

Research Advisory Committee on Gulf War Veterans' Illnesses

May 15-16, 2006, Committee Meeting Minutes

U.S. Department of Veterans Affairs
Washington, D.C.



DEPARTMENT of VETERANS AFFAIRS

**Research Advisory Committee on Gulf War Veterans' Illnesses
VA Eastern Kansas Healthcare System (T-GW)
2200 S.W. Gage Blvd. Topeka, KS 66622**

I hereby certify the following minutes as being an accurate record of what transpired at the May 15-16, 2006, meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses.

/signed/

James H. Binns

Chairman

Research Advisory Committee on Gulf War Veterans' Illnesses

Table of Contents

Attendance Record..... 5

Abbreviations 6

Agenda 7

Welcome, introductions, and opening remarks..... 9

The Gulf War and its aftermath..... 11

The Gulf War and Gulf War Illnesses: An Overview of Research Reviewed by the RAC-GWVI.. 13

Toxicological Studies Evaluating Synergism between Gulf War Exposures..... 14

UT Southwestern Research on Gulf War Syndrome..... 16

Biological Mechanisms Potentially Associated with Gulf War Illness: Neuroinflammation/Cytokine Activation in Response to Toxic Exposure 17

Neuroplasticity and Gulf War Veterans Illnesses 19

The Cause(s) and Potential Treatments of Chronic Multisymptom Illnesses Following the First Gulf War..... 21

Committee Discussion: Biological Mechanisms Potentially Associated with Gulf War Illnesses..... 25

Public Comment – Day 1 28

Day 2..... 31

Gene Expression Profiles Following Sarin Exposure 32

“A Chronic Fatigue Syndrome Related Proteome in Cerebrospinal Fluid” 36

Update on Research in Persian Gulf War Veterans Illnesses – May 2006 39

Current Gulf War-related research at VA..... 39

Plan for Gulf War Illness and Chemical Exposure Research Program..... 42

Federal Advisory Committee Ethics Training 48

RAC Committee Business 48

Public Comment – Day 2..... 49

Appendix A..... 51

Presentation 1 – Joel Graves 51

Presentation 2 – Lea Steele..... 57

Presentation 3 - Mohamed Abou-Donia 85

Presentation 4 – Robert Haley..... 111

Presentation 5 – Jim O’Callaghan 140

Presentation 6 – Floyd Bloom 159

Presentation 7 – Dan Claww..... 174

Presentation 8 – Mohamed Abou-Donia 189

Presentation 9 – James Baraniuk 225

Presentation 10 – Beatrice Golomb..... 243

Presentation 11- Robert Haley 268

Appendix B **273**

Public Submission 1 – Kirt Love..... 273

Public Submission 2 – Denise Nichols..... 274

Attendance Record

Members of the Committee

James H. Binns, Chairman

Adrian Atizado

Carrolee Barlow

Floyd E. Bloom

Daniel J. Clauw

Beatrice A. Golomb

Joel Graves

Robert W. Haley

Anthony Hardie

Marguerite L. Knox

William J. Meggs

Mary D. Nettleman

James P. O'Callaghan

Steve Smithson

Lea Steele

Hugh H. Tilson

Consultant to the Committee

Jack Melling

Committee Staff

Laura Palmer

Barbara LaClair

Guest Speakers

James Baraniuk

Mohamed Abou-Donia

Abbreviations

AChE	Acetylcholinesterase
AFIP	U.S. Armed Forces Institute of Pathology
ALS	Amyotrophic Lateral Sclerosis
CAA	Cerebrovascular Amyloid Angiopathies
CBT	Cognitive Behavioral Therapy
CDC	Centers for Disease Control
CFS	Chronic fatigue syndrome
CNS	Central Nervous System
CRADO	Chief Research and Development Officer (VA)
DEET	N,N-diethyl-meta-toluamide
DoD	U.S. Department of Defense
FACA	Federal Advisory Committee Act
FY	Fiscal year
GWV	Gulf War illness
HPA	Hypothalamic-pituitary-adrenal axis
IOM	Institute of Medicine
IRB	Institutional Review Board
MOU	Memorandum of Understanding
MS	Multiple Sclerosis
MVC	Motor vehicle collision
NIH	National Institutes of Health
NGWRC	National Gulf War Resource Center
OPIDN	Organophosphate-induced delayed neurotoxicity
ORD	Office of Research and Development (VA)
PB	Pyridostigmine bromide
PET	Positron emission tomography
PMSF	Phenylmethylsulfamyl fluoride
POMS	Profiles of Mood State
PTSD	Post traumatic stress disorder
RAC-GWVI	Research Advisory Committee on Gulf War Veterans' Illnesses
RFA	Request for Applications
SPECT	Single photon emission computed tomography
TCCD	Transcranial Color Coded Duplex Sonography
VA	U.S. Department of Veterans Affairs
WRIISC	War-Related Illness and Injury Study Center (VA)
WTC	World Trade Center

**Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses
May 15-16, 2006**

**Agenda
Monday, May 15**

Meeting Location: 7th Floor Conference Room, American Legion Headquarters
1608 K St., N.W., Washington, D.C.

8:00 – 8:30	Informal gathering, coffee	
8:30 – 9:15	Introductory remarks, introduction of members	Chairman Jim Binns
9:15 – 10:15	The Gulf War and its aftermath	Mr. Joel Graves Mr. Anthony Hardie
10:15 – 10:30	Break	
10:30 – 11:30	Overview of research on Gulf War illnesses	Dr. Lea Steele
11:30 – 12:15	Toxicological studies evaluating synergism between Gulf War-related exposures	Dr. Mohamed Abou-Donia
12:15 – 1:15	Lunch	
1:15 – 2:15	Studies of neurological function in Gulf War veterans	Dr. Robert Haley
2:15 – 3:00	Biological mechanisms potentially associated with GWI: Neuroinflammation/cytokine activation in response to toxic exposures	Dr. James O'Callaghan
3:00 – 3:15	Break	
3:15 – 3:45	Biological mechanisms potentially associated with GWI: Neural plasticity	Dr. Floyd Bloom
3:45 – 4:30	Biological mechanisms potentially associated with GWI: Neural dysfunction underlying multisymptom illnesses	Dr. Daniel Clauw
4:30 – 5:30	Discussion: Biological mechanisms potentially associated with GWI	
5:30 – 6:00	Public Comment	

**Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses
May 15-16, 2006**

**Agenda
Tuesday, May 16**

Meeting Location: Room 230, VA Headquarters
810 Vermont Ave., N.W., Washington, D.C.

8:00 – 8:30	Informal gathering, coffee	
8:30 – 9:15	Secretary addresses the committee	Secretary James Nicholson
9:15 – 9:45	GWV and CFS-related proteome in human cerebrospinal fluid	Dr. James Baraniuk
9:45 – 10:15	Gene expression profiles following sarin exposure	Dr. Mohamed Abou-Donia
10:15 – 10:30	Break	
10:15 – 10:45	Discussion: Use of microarray technologies in GWV research	
10:45 – 11:30	Update: Recently published research relevant to Gulf War illnesses	Dr. Beatrice Golomb
11:30 – 12:30	Lunch	
12:30 – 1:15	Gulf War illness research at U.T. Southwestern	Dr. Robert Haley
1:15 – 1:30	Current Gulf War-related research at VA	Dr. Bill Goldberg
1:30 – 2:00	Discussion	
2:00 – 2:15	Break	
2:15 – 2:45	Federal Advisory Committee ethics training	Mr. Jonathan Gurland
2:45 – 3:00	RAC committee business	Chairman Binns, Dr. Steele
3:00 – 3:30	Public Comment	
3:30	Adjourn	

The first day of the meeting (May 15, 2006) was held at the American Legion Headquarters, 7th Floor Conference Room, 1608 K. St., NW, Washington, DC.

Welcome, introductions, and opening remarks

James H. Binns, Jr., Chairman

Chairman James Binns called the meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses (hereinafter referred to as "the Committee") to order at 8:31 a.m.

Chairman Binns welcomed the Committee members, guest speakers, Department of Veterans Affairs (VA) and other Federal research managers, and members of the public. He extended special thanks to Mr. Steve Smithson and the American Legion for providing their facility for the day's meeting. He explained that, due to a sudden change in the Secretary's schedule, the Committee had needed to find alternate meeting space for this day's meeting. He noted, however, that the Committee would be holding its meeting at the VA Headquarters, Room 230, 810 Vermont, NW, on the following day.

