontal axis of graph A, rotational speeds of .01, .02, .04, .08 and .16 are measured in Hz. On the horizontal axis of graph B, the conditions 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* 1999, in press.)

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 7, 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 7, graph B). Interaural asymmetry of caloric responses appeared greater in veterans with syndrome 2 than in controls (p=.07).

When saccadic eye movements were evaluated by ENG, veterans with syndrome 2 had either an abnormal saccadic accuracy or velocity (Figure 7, 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 7, graph D).

Interaural asymmetry of the auditory brainstem response (ABR) was manifested as wave 1-3 interpeak latency differences between ears that were greater in cases than in controls (p=.02). This was true of syndromes 1 (p=.005) and 2 (p=.07) but not in syndrome 3.

Abnormalities were also apparent in platform posturography. Veterans with syndrome 3 demonstrated significantly lower response strength from both the right and left forward components of the platform than controls (p=.03 and .09, respectively) (Figure 7, graph F). Veterans with syndrome 2 had lower vestibular ratio than controls (p=.10) (Figure 8, graph G). The two groups did not differ in the somatosensory, visual and visual preference ratios, and no subject displayed a malingering pattern. In the 23 case subjects reductions of the vestibular ratio were correlated with prolongation of ABR wave I-V latency on the right (Pearson's r= -.47, p=.02) and on the left (Pearson's r= -.37, p=.08).

An overall measure of organic brain dysfunction is the Halstead-Reitan neurologic impairment scale.[82] 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 1 (impaired cognition) had a slightly elevated mean (±SEM) score just above normal (0.43±0.09, p=.3); whereas, those with syndromes 2 (impaired cognition) and 3 (arthro-myo-neuropathy) had evidence of substantial organic brain dysfunction (0.59±0.06, p=.006 and 0.54±0.10, p=.09, respectively).[23] 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.[83]

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.[81] These differences, however, are subtle and difficult to detect, as has generally been found in patients with documented neurotoxic brain injury.[84] 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 has until recently been overlooked.

Evaluation of Neurologic Function: Study of a Monozygotic Twin Pair

We studied a 48 year old white male 27-year veteran officer of U.S. Army Special Forces, who developed a debilitating neurological condition shortly after the Gulf War, and his identical, non-military twin.[85] 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 moderate fatigue. For several months at a time, he experienced paroxysms of coughing, severe myalgias, hot flashes and night sweats, and worsening of fatigue, triggered by exposure to fumes. Evaluation in the CCEP 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.

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. High resolution brain SPECT scans found reduced blood flow in the right putamen and left temporal lobe. 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.[86,87] 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.

Postwar this officer developed chronic organic brain dysfunction, not found in his twin, that was not detectable by standard medical testing. The results of these tests have been shown to be more similar in monozygotic twins than in dizygotic twins and unrelated subjects. Small studies in twins should be more effective in elucidating the subtle neurologic deficits in Gulf War veterans than larger studies comparing unrelated groups of veterans. This case report also provides the first detailed clinical description of Gulf War syndrome and refutes the oft repeated concern that veterans with the syndrome are merely opportunists seeking financial retirement benefits.

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,[22] 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.[74]

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).[88-91] 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.[24] 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.[92]

Syndrome 1 ("impaired cognition") was 8 times more common in veterans who reported having worn pet flea-and-tick collars to repel insects during the war (**Table 3**).[24] 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

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*≤.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).

Table 3. 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 and Kurt. *JAMA* 1997; 277: 231-237)

during the Gulf War involved security (Table 3). 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.

Syndrome 2 ("confusion-ataxia") 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 3). 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 did increase with the scale of advanced side effects from PB (X^2 for trend p<.0001; Table 3).

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 near Khafji on 20 January 1991.

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 4**). 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 4). 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).

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

Table 4. 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)

The prevalence of **syndrome 3** ("arthro-myo-neuropathy") 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 3). 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).

Synergism 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 these findings suggest that each of our three syndromes may be 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.

Tests of Biological Plausibility in Animal Experiments

To test the biological plausibility of the epidemiologic finding, 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.[93,94] The implicated chemicals, pyridostigmine, 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 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 8**). 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. 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 (**Figure 9**). 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.