Chairman Binns stated that, although this meeting marked the start of the Committee's fifth year of work, it was a new beginning in many important respects. He noted that half of the Committee members were new, and the Committee would benefit from their new insights and expertise. He noted that this change in membership also reflected a new phase in the working of the Committee. He stated that, up until this point, it would be fair to say that the Committee has focused largely on past research and on general questions having to do with the scope and nature of Gulf War illnesses. This information, including the Committee's findings to date, would be briefly reviewed that morning for the benefit of new members. He stated that the Committee's new goal was to move beyond these general questions and address the specific mechanisms involved in these illnesses and the specific research studies needed to answer these questions. With this goal in mind, he expressed his pleasure in having the new members' participation in these discussions.

Chairman Binns noted that the Committee's charter provided a clear standard by which the Committee was to measure Federal Gulf War research. He stated that this standard was whether the research made a difference to the health of ill Gulf veterans. He acknowledged that to date, despite the tens of millions of dollars spent and the many years that have gone by, it had not. He noted that this was the case, despite the most recent findings of the VA's ongoing longitudinal survey that 25% of veterans deployed during the Gulf War have chronic multisymptom illnesses over and above the base rate of nondeployed veterans of the same era.

Chairman Binns stated that a new element in Gulf War illness research was VA's establishment of a Gulf War illnesses research center at the University of Texas Southwestern last month. This center will manage a \$15 million program, created by Senator Kay Bailey Hutchison (R-TX) in the FY2006 VA appropriations bill. Senator Hutchison had stated that the center would be funded at this level for five years. Chairman Binns said he had been pleased to be invited by VA to attend the public event in Dallas at which the new center had been announced.

While the video of the press conference was being cued, he noted that Senator Hutchison had generously given credit to the Committee for its recommendations that led to this work and decision. He stated that she was referring to the Committee's 2004 report, which recommended that VA spend at least \$15 million per year for four years on Gulf War illness research. He stated that the Committee did not recommend that it be focused in one location, i.e., at UT Southwestern. However, he understood the decision by Senator Hutchison and VA Secretary R. James Nicholson. They have observed, as have Committee

members who have served for the past several years, that when VA leadership has made a commitment to the Gulf War illnesses research, it has proven difficult to see these commitments carried out within the conventional confines of VA research policy and procedures. He welcomed the bold step by Senator Hutchison and Secretary Nicholson to place research management and program funds in the hands of people who were committed to the problem. He acknowledged that this followed a different funding model than the one most academicians and federal research managers were accustomed to, i.e., bidding and inviting proposals on individual research studies proposed by individual researchers. He reminded those that were used to the usual scientific process that every piece of equipment in their laboratories and clinics had been developed using a business model, that is, an approach that was more centralized and targeted to achieve specific objectives. He expressed hope that this model would be more successful than the past one in this particular case. Videotaped portions of comments made by Senator Hutchison and Dr. Jonathan Perlin, VA Under Secretary for Health, at the April 21, 2006, Dallas, TX, press conference were played for the audience.

Chairman Binns stated that the UT center would not be the only place at which federally-funded Gulf War illness research would be conducted. VA would continue to sponsor Gulf War illness research studies, managed from VA's Office of Research and Development (ORD). In addition, the Department of Defense (DoD) would be investing \$5 million in FY2006 in Gulf War illnesses research. He welcomed the managers of these federal programs to the meeting.

Chairman Binns noted that there was a new committee, a new mission, and a new research center. At that point, he turned the focus to the first element of this equation: the new committee. He asked Committee members to introduce themselves and provide a few comments on their research and/or professional interests that bear on Gulf War illnesses.

Chairman Binns commented that the Committee was made up of very accomplished individuals, and he appreciated their choosing to be there today. He stated that it had been fifteen years since the Gulf War, and this was a long time. It had been four years since the Committee started its work, which seemed like a long time as well. Fifteen years was a long time to invest in medical research without having a diagnosis or treatment to show for it. It was a long time to listen to previous generations of government bureaucrats minimize the importance or even the existence of the problem. It was also a long time to be sick.

Chairman Binns, however, was optimistic for three reasons: (1) the Committee had never before had this amount of talent to apply to Gulf War illnesses research. (2) the management of Gulf War illnesses research had never before been in the hands of individuals who believed there was a problem and were dedicated to solving it. He stated that not only was he referring to Dr. Robert Haley at UT Southwestern, but also Dr. Joel Kupersmith, VA Chief Research and Development Officer (CRADO), his colleagues, including Dr. William Goldberg, and leadership of the Congressionally-directed medical research program at DoD. (3) He believed that this was the beginning of a sea change when it came to thinking about the chronic multisymptom illnesses that plague modern society. He noted that the Centers for Disease Control (CDC) had just announced evidence that there was a real physiological basis for chronic fatigue syndrome (CFS). He noted that this was similar to the work Dr. Daniel Clauw and his colleagues have been doing for fibromyalgia. Chairman Binns stated that word was getting around VA that Gulf War illness was not simply a new name for battlefield stress or what happens after every war. He went on to say that it is hard seeing history being made when you are in the middle of it. It isn't like the movies where it is perfectly scripted and the background music comes up to tell you that something important is happening. But something important is happening. The Committee has the potential to play an important role in this history. The Committee is not a typical advisory committee that produces reports that sit on shelves. The recommendations made by the Committee go directly to the programs that have the

resources to apply to these problems. Chairman Binns doubted that anyone would ever make a movie about a Committee, but to help produce a research program that could make a difference to the health of ill Gulf War veterans, with implications for other ill individuals, was surely a task worthy of the Committee's best efforts.

Chairman Binns thanked Dr. Lea Steele for organizing an exemplary program for the next two days. He noted that the agenda was full, and asked everyone to please observe the time limits given so that everyone had a chance to present.

Dr. Steele went over certain logistical aspects of the meeting, including asking that everyone speak into a microphone. She noted and reviewed the contents of the Committee's binders.

The Gulf War and its aftermath

Rev. Joel Graves, Gulf War veteran, hospice chaplain

Mr. Anthony Hardie, Gulf War veteran, Executive Assistant, Wisconsin Dept Veterans Affairs

Mr. Joel Graves gave a brief presentation describing his experiences during the Gulf War, and outlining his belief that sarin gas exposure was a primary cause of Gulf War veterans' illnesses. ([See Appendix A – Presentation 1.](#)) He stated that he became severely ill during his deployment and afterwards, so sick that he almost resigned his commission. He stated that he had retired early because of his illness. He noted that he currently wasn't as sick as when he was first appointed to the Committee.

Chairman Binns asked Mr. Graves if he would share with the Committee what his symptoms had been since the war and how it affected his various career moves. Mr. Graves stated that when he was in the military, he was rather "studly", e.g., always maxed the PT test, could physically outperform junior colleagues, etc. When he came back from the war, the areas where he had been the strongest were the areas in which he was failing. He had two surgeries, developed arthritis, memory loss, etc.

Mr. Graves commented that his memory loss had been profound and debilitating. He related a story about trying to purchase an oil filter for his truck, and not being able to remember the filter model number even though he had just read it in the manual hanging from the wall. He ultimately had to tear the manual off the wall and directly compare the numbers in the manual with the ones on the product.

Mr. Graves shared another story about his time as an S4 logistics officer, responsible for organizing troop movement to Germany. During this operation, he wrote everything down, created an elaborate appendix to his report, and shared information with everybody. During the deployment, many individuals would simply rip out his appendix because it had everything one could possibly need to know. He even received a medal for creating this document, but he was doing it to protect himself. Afterwards, he found himself missing meetings and other commitments. He didn't even remember someone telling him that he needed to be at these places. He ultimately was "called on it", and retired from the service.

Mr. Graves shared another story about talking with his grandmother back in 1994 about his memory loss. She recommended magnesium and lecithin supplements. He started taking these and his memory improved; when he stopped taking them he got "stupid" again. After being on the Committee for two years, he found himself talking to a researcher about this. The researcher confirmed that there was a physiological basis for these supplements. He had found that either one separately had a little bit of effect, but the two together are "magic." He stated it wasn't a perfect solution, especially if he encountered a stressful situation, but felt that he was simply managing the problem. Mr. Graves also indicated that he didn't heal well from injuries. He acknowledged that part of the problem was that he

was getting older. But he had noticed the problem just after the war. Things didn't work the same as before the war.

Dr. Haley asked why Mr. Graves took "ranger pills", or 800 mg. Ibuprofen pills. Mr. Graves stated it was for muscle aches and pain. He stated that he also had arthritis in his neck, back and feet. The pills helped take the edge off the pain.

Dr. Steele introduced Mr. Anthony Hardie, a new member of the Committee and a 1990-1991 Gulf War veteran.

Mr. Hardie stated that when he joined the military, he hoped he would receive language training. He was ultimately sent to an intensive language school to learn French, and was assigned during the Gulf War to be a linguist for a special intelligence liaison unit with the Joint Forces Command East. This unit was free roaming compared to other units, and he found himself working throughout much of Kuwait and Southern Iraq.

Mr. Hardie described several of his experiences in the Gulf War that led him to believe that he had possibly been exposed to chemical warfare agents. He also described exposures to depleted uranium, oil well fires, and vaccinations, as well as his experiences witnessing battlefield casualties. He discussed several health concerns that developed in the Gulf; some of which continue to the present day. Before the Gulf War, he was very athletic and competed in cross-country running. Upon his return, he found himself dealing with fatigue, memory loss, respiratory ailments, chronic sinusitis and other infections, etc. He discussed his difficulties in finding help through the VA system for his ailments. He was told by the Gulf War Coordinator for a VA hospital that there was nothing wrong with Gulf War veterans. It was "just in their heads." He sought answers outside the VA, and found through the Internet and through his work as a Congressional staffer that there were other veterans with similar problems. It was only when he began to be referred for medical issues that could not be explained by psychiatric/psychological illnesses that he began to receive more serious treatment from the VA. He believes that Gulf War veterans had unique exposures, and aren't simply experiencing what all veterans have experienced since the Peloponnesian Wars.

When asked what vaccinations he had received, Mr. Hardie stated he didn't know. They were told that it was "secret" and he has not seen his medical records since he returned from the Gulf. Mr. Graves indicated that he had kept copies of his medical records, and knew that he had received vaccinations and pyridostigmine bromide (PB).

Dr. Golomb asked if Mr. Hardie had been exposed to various pesticides, including permethrin uniforms and those sprayed on bedding. Mr. Hardie stated that both were applicable to him, especially during his tours in West Africa.

Dr. Haley noted that some veterans reported side effects following the ingestion of PB. He asked Mr. Hardie if he had seen similar reactions in his colleagues and whether the reaction was immediate or not. Mr. Hardie indicated that the reaction was immediate and got progressively worse over time. He described the reaction as being flu-like symptoms.

Dr. O'Callaghan asked whether the special operations teams were more likely to receive a wider range of exposures than other troops. Mr. Hardie stated that he believed this to be true. He suggested that it would be interesting to look at special operation soldiers to see if there were any commonalities among them.

Dr. Golomb noted that Special Forces troops would also have been more physically able than the regular troops. Mr. Hardie agreed.

Chairman Binns thanked Mr. Graves and Mr. Hardie.

The Gulf War and Gulf War Illnesses: An Overview of Research Reviewed by the RAC-GWVI

Lea Steele, PhD

Scientific Director, Research Advisory Committee on Gulf War Veterans' Illnesses

Dr. Steele provided an overview of the Gulf War and research on Gulf War illnesses, and highlighted key findings and conclusions in these areas. ([See Appendix A – Presentation 2.](#))

Dr. Hugh Tilson asked Dr. Steele to comment on the findings of any epidemiological studies of the local population and other coalition nation troops. Dr. Steele stated the Committee staff had undertaken a review of information available on troops from other countries, and that U.S. and U.K. troops looked similar in their exposures and manifestation of illnesses. It appears that some other coalition troops had similar problems, but at lower rates. This would include Canada, Australia, Denmark, etc. She stated that there were very few studies with regards to the health effects on local populations. She noted that there was one study looking at the Saudi National Guard hospitalization rates. As for the local civilian populations, there had been a few clinical studies looking at hospitalization rates. There is also an ongoing study of the local Kuwaiti population by the Harvard School of Public Health. They have identified a 30% increase in mortality rate among older Kuwaitis. The cause is unknown. The Harvard researchers will be looking at multisymptom illness among younger Kuwaitis. She noted there were rumors about an increase in birth defects and leukemia in the southern part of Iraq, possibly due to spent depleted uranium. However, there have been no published, peer-reviewed studies examining these claims. There had been conferences in Kuwait about possible health problems. The conference transcripts indicated that Gulf War illness symptoms were not being seen in the local population, but this was largely speculation.

Dr. William Meggs thanked Dr. Steele for condensing four years of Committee discussion into a clear and concise one-hour synopsis. He noted that, based upon this distillation, pesticides, PB and nerve agent exposure stand out as possible causes of the Gulf War illnesses. From a toxicological standpoint, these are all from the same class of compounds. He noted that there are both anecdotal reports and solid literature documenting that individuals poisoned with organophosphates develop similar symptoms. Because of this, he was happy to see the appointment of several neuroscientists to the Committee. He commented that the real problem facing the Committee was looking for treatments, as it is possible that these veterans have had permanent brain damage that cannot be reversed. Dr. Steele noted that if the specific pathophysiological processes could be identified, it might be possible to find a way to treat or reverse the damage.

Dr. Floyd Bloom asked whether the gender or ethnic background of the troops were risk factors for developing Gulf War illnesses. Dr. Steele stated that crude analyses show a somewhat elevated rate in minority populations. However, once adjustments are made for troop locations and rank, this association is greatly reduced. She noted that more minorities were enlisted and served at the front. She stated that there was not the strong association between Gulf War illnesses and gender, as seen in the civilian population with chronic fatigue syndrome and fibromyalgia. She noted that 7% of the troops had been women, which was the highest proportion of women serving in a war zone up to that time. However, women veterans were experiencing multisymptom illnesses at only slightly higher rates than men, if at

all. She noted that there a couple of studies showed somewhat higher rates in women veterans; however there was not the striking association seen in the civilian population.

Dr. Haley commented that the French had a unique experience in the Gulf, and asked Dr. Steele to comment on the findings among French Gulf War veterans. Dr. Steele noted that French troops were positioned on the outer perimeter of the ground war, never getting close to the action in Iraq and Kuwait. It has been said that the French troops did not receive anthrax shots or take pyridostigmine bromide (PB), and the French government has consistently held that chronic multisymptom illnesses were not being observed in their veterans. Dr. Steele reported that the first epidemiologic study looking at French Gulf War veterans had been recently published, and it had been hoped that this study would shed light on the illness experience of French Gulf War veterans. She stated that the study found high symptom reporting among veterans, possibly due to the way in which questions were asked. She noted that the study did not include a control group for comparison purposes. Dr. Golomb noted that the French study also didn't relate health outcomes to exposures.

Dr. Golomb commented that there were a couple of other epidemiological studies that showed a significant association with the anthrax vaccine, albeit much smaller than the association with acetylcholinesterase (AChE) inhibitor exposure after adjustment. She stated that she had an undergraduate student working on a project examining the attributable risk of illness in Gulf War veterans. She stated that the preliminary data suggested that PB was the clear leader in terms of attributable risk.

Mr. Kirt Love, an audience member and Gulf War veteran, asked if Dr. Steele had any interaction with the French Gulf War veteran community. Dr. Steele stated that she had reviewed their printed literature and had contacted the French scientist who conducted the epidemiologic study. Mr. Love commented that French Gulf veterans had experienced similar problems with their government not acknowledging a problem nor conducting research in this area. Dr. Steele stated that she understood that about 200 disability claims had been put forward by French Gulf War veterans.

Chairman Binns thanked Dr. Steele for her presentation. He also thanked her and the Committee's staff for their hard work.

Toxicological Studies Evaluating Synergism between Gulf War Exposures

Mohamed Abou-Donia, PhD

Professor, Duke University Medical Center

Dr. Abou-Donia gave an overview of his research looking at the neurotoxic effects of PB and various pesticides (N,N-diethyl-meta-toluamide (DEET), permethrin, chlorpyrifos, etc.), alone and in combination. ([See Appendix A – Presentation 3.](#))

Following Dr. Abou-Donia's presentation, Dr. Golomb stated that she had been thinking along the same lines in terms of oxidative stress and mitochondrial injury. She noted that the primary organ targets for mitochondrial injury were those that had aerobic requirements, with symptoms predominant in muscle or brain. It might also account for the other symptoms reported by Gulf War veterans, e.g., gastrointestinal, etc. However, because these systems are less aerobically demanding, their involvement was more variable. She believed that this was a very important direction in research.

Dr. Abou-Donia noted that, after one month, the animals looked normal and that the cellular apoptosis had not been limited to the brain, but also occurred in the intestines, liver, and testes. However, these findings were complicated by the aging process in the animals.