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,[63]

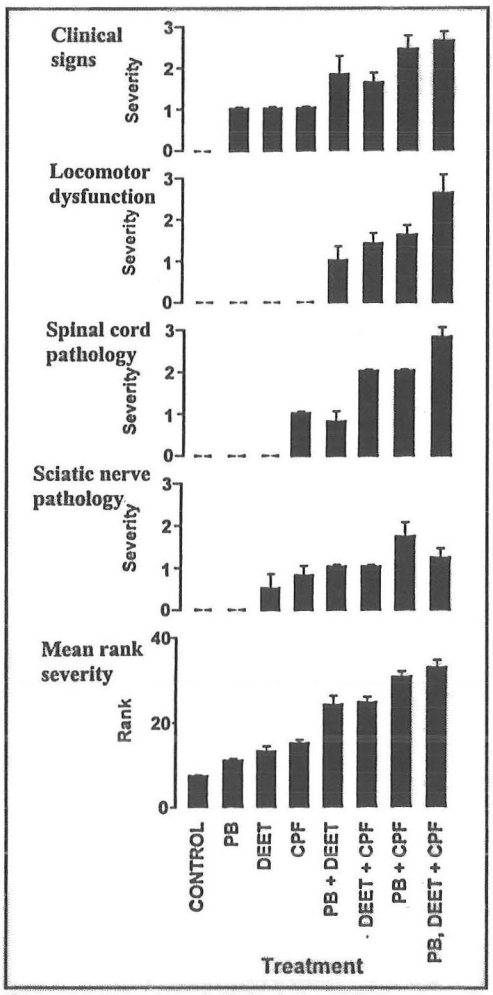

Figure 8. Severity of clinical signs, locomotor dysfunction, histopathological changes in spinal cord and sciatic nerve and mean rank of hens following daily administration of pyridostigmine bromide (PB), DEET, and chlorpyrifos (CPF), either alone or in combination. (From Abou-Donia et al. *Fundament. Appl. Toxicol.* 1996; 34: 201-222.)

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.[95-97] 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.[95] and Willems et al.[96] 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,[95] 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.

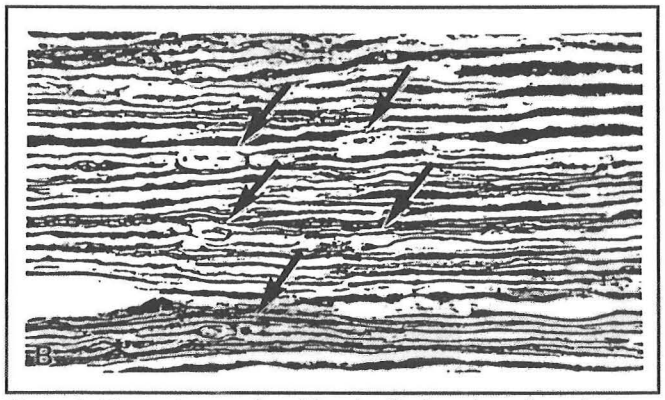

Figure 9. Photomicrographs of longitudinal sections through the lateral columns of the spinal cord (x100) from hens treated with pyridostigmine bromide, DEET and permethrin daily for six weeks. There are axonal varicosities and fragmented axons typical of organophosphate induced delayed neurotoxicity (from Abou-Donia et al. *Toxicol Environ Health* 1996; 48: 35-56)

In 1993, however, Husain et al. reported that unprotected mice given 10 daily inhalation exposures to sarin at a low concentration not causing cholinergic signs developed typical OPIDP with ataxia from histologically proven axonal degeneration of the spinal cord beginning 14 days after the first exposure.[98] Subsequently, they duplicated the finding in hens with 10 daily subcutaneous injections of 0.1 LD_{50} of sarin.[99]

Limited reports of accidental exposures in humans support the findings of Husain et al. 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.[100,101] In 1974 Sidell reported persisting psychiatric symptoms in 2 workers of the Edgewood Arsenal accidentally overcome by sarin and soman, respectively.[102] 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.[87] Burchfiel and Duffy experimentally produced the same EEG abnormalities by experimental administration of low-level sarin to primates.[86]