Dr. Mary Nettleman thought this was a very important point, because Dr. Abou-Donia's research points to possible treatments or prevention of Gulf War syndrome. She noted that this might be an animal model for studies where once the exposure is stopped, one could evaluate long-term effects on the animal, as well as potential agents that might affect the long term course. Dr. Abou-Donia stated that his presentation on the next day would provide some insight in this area.

Dr. Golomb stated that there were studies showing that the use of antioxidants pre- and post-exposure to organophosphates does attenuate chronic injury. She also commented on finite regenerative potential, which represents additional mechanisms at the peripheral level that can impact muscle. She stressed that muscle and brain tended to be the primary areas affected in Gulf War veterans.

Dr. Carolee Barlow stated that the most compelling aspects of Dr. Abou-Donia's work were that he had: (1) defined a time course for injury and combinations of injury; and (2) developed information that might provide the basis for a biomarker to track the progress of the disease. It would be incredible if this could be translated into additional studies/models that would provide better understanding of the timing of the exposure, e.g., how much PB, how long of exposure to PB is required, and the time course for returning back to normal after a state of oxidative stress. It would be great to identify a peripheral marker of these processes and to look at the genetic underpinnings that could explain why one individual was affected while another was not.

Dr. Barlow asked how much of Dr. Abou-Donia's efforts would be focused on developing this as a model, using the urinary biomarker he had identified. Dr. Abou-Donia commented that he hoped to do this, and wished that Dr. Barlow had been sitting on the study sessions for his grant proposals. He stated that gene expression was altered after exposure to these chemicals. He indicated that there were many questions that needed to be answered in this regard. Based upon her research experience in gene expression in the central nervous system (CNS), Dr. Barlow commented that it was difficult to translate these findings to a patient population. She noted that Dr. Abou-Donia had some evidence that there was a urinary biomarker and potentially a blood-based biomarker. She believed transferring this knowledge into a clinical setting could have promise.

Dr. Meggs commented that a general principle in occupational and environmental medicine was that people who withstood continued exposures have a progression of the disease, while those who get away from the exposure stabilize and perhaps improve. He stated that CDC has shown that all humans are walking around with organophosphates in their blood. Thus, it may be a very bad thing for the veterans to use DEET, while eating organic foods might be a good thing. Dr. Abou-Donia agreed.

Dr. O'Callaghan noted there were a fair number of animal studies involving known neurotoxic agents and the use of antioxidant therapy in the diet/water. He stated these were not terribly easy studies to work out in animal models, and the data were mixed with respect to humans. He noted his impression that antioxidant and anti-inflammatory treatments as a way to suppress reactive oxygen species hadn't worked out so far. Dr. O'Callaghan then referred to a comment by Dr. Abou-Donia about old data indicating neural pathology relating to DEET exposure in humans. Dr. O'Callaghan inquired as to which areas of the brain were affected. Dr. Abou-Donia stated that he knew of 18 cases of neurological deficiencies in children overexposed to DEET in utero. Dr. O'Callaghan asked if the brains were examined post-mortem. Dr. Abou-Donia stated that they were not.

Dr. Bloom noted that free oxygen radicals were believed by many to be the cause of a lot of neurological damage and that this area is among the most contentious in neuroscience. However, the second most contentious area involved agents that open the blood-brain barrier. He noted that Dr. Abou-Donia had two papers in his bibliography where a blood-brain barrier opening was claimed. He asked Dr. Abou-Donia to speak to this. Dr. Abou-Donia discussed his research related to the effect of sarin exposure on the blood-brain barrier.

Mr. Steve Smithson asked for clarification as to the number of troops who were given PB. Dr. Steele stated that PB distribution and usage were different, but that the proportion of people who reported taking PB and now have GWI is greater than the general proportion with GWI. Fifty to sixty percent of the U.S. troops were reported to have taken any PB, and a smaller proportion took a very large dose. Dr. Golomb commented that the official estimate was 250,000 U.S. troops took PB in the Gulf War. However, due to the poor documentation, it was difficult to know where it lies between that and the self-reported rates, which are a little bit higher.

Chairman Binns thanked Dr. Abou-Donia.

The meeting adjourned at 12:17 p.m. for lunch.

The meeting reconvened at 1:17 p.m.

UT Southwestern Research on Gulf War Syndrome

Robert Haley, MD

Professor, UT Southwestern School of Medicine

Dr. Haley provided an overview of UT Southwestern's past and current research into Gulf War illnesses. ([See Appendix A – Presentation 4.](#))

Dr. Barlow asked Dr. Haley to comment in more detail on his mood disorder questionnaire, in particular the specific disorders they assessed. Dr. Haley stated that he had a cognitive neuroscientist reviewing findings from a number of assessment instruments, and hopes to correlate those findings with their objective neurological findings.

Dr. Steele asked if Dr. Haley's group had evaluated anything other than changes in mood or cognition accompanying physostigmine infusion. Dr. Haley stated they had used POMS (Profiles of Mood State) questionnaires every 15 minutes during the infusion and full neuropsychological batteries at other times during the week-long testing of each veteran. He noted that this was a small study, with the objective of generating hypotheses about Gulf War syndrome. He stated that his group believes that the amygdala is the main brain region affected in veterans with Gulf War illnesses.

Dr. O'Callaghan asked if Dr. Haley had looked at the serum cortisol levels in these veterans. Dr. Haley stated that they had collected 7 a.m. cortisol levels, but hadn't examined if there was a relationship with Gulf War illnesses. He indicated that they would do this analysis.

Mr. Kirt Love asked Dr. Haley if he would be looking at anything involving glutamate or glutamate receptors in conjunction with the amyotrophic lateral sclerosis (ALS) studies on glutamate toxicity. Dr. Haley stated that, in the next phase of their work, he had nuclear medicine experts interested in doing SPECT and/or PET imaging of several different types of receptors. Dr. Haley stated that the tendency is to say this is a cholinergic receptor abnormality. However, it is not known if this is the case, so imaging

of all receptors, in both human and animal studies, would be the next step in the research process. Dr. Golomb agreed that although there was evidence that cholinergic receptors are affected by organophosphates, this wasn't necessarily the only mechanism for cholinergic dysregulation.

Dr. Oliver Lawless, an audience member, asked about measuring antibodies to cholinesterase receptors. Dr. Haley stated that there was no difference between case and controls.

Dr. Steele thanked Dr. Haley.

Biological Mechanisms Potentially Associated with Gulf War Illness: Neuroinflammation/Cytokine Activation in Response to Toxic Exposure

James P. O'Callaghan, PhD

Head, Molecular Neurotoxicology Laboratory, Centers for Disease Control and Prevention

Dr. O'Callaghan provided an overview of how toxic insults to the immune and endocrine systems result in neuroinflammation and cytokine activation, and how this might relate to Gulf War illnesses. ([See Appendix A – Presentation 5.](#))

Dr. Meggs noted that there was a lot of interest in neurokinins and substance P playing a role in chronic pain, chemical sensitivities, etc. He wondered how they might fit into what Dr. O'Callaghan had presented. Dr. O'Callaghan discussed normal signaling in the basal ganglia and processes relating to relationships between dopamine, neurotensin and substance P.

Dr. Haley noted that from the perspective of an ill veteran, symptoms wax and wane. He asked Dr. O'Callaghan if he might hypothesize how this might be happening in relation to cytokines, microglia, etc. Dr. O'Callaghan stated that, in the peripheral immune system, TNF-alpha mediated responses can make people feel sick. This is seen after infection and is believed by some to be a component of depression. He stressed that it should not be forgotten that rewiring of the brain can result in profound, long lasting alterations on any of these measurements. He stated that there was a need to develop some kind of challenge test to tease out these answers and thought it would be important to evaluate HPA function in response to challenge in ill Gulf War veterans using dexamethasone testing. Dr. Steele noted that there were a couple of VA-funded studies currently underway looking at dexamethasone and HPA function in ill Gulf War veterans.

Dr. Steele asked Dr. O'Callaghan to comment on preliminary indications of peripheral cytokine activation in Gulf War veterans and their potential link with central processes/glial activation. (1) If cytokines were elevated in the brain, what would be seen in the periphery?; and (2) Is there any way to know if there is glial activation in human beings? Dr. O'Callaghan stated that there were extensive neuroimaging methods to detect glial activation, but they were not easy. Another problem is that not enough is known about these signals and what they do normally in the brain. However, from the data, it doesn't look like severe inflammatory disease is involved here. It may be more subtle than glial activation responses occurring normally in the CNS. The observed autonomic symptoms support this. It may have nothing to do with glial activation in the damage sense. If those events occurred and if there was subtle or bad brain damage in Gulf War veterans, those glial activation signals would be gone, but the changes that occurred in the brain or periphery, not to mention plasticity responses, could be there. Dr. Steele asked if this might include an activation of cytokines in the central nervous system that wasn't seen in the periphery. Dr. O'Callaghan indicated this was possible.

Dr. Barlow asked, if there was increased risk related to differences in the metabolizing enzyme paraoxonase (PON) and an abnormal centrally mediated response to physostigmine, whether there had been any evaluation of the patient's cells in the periphery that were responsive to these types of compounds. Dr. O'Callaghan didn't think so. Dr. Barlow stated this would seem to tie back to TNF-alpha involvement. She noted that if one had a group of patients who are unable to clear a toxin and also had a reduced ability to respond appropriately to the toxin, one could imagine setting up a system to test autonomic and peripheral nervous system effects, as well as central effects, targeting the same portions of the brain being discussed.

Dr. Barlow asked Dr. Bloom to comment on the late stages of acetylcholinesterase abnormalities in Alzheimer's disease, and how this might ultimately impinge on other systems outside the hippocampus. Dr. Bloom stated that, overall, cholinesterase differences were nonpredictive of Alzheimer's disease. If limited to plaques in the hippocampus and measuring cholinesterase there, there is some predictability. However, general brain cholinesterase activity is not a good indicator. Early data suggested this might be the case, but as the "n" expanded the correlations became non-meaningful.

Dr. Bloom referenced the neuroscience research conducted on neuro-AIDS. He noted that most patients do not present with neuro-AIDS today because the high activity, antiretroviral agents have largely eliminated this phase of the illness. However, when it was a problem, a group of HIV-positive Navy personnel were hospitalized in Florida Canyon for observation, allowing researchers to study the sailors' 24-hour sleep patterns. Since researchers didn't understand why we sleep, they were looking for other clues relating to correlates of sleep patterns. They found that in healthy individuals and still healthy HIV-positive sailors, there was a strong correlation between slow wave sleep during the night and peristaltic movement of the intestinal tract. This correlated nicely with cytokine patterns in the blood stream during the night. There was a loss of the high correlation between the brain's pattern and the gut's pattern as the individuals got sicker and sicker. There is a lurking idea in the immunology of sleep field that part of what sleep is for is to relax the intestinal tract. This allows the lymphoid tissues of the intestinal tract to sample what they have been challenged with during the day and react to the foreign proteins. If this peristaltic maneuvering is a way of presenting the appropriate antigens to the immune system, there is a correlation, but which way it is going, e.g. gut to brain or brain to gut, is not clear. As Dr. O'Callaghan pointed out, the visceral afferent nerves of the vagus have more partners taking information back to the brain than taking information from the brain to the gut. The gut is even more autonomous than the autonomic nervous system, having its own intrinsic nervous system. Since there is a dyspeptic syndrome described in Gulf War veterans, it would also be important to study the function of the gastrointestinal nervous system.

Dr. Haley noted that there were several anecdotal reports of veterans who responded to glucocorticoids. He stated that he had an ill Gulf War veteran with "Syndrome 2", who was tremendously impaired with fatigue, but responded to glucocorticoids (prednisone) with dramatic results. He asked Dr. O'Callaghan how this might be explained. Dr. O'Callaghan stated that it might not be surprising to see a clinical response to glucocorticoids, if the problem related to inflammation. However, he had been referring earlier to outstanding questions relating to abnormalities in the hypothalamic-pituitary-adrenal axis (HPA), including a possible disconnect with the CNS. He had discussed with Dr. Steele the need for a test of the HPA response in ill Gulf War veterans, and had understood there had not been trials of low-level glucocorticoid therapy in Gulf War veterans.

Dr. Clauw commented that high dose glucocorticoids have many effects, including many above and beyond immune effects. However, when high-dose glucocorticoids have been given to CFS and fibromyalgia patients, the effects aren't sustained. He stated that wonderful theories were being discussed, but no one in the rheumatology community thinks the immune system is playing a prominent

role in fibromyalgia. He indicated that it is hard to get scientific unanimity, and it is important to be careful and balance the different changes seen in this illness. He stated that fibromyalgia, originally called fibrositis, was originally thought to be a peripheral inflammatory disease. There is overwhelming evidence now, however, that these symptoms are not immune-related, do not respond to the usual immune therapies, and have alternative mechanisms. Dr. O'Callaghan commented that the issue of neuroinflammation had not yet been thoroughly evaluated.

Dr. Nettleman commented that many of the things the Committee had been hearing about Gulf War illnesses and exposures involved a lack of responses seen in normal animals. Yet when studies were conducted with Gulf War veterans, using a hugely expensive battery of tests, researchers often had not included normal control groups. The lack of normal controls appeared to have limited the usefulness of some research findings in both human and animal studies.

Dr. Meggs commented there was a hen study showing that triamcinolone blocked organophosphate-induced delayed neuropathy (OPIDN), if given early on. Dr. Abou-Donia stated that it had been known for many years that if a phenylmethylsulfonyl fluoride (PMSF) inhibitor was administered within one hour of exposure, OPIDN could be blocked. However, he was not aware of glucocorticoids doing the same thing.

Chairman Binns thanked Dr. O'Callaghan.

The meeting adjourned at 3:19 p.m. for a break.

The meeting reconvened at 3:30 p.m.

Neuroplasticity and Gulf War Veterans Illnesses

Floyd Bloom, MD

Neurome, Inc., and The Scripps Research Institute

Dr. Bloom discussed neuroplasticity and its potential relevance to Gulf War illnesses. ([See Appendix A – Presentation 6.](#))

Dr. Haley commented that the information Dr. Bloom presented could provide insights into how, if low-level sarin exposure was the cause of Gulf War illnesses, the initial exposure could have resulted in a sustained effect. Dr. Bloom indicated that it could relate to inhibition of acetylcholine receptors in the locus coeruleus, followed by sustained alterations that could include altered responses to novel events in the environment. He described research in monkeys and rats given alcohol and exposed to novel sensory events. The synchrony between the novel sensory event and the discharge of the locus coeruleus decreases as the animal's blood alcohol level increases.

Dr. Haley asked what if the initiating insult involved a prolonged stimulus, e.g. an initial sarin exposure that persisted for an extended period in the brain. Dr. Bloom stated the locus coeruleus could adapt and overcome the effects of this exposure. Dr. Haley commented that this could mean that by the time the exposure disappears, the individual would have established a hardwired plasticity response that might be with that person forever. Mr. Graves asked if this would be chronic. Dr. Haley stated that it would, because it was unlikely that one would undergo reverse plasticity. Dr. Bloom said he didn't know if changing the equilibrium between the activity of the target cells and the amount of norepinephrine would result in permanent changes in places like the amygdala, which is also innervated by the locus coeruleus.

This might result in an individual reacting differently to stressful events in the environment around them.

Dr. Haley commented that a plasticity “reset” might explain an abnormally low baseline blood flow in other areas of the brain. He stated this could make it easier to understand an aberrant response to physostigmine stimulus. Dr. Bloom noted that blood flow studies could be conducted to evaluate changes in blood flow to the somatosensory cortex in relation to stimulation by a sensory event. He added that it was important to keep in mind that the three main areas of cholinergic neurons in the brain include the parabrachial set across the locus coeruleus, those in the substantia innominata (which projects in part to the cortex and the hippocampus), and the large neurons of the striatum. Each of these areas have different combinations of muscarinic and nicotinic receptors. He thought it should be possible, through monkey or post-mortem studies, to determine which areas are likely to be most dysregulated in ill Gulf War veterans.

Mr. Graves noted that some of the symptoms from organophosphate exposure had a cascading effect. He wondered if the plasticity model might suggest that if an individual is exposed to a toxin, changes ensue and perhaps evolve into things like ALS or multiple sclerosis (MS) while trying to repair the damage. Dr. Bloom stated that he wasn’t able to relate what he had just described to ALS or MS. Mr. Graves stated that it just makes one wonder if all these areas are “talking to each other.”

Dr. Bloom commented that the reason he asked about ethnic differences earlier was that neurological illnesses tend to be shaped by the ethnic background of the genome. Extrapyramidal motor symptoms present differently in African-Americans than Caucasians. He stated that neurology and psychiatry textbooks are largely based upon the symptom and sign profiles of Caucasians. So when physicians and researchers encounter the same disease, presented differently in individuals with different ethnic background, the tendency is to call it something different while it might not be a different illness at its root cause. Dr. Bloom stated that John Heiden, a neuropathologist at the National Institutes of Health (NIH), has written extensively about this area.