These findings are consistent with a large scientific literature documenting the chronic neurologic and behavioral sequelae of exposure to certain organophophate pesticides.[103-108]

A substantial body of clinical research testing of subjects exposed to sarin in the 1995 terrorist attack in the Tokyo subway 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).[109] 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 sensory nerves, indicating that sarin produces OPIDP at a much lower dose than suspected from prior studies.[95-97] Morever,

although all acutely exposed individuals were found 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.[109-114] These findings, uncorrelated with psychological sequelae such as PTSD, directly parallel findings of the UT Southwestern seabees studies.[23,81]

This body of literature supports the biologic plausibility of the neurologic damage resulting from low-level exposure to synergistic combinations of organophosphate 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.

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 the 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:[115-117] 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).[118] 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.[119,120]

The type Q allozyme, present in homozygous Q and heterozygous QR individuals, has higher hydrolytic activity against a wide range of agents including sarin, soman and diazinon but lower activity against paraoxon, the metabolite of parathion.[119,120,121] 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.[115,122] Quantitative differences may predict susceptibility to acute toxicity in animals,[116,117,122-124] and dose-response curves for organophosphate toxicity are very steep,[123] suggesting that small differences in hydrolytic rates below a critical threshold could account for large differences in toxicity.[121] 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 who have the R allele (homozygous R or heterozygous QR) of the PON1 gene than in those who do not (homozygous Q).[125]

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.[126] These determinations were made in the laboratory of Dr. Bert La Du at the University of Michigan Medical School.

Independent variable	Symptom complex 2 vs controls: Odds ratio (95% CI)[a]	p	All ill veterans vs controls: 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

Table 5. 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).

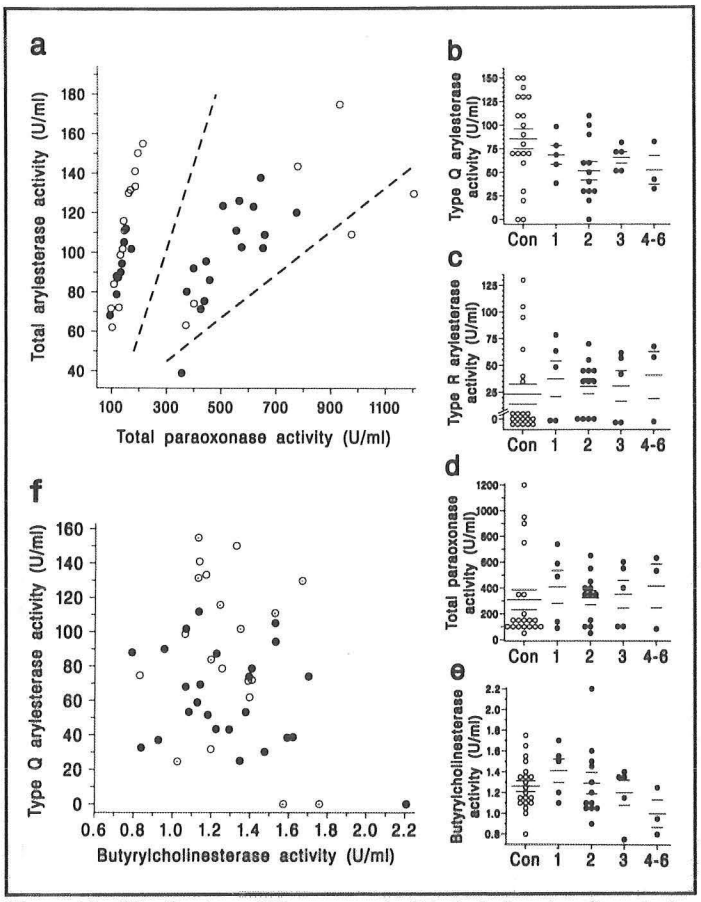

Figure 10. Distribution of ill veterans (solid circles) and well controls (open circles) by measures of paraoxonase/arylesterase 1 (PON1) and butyrylcholinesterase (BChE) genotypes and plasma allozyme activity. In **a**, plotting cases and controls by total paraoxonase activity and total arylesterase 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)

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) (**Figure. 10a** and **Table 5**).[126] Since only 9% of Caucasian populations have the homozygous R genotype,[125] 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 10a), 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 (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), and mean BChE activity did not differ between ill veterans (1.29±0.06) and controls (1.29±0.05).