Dr. Golomb commented that acetylcholine was an important regulator of blood flow.

Dr. Barlow suggested it might be useful to speculate about why specific areas like the dentate gyrus were affected by a cerebellar lesion. She asked this because, in patients with epilepsy, it is believed that substantial rewiring is thought to cause the long lasting deficits that occur over time in these patients, even when they are adequately treated. Dr. Bloom stated that when they did this study, they didn’t know that a single locus coeruleus neuron could innervate multiple peripheral fields. They did follow-up studies eight years later, and found that most locus coeruleus neurons would innervate more than one terminal field. The ones that innervated the cerebellum also innervated the spinal cord, and some went forward into the metencephalon.

Dr. Barlow noted that the ratio seemed higher in the hippocampus than in the cerebellum. She asked if this suggested that the hippocampus was somehow unique in its overall plasticity. Dr. Bloom stated that they had only found innervation in the three fields indicated (cerebellum, dentate gyrus and hippocampus) but that more is known today and it might be possible to redo this study with newer techniques. He added that the idea of epilepsy was important because one could have a lot of underlying brain damage that could change one’s response thresholds in epilepsy, but not be obviously affected if one lives in a protected environment without chronic stressors.

Chairman Binns thanked Dr. Bloom.

The Cause(s) and Potential Treatments of Chronic Multisymptom Illnesses Following the First Gulf War

Daniel J. Clauw, MD
Professor of Internal Medicine, University of Michigan
Director, Chronic Pain and Fatigue Research Center

Dr. Clauw provided an overview presentation describing potential causes and treatments of chronic multisymptom illnesses in Gulf War veterans and the general population. ([See Appendix A – Presentation 7.](#)) He said, in his introduction, that as a clinician he was not able to distinguish the symptoms reported by Gulf War veterans from those reported by individuals with fibromyalgia or CFS. He stated that the Committee seemed focused on exposures as causing Gulf War illnesses. He did not believe, however, that it was possible to conclude that sarin had caused Gulf War illnesses in the majority of ill veterans since the overwhelming majority of individuals who developed these illnesses did not come into contact with sarin. He also pointed out that individuals in the civilian population that have developed these illnesses but have not come into contact with sarin.

Following Dr. Clauw's presentation, Dr. Meggs asked whether Dr. Clauw's motor vehicle collision (MVC) study controlled for whether individuals had been at fault in the accident. Dr. Clauw said they were collecting these data, but had not controlled for it. He indicated that there were differences between countries with respect to rates of fibromyalgia or regional pain after a MVC and that research indicates that these differences are not related to insurance or disability status. He thought the differences related to how the health care systems operate. In the U.S., many people are told to rest after MVC, which may be the best way for acute pain to develop into chronic pain. Dr. Clauw stated that, while Gulf War veterans might not want to hear this, exposure to nerve gas or any number of other exposures might have triggered acute symptoms, but the troops' lack of regular activity while waiting for the next "bad thing to happen" might have contributed to their chronic problems. He suggested that the troops might not have been as aerobically active during this period of time as they were normally. He added, however, that this was a very complex issue and that any attempts to oversimplify it would be naïve.

Mr. Graves stated that this line of thought could have parallels with Dr. Haley's findings related to PON1. He stated that, from his own experience of being exposed to sarin gas, some individuals got very sick, while others were not affected. He stated that the majority had vertigo, nausea, etc. He agreed with Dr. Clauw in theory, but there was a point in time where Gulf War veterans were poisoned. After his initial exposure, he didn't feel bad. He kept running, exercising, and doing everything he normally did. However, as time went by, he felt as if he was ratcheting down, e.g., lower physical test scores, couldn't finish a round of golf, etc. He didn't know what was wrong until he joined this Committee.

Dr. Golomb stated that she agreed with Dr. Clauw that Gulf War illnesses were not caused solely by sarin, and the similarities with other chronic multisymptom illnesses are very important to look at. However, she didn't agree with the idea to not separate exposures and to call everything a "stressor." She stated that the epidemiology clearly showed that there are elevations in the rates of illnesses in people with specific exposures. Gulf War veterans have higher illness rates compared to veterans with other deployments that are thought to be associated with similar work and psychological stressors. She stated that, as a Gulf War veterans' research advisory committee, the Committee needed to look at the factors specific to Gulf War veterans. She noted that there was some evidence that suggests similarities with other chronic multisymptom illnesses. She referred to an earlier study that examined blood flow responses in ill Gulf War veterans, and compared these rates to patients with chronic fatigue or organophosphate pesticide exposure. She stated that it is important to consider both the similarities and differences of these chronic multisymptom illnesses, and that the Committee needed to consider effects of Gulf War exposures.

Dr. Golomb also commented that animal studies could be used to evaluate questions related to lack of exercise following injury. She noted that one of the problems with AChE inhibitor exposure is a mismatch between cell energy supply and demand leading to cell death. In animals, exercise appears to enhance damage due to exposure. She noted Gulf War veteran committee members' anecdotal comments about no longer being able to do the strenuous physical activities they had previously been capable of.

Dr. Clauw said that he didn't want to toss out the whole issue of exposures. He stated, however, that he hadn't heard anything earlier in the day that looked at alternative causes and wanted to bring balance to the discussion. He believed that a lot of emphasis had been placed on a single theory, i.e., nerve gas and sarin, and that this theory had problems. He stated that the chronic multisymptom illness theory he discussed was not necessarily the whole picture, the Committee should look broadly at the fact that there were 700,000 troops, and these individuals' biggest risk of developing Gulf War illnesses was being deployed. He stated that most troops were not exposed to sarin, nor did they take PB. If the Committee was going to look at Gulf War veterans, the Committee needed to look at all 700,000 Gulf War veterans and not just the 100,000-150,000 troops that might have been exposed to sarin and might have developed a problem associated with this exposure. He stated, however, that processes related to exposures might not have been fundamentally different than what he had discussed, i.e., exposure to sarin could have reset the autonomic baseline tone in a veteran, causing him or her to develop the same fundamental problems seen in chronic multisymptom illnesses. Dr. Golomb commented that the views expressed earlier, with regards to sarin being a primary cause of Gulf War illnesses, were not necessarily reflective of the views of the entire Committee.

Dr. Jack Melling asked if Dr. Clauw's MVC research would look at whether there were differences in the outcomes for pre-accident non-exercisers and exercisers. He stated that, based upon the hypothesis put forth by Dr. Clauw, one would expect the non-exercisers would be less likely to develop these chronic multisymptom conditions. Dr. Clauw stated that they were looking at this issue, and that there was some evidence of this in chronic fatigue and fibromyalgia. He commented that it was unusual for a "couch potato" to develop chronic multisymptom illnesses. He stated that the earlier accounts by Gulf War veteran committee members regarding how active they were prior to the war resonated with the accounts of individuals with chronic multisymptom illnesses. Many people, early in life, figured out that they feel better with exercise and high levels of activity. However, an exposure to a stressor can disrupt things, especially in relation to the autonomic nervous system.

Mr. Hardie commented that he found Dr. Clauw's discussion about the interaction between sleep and chronic pain to be very interesting. Mr. Hardie stated that he had experienced significant sleep problems following the Gulf War. However, after instruction in good sleep hygiene and some pharmacological assistance, his pain has been greatly diminished. He didn't believe that his chronic infections are related to CFS or fibromyalgia, and they have not abated over time. With regards to exercise deprivation, his experience was the opposite of what Dr. Clauw suggested, i.e., there was a cause-effect relationship in which exercise deprivation resulted in chronic illnesses. Mr. Hardie believed that the opposite was the case for him, i.e., the illnesses reduced his level of exercise.

Dr. Clauw stated that this happens in many individuals with fibromyalgia and CFS. Exercise intolerance is a hallmark of chronic multisymptom illnesses. He said the exercise aspect of their sleep deprivation study was just a model which allowed them to study controls. The researchers used these models for two reasons. First, individuals in MVCs were a convenient sample; the researchers did not cause the accidents but would be able to study the after-effects. And sleep deprivation studies provide a model which allows something to be imposed on healthy, normal volunteers that causes acute, somatic symptoms, but not necessarily in all participants. Dr. Clauw stated that the military was very interested in sleep deprivation

with respect to performance. Such studies have been conducted at Walter Reed Hospital for the last 20 years. They have found that some people can be deprived of sleep for weeks without detriments in performance, while other people may be deprived of sleep for a night or two and have tremendous detriments. Dr. Clauw noted that there must be something that causes inter-individual variability with respect to how each person responds to common occurrences. Dr. Clauw commented that researchers were limited in their ability to figure out what caused Gulf War veterans' illnesses due to the amount of time that had passed since veterans developed acute symptoms.

Dr. Golomb stated that it was important to consider effect modification by the HPA axis, but other associations and effect modifications in general were also important. She noted earlier discussions of different vulnerability factors of veterans related to exposures during the Gulf War. She provided the example of veterans with particular butyrylcholinesterase genomes in relation to effects of PB. Dr. Clauw stated that he wasn't sure why these factors wouldn't have similar effects on autonomic function. He didn't believe that it was accidental that when you look at all the exposures, the two found to be significant were sarin and PB. These exposures affect the autonomic nervous system, while other toxins, after adjustment, weren't found to be associated with illness. It is these toxins that might make ill Gulf War veterans look like those people who have a higher rate of developing pain, posttraumatic stress disorder (PTSD) and/or depression after a MVC. Dr. Clauw didn't believe what he was proposing was at odds with the notion that PB or sarin could have accelerated or caused illness in some individuals. However, it is sometimes hard to get to the bigger picture, i.e., that there are probably a lot of different ways that people could get to the same common final pathway. Dr. Golomb agreed that these viewpoints were not incongruent, but was pointing out that there might be more than one effect modifier or group of factors associated with illnesses in Gulf War veterans. Whether those factors lead to HPA changes is an additional question.

Dr. Barlow asked if Dr. Clauw's study was also looking at any potential treatments for MVC patients with an increased risk of developing symptoms. She noted that this might be beneficial for today's active troops. Dr. Clauw stated that the study he described didn't include this aspect. They hadn't wished to create confounding issues, and the cost would have been prohibitive. However, they currently had a proposal for a randomized study under review that would give low-dose beta blockers to the group of patients who were at a high risk of developing symptoms. He also noted that polymorphisms in beta 2 and 3 adrenergic receptors could actually predict a huge variance in pain and pain development. He stated that the military could use this data in several ways. Having low heart rate variability or hypoactivity of the HPA axis could be the equivalent of the World War I and II enlistment preclusion for those with flat feet. He didn't know if the military would want to develop a gene chip that could identify the polymorphisms of the individuals who were most vulnerable, but he knew that the technology to do this would be available in the next 5-10 years. The military will have to decide whether it will use this information to determine enlistees' task assignments.

Dr. Haley referenced Dr. Clauw's statement that many deployed Gulf War veterans were not exposed to sarin or PB. Dr. Haley stated that this implies that these exposures were only two of many things that might have caused Gulf War veterans' illnesses. However, this doesn't take into account the fact that two or three of these risk factors account for virtually all the cases of chronic multisymptom illness. Dr. Golomb pointed out that this was especially the case with the excess rate of chronic multisymptom illnesses above baseline. Dr. Haley stated that nobody had calculated the population attributable risk proportion. Dr. Golomb stated that she had attempted to look at this, but the problem was that most studies have odds ratios instead of risk ratios. Therefore, one could only look at the relative attributable risk. Dr. Haley commented that his group would be able to look at this in their study. He stated that Dr. Clauw had made a good point, i.e., that it was unknown what percentage of the chronic multisymptom illnesses in excess of the non-deployed population were attributable to these risk factors.

Dr. Clauw agreed that additional analyses needed to be done. He stated that it was his understanding that there was a fair amount of overlap between individuals who were or might have been exposed to sarin and those who took PB. Both of these groups would include people in the theater of operations. He stated that there were probably 450,000 troops that were not exposed to either. If the odds ratios were only 2 or 2.5 for people developing chronic multisymptom illnesses after those exposures, he said that there were a whole bunch of people who developed these illnesses that didn't have either exposure. Drs. Clauw, Haley and Golomb agreed that this was a question that could be answered empirically. Dr. Golomb commented that it was estimated that 250,000 troops took PB during the Gulf War. She noted that, if the excess fraction of the population was 25-30%, than this could explain it alone. She indicated that she didn't necessarily believe this was the case, but it could explain it.

Dr. Haley went on to reference Dr. Clauw's comments about the overlap between these syndromes. Dr. Haley stated that there were some common Gulf War veteran symptoms that were not typical of the overlap with fibromyalgia, CFS, etc. The question is whether there are biomarkers that would substantiate a real difference, e.g., temperature thresholds, quantitative sensory test, etc. Dr. Haley noted that ill Gulf War veterans had increased temperature sensitivity thresholds. He noted that there were two studies that showed an increase in temperature and pressure pain thresholds. Dr. Haley stated, however, that there appeared to be a significant decrease in these thresholds in fibromyalgia patients. Dr. Haley acknowledged that Dr. Clauw had done a study which showed a decreased threshold in ill Gulf War veterans, and this would suggest classifying their symptoms more like fibromyalgia. Dr. Haley stated the question of whether there was an increase or decrease in pain thresholds of ill Gulf War veterans needed to be addressed. Also, there was a need to do biomarker studies looking at ill Gulf War veterans, along with individuals who have well-defined CFS and fibromyalgia. This would allow comparison and differentiation between the conditions.

Dr. Clauw stated that he would have to agree to disagree about the similarities and differences of symptoms between these chronic multisymptom illnesses. He stated that the only symptom that was different was skin rash. Dr. Steele stated that it is not that these symptoms aren't experienced individually by people with these various illnesses, but rather in the proportion of people with the different syndromes who report different symptom types, for example, diarrhea or skin rash. She stated that some of these symptoms are more prevalent in Gulf War veterans than in other chronic multisymptom illnesses.

With respect to controls for functional neuroimaging studies, Dr. Clauw stated that it seemed this would depend on what the purpose of the study was, i.e., whether you were doing functional neuroimaging to determine what is going on in a person's brain or the particular cause of brain damage. If the purpose was to determine if someone had brain damage due to a toxin, e.g., sarin, he would look for control subjects within the general population who couldn't have been exposed to sarin with the exact same symptom complex as the individuals who could have or were exposed to sarin. He offered to help identify these control subjects out of his fibromyalgia population. He stated that his studies are designed to take someone with fibromyalgia and figure out what is "wrong" in the brain of someone with the disease. Thus, the kinds of controls he utilized in his studies would be different from controls utilized by Dr. Haley.

Dr. Haley stated that this then begged the question of what caused CFS and fibromyalgia. For example, CFS and fibromyalgia were virtually unknown before a few outbreaks in the late 1950s and 1960s. He pondered whether these syndromes might be due to widespread organophosphate pesticide exposure damage from home and agriculture exposures. If this was the case and a study used fibromyalgia patients as putative "non-organophosphate-exposed" controls, it might appear to show that they were the same

thing, i.e., prove that it wasn't organophosphate exposure. However, it might actually be proving that it was really organophosphate exposure. Dr. Haley stated that one had to know what the cause of fibromyalgia is before it was used as an etiologic control.

Dr. Clauw responded by saying that there was no evidence that there has been an increase in the prevalence of fibromyalgia and CFS. There have been population studies going back many years that have shown no change in the prevalence of these conditions. Dr. Clauw stated that the rate of fibromyalgia was just as high in Bedouins in Israel and the Amish in rural Canada, areas in which people haven't been exposed to these types of toxins. He stated that there was overwhelming evidence that these conditions have been around forever. They just have been called different names. He stated that there was no evidence that these were new illnesses or increasing in frequency. It is our labels and awareness of the problem that has changed. He commented that he would like to see a study identify the exact same functional imaging abnormalities in people with fibromyalgia who have the same symptoms as Gulf War veterans, because they could then say the only hypothesis to disprove was whether somehow there was widespread organophosphate or sarin poisoning in the general population. However, until this study is done with this control, Dr. Clauw had a problem inferring the cause of the illness by identifying functional neuroimaging abnormalities.

Dr. Golomb did not dispute Dr. Clauw's position that chronic multisymptom illnesses in Gulf War veterans were similar to other chronic multisymptom illnesses. She noted that there were many factors in common. However, there is a population, Gulf War veterans, who have an increased risk of developing these conditions so an important question is how we look at the exposures that are different in Gulf War veterans. Dr. Golomb stated that one should begin by identifying biomarkers that may be the same in Gulf War veterans and those with other chronic multisymptom illness, and then do animal exposure studies to identify which biomarkers are generated and associated with autonomic function and other measures in these animals. These would be ways to approach the etiologic issues of this dilemma. Dr. Clauw agreed.