The type Q arylesterase activity, however, was significantly lower in the ill veterans (mean 62±6) than in the well controls (mean 88±11), 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 (**Table 6**), than for syndromes 1 (mean 72±11) and 3 (mean 69±7) (Figure 10b). 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).

Clinical group[a]	Symptom complex name	Mean Halstead Impairment Index (SE)[b]	Percentage unable to work (SE)	Risk factors having relative risks >4 with $p < 0.001$
No post-war health problems	—	0.38 (0.05)	3 (2)	—
Symptom complex 1	"Impaired cognition"	0.43 (0.09)	17 (11)	Wore pesticide-containing pet flea collars; worked as a security guard
Symptom complex 2	"Confusion–ataxia"	0.59 (0.06)	52 (11)	Perceived chemical nerve agent exposure; excessive side effects from pyridostigmine tablets
Symptom complex 3	"Arthro-myoneuropathy"	0.54 (0.10)	14 (7)	Wore insect repellant with high concentrations of DEET; excessive side effects from pyridostigmine tablets

Table 6. Summary of epidemiologic findings on three primary Gulf War neurologic syndromes identified by factor analysis of symptoms in members of a Naval Reserve battalion who served in the Gulf War. (From Haley et al. *Toxicol Appl Pharmacol* 1999; 157: 227-233).

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 10f). There was a possible contribution from low levels of BChE activity as well (Figure 10f). 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 5). This association was strongest with syndrome 2 (Table 5), the condition previously demonstrated to have the highest rate of occupational disability,[22] the most severe neurologic impairment,[23] and the strongest epidemiologic associations with risk factors of wartime environmental chemical exposure[24] (Table 6). Being in the lowest quarter of BChE activity also predicted illness, but the difference was not statistically significant (Table 5).

The PON1 Q/R polymorphism has provided a very 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. At present, this is a very short list, headed by sarin, the chemical nerve agent that was known to be in the Iraqi arsenal that U.S. planes bombed and which was detected among our troop concentrations by the independent Czechoslovakian CW experts.[3] The facts surrounding exposure of U.S. troops to sarin are hotly disputed by DoD officials who have investigated the issues, but the basic evidence for and against such occurrences remains largely secret.

Ongoing Research to Demonstrate the Brain Damage

Our large UT Southwestern collaborative research team has recently completed a second clinical case-control study to try additional testing strategies that might provide more sensitive and specific tests for the subtle brain damage underlying Gulf War syndrome. Among the many strategies being tried are new brain imaging techniques developed to measure abnormalities in other brain diseases not evident on brain MRI. Most promising at present are magnetic resonance spectroscopy and SPECT imaging of regional cerebral bloodflow analyzed by statistical parametric mapping (SPM). We have collected additional clinical measurements in cases and controls to try to explain how the brain injury produces many of the most troubling symptoms. And we have performed an initial exploratory clinical trial of common psychoactive medications to identify treatment options.

Criticisms of the Research

The research publications from our work have evoked strongly critical reactions from medical officials in the Departments of Defense and Veterans Affairs[46,127-131] as well as from researchers commenting on behalf of major chemical companies.[132] We believe that the most frequently mentioned criticisms of possible selection bias, recall bias, multiple hypothesis testing, and small sample sizes were neutralized by design features or analytic findings thoroughly described in our papers.[22-24,81,83,125] We have consistently acknowledged, however, that to date our studies, focused on members of a single seabees battalion, have only raised a promising theory that must be tested by further replication. Initial replication efforts of our case definition have confirmed our findings.[6,72,73,79] We have proposed a national replication study to be done by an independent research organization to our specifications in random samples of the Gulf War-era deployed and nondeployed populations. The essential collaboration with the Department of Defense is presently under discussion.

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