Chairman Binns thanked Dr. Clauw.

Committee Discussion: Biological Mechanisms Potentially Associated with Gulf War Illnesses

Dr. Meggs commented that, during the Committee's discussion, adaptation syndromes and maladaptation issues should be kept in mind.

Dr. Steele asked Committee members for their thoughts on other possible biological mechanisms that might explain Gulf War illnesses, as well as ideas about different avenues of research that would be fruitful in understanding Gulf War veterans' symptoms. She stated that the Committee wanted to be as broad as possible when it came to potentially high value targets for exploring specific biological mechanisms.

Dr. Barlow stated that she was impressed by the data on the striatum, along with the veterans' comments about lack of attentiveness and the inability to form short-term memory. She asked if Ritalin had been considered as a possible treatment. Dr. Steele stated that there had been very few treatment studies for Gulf War veterans, and didn't believe that Ritalin had been tried with other chronic multisymptom illnesses. Dr. Clauw stated that there were anecdotal clinical stories about using Ritalin for chronic multisymptom illnesses, and it didn't seem to work. He stated that milder stimulants seemed to work better than amphetamines. He noted that almost all the drugs used to treat these illnesses raise the idea

that the cause involves some combination of norepinephrine, serotonin and dopamine. Thus, it isn't accidental that the focus is centered on the autonomic nervous system and the stress response.

Dr. Clauw commented that, when the VA and DoD funded two large treatment studies in the late 1990s, there wasn't a drug that could be given to someone with chronic multisymptom illnesses. The research group, of which he was a member, had to concede that the only options were cognitive behavioral therapy (CBT) and exercise. The study did not include any treatment that would fundamentally change the amount of pain, fatigue, or other symptoms, and ultimately didn't work. He noted that, because of the intensity of the treatment required, the effects that were seen were very modest. He finds this in his own clinical practice. If these treatments (CBT and exercise) are used alone, they don't work nearly as well as if the patient's pain and symptoms are brought under better control with pharmacologic therapy. The next step is to educate the patient that, in addition to what caused his or her pain, fatigue, and other symptoms in the first place, there have been other things that may have contributed to their continuing illness. He noted that every CBT program has a sleep hygiene component, which is evidence that something simple can have a big difference in how someone feels. But a problem they have when it comes to convincing people to try CBT is that the patient may think the physician is saying it is a psychological problem. Dr. Clauw stated that he has never thought these chronic multisymptom illnesses were psychological problems. He believes that these patients are very sick, but the patient and researchers have to realize there is more to it than just what is going on in the brain and spinal cord. The only way you can help people with chronic multisymptom illnesses improve is to understand how these illnesses mess up patients' lives in multiple ways.

Dr. Barlow noted that it was just as informative if a patient didn't respond to these drugs. It would provide insight into what they don't have. She said that this was a situation where the cause could be a host of different things. One could probe what might or might not be the cause of the illnesses by testing various available drugs that are relatively safe. If a short trial of Ritalin in a small group of patients clearly showed no response, it would be telling as to what most likely wasn't the problem. Dr. Nettleman commented that this could show what the problem might not be, but considering Dr. Haley's data showing syndromes within syndromes, it becomes more complex.

Dr. Meggs stated that there were early anecdotal reports that VA physicians were trying all types of drugs on these veterans, but this general clinical approach didn't really find anything that clearly worked. Self-reported veteran data indicates that few of these treatments provided a benefit. Dr. Clauw stated this sounds just like the fibromyalgia patients he sees, and one has to be careful about the inferences of this information. Dr. Meggs agreed, but commented that if there was a magic bullet, it might have been found by now. Dr. Clauw noted that the particular class of drugs he discussed earlier works for 45% of individuals in a clinical trial, while 20% of the placebo group will also get better. Thus, a subset of about 25% was helped by the drug. It would be better if one could show that these drugs didn't have to be given shot-gun and there was some baseline abnormality, like low heart rate variability, that would predict the responsiveness to a dual re-uptake inhibitor. Dr. Meggs asked whether these data reflected the percentage of patients cured versus those showing pain improvement. Dr. Clauw stated that the percentages he was referring to were those patients who reported a 50% improvement, which was significant.

Chairman Binns interjected that he didn't want to discourage discussion about possible treatment options, but suggested that the Committee spend the remaining discussion time by having each Committee member express their thoughts about possible biological mechanisms worthy of study, keeping in mind that the goal is to develop a treatment.

Dr. Bloom commented that there seemed to be genetic factors involved here that could help to determine vulnerabilities and diatheses with respect to the types of events precipitating these illnesses. He noted that there was considerable money being spent on DNA banks. He suggested that with today's technology, one could begin to probe for candidate genes that would show a difference between vulnerability haplotype maps and resistance haplotype maps. In addition, one would also want to probe whether other family members of the veterans have aspects of this circle of illnesses. He noted that if, for example, beta-blockers were the answer and a certain polymorphism of beta receptors was required to be responsive or unresponsive, the results might seem negative with regards to a medication when they actually would be positive. He believed that this area of study would benefit the current veterans, as well as those who might be exposed to such events in the future.

Dr. Tilson commented that the steep learning curve he faced with this particular area of research was exhilarating. He indicated that he had learned a lot from the day's presentations. However, he wished to learn more about this diathesis and what is known about the natural history of people with fibromyalgia, e.g., health background as children and young adults. Knowing these patients' history might provide tips for proper screening. Dr. Tilson suggested that the Committee may wish to recommend these types of studies. This type of research would help provide insight into both treatment and prevention options.

Dr. Nettleman noted the discussion about the temporal relationship between the initial exposure and what evolved fifteen years later. She stated that, in the future, pre-exposure data needed to be acquired. Also, with regards to DNA banks, researchers will need to look at the baseline in the population and the association between the onset of these symptoms and the prevalence of specific genes.

Dr. Melling stated if Gulf War illnesses and fibromyalgia had similar underlying issues/mechanisms, it would be worth investigating familial relationships between those who suffer from CFS and fibromyalgia and those individuals who manifest Gulf War illnesses.

Dr. Golomb agreed that diathesis/exposure relationship research was important. She also noted that autonomic function was an important marker in all of these conditions, but she would hesitate to attribute all of the symptomology to this. Oxidative stress and mitochondrial function should also be investigated.

Dr. Steele agreed that oxidative stress might be a factor and should be reviewed further. She commented that, while drafting the 2006 report, it has become clear to her that one did not have to lump or split, that is, there were likely important things to be learned from both similarities and differences between Gulf War illnesses and other chronic multisymptom illnesses. She stated that the Committee did not have the luxury of abandoning the pursuit of what caused Gulf War illnesses. Part of the Committee's charge is to understand why veterans are ill, how it relates to their military service, and help prevent these types of illnesses in the future. Further, she noted that it was difficult to determine how to treat these illnesses because none of them were well characterized pathophysiologically. In terms of mechanisms to explore, she also noted that there was an interesting body of work looking at microsomes from human liver cells and how one exposure can inhibit the cells' ability to metabolize other concurrent exposures. She suggested that the Committee may want to investigate this body of research.

Dr. Haley commented that everything being discussed by the Committee came back to case definition. It will be hard to learn anything, for certain about anything until there is a way to classify these individuals on an objective basis. He stated that the highest priority in human Gulf War illnesses research is to figure out how to classify these patients. He noted that the first classification would likely be imperfect, but progress would be in fits and starts until this was worked out. He stated that brain imaging techniques were improving and hopefully would help answer these questions.

Dr. O'Callaghan agreed that mining samples for genetic susceptibility was an important direction for Gulf War illnesses research. He reiterated his earlier point about functional responsiveness of the HPA axis, especially in light of Dr. Clauw's comment about low baseline responses. He noted that the Committee hadn't discussed much about the circuitry that develops if you don't treat pain, e.g., the development of "bad" plasticity. He wondered if there were data on interventions that were done in situations like this, and whether this could be tied in with sleep hygiene.

Mr. Hardie stated that he appreciated hearing all the perspectives that day, and was encouraged by how far the research had come. However, there was still a ways to go. He wasn't sure what further epidemiological studies would be appropriate, but noted unique exposures in the Gulf War, including inhaled or ingested depleted uranium dust, particulate matter from oil well fires, vaccinations, PB, etc. He does not believe that there was one sarin exposure or that sarin was the only chemical warfare agent to which Gulf War veterans were exposed. He knew other veterans who had personally witnessed chemical munitions before detonation, chemical alarms and detections that were verified, etc. He is a firm believer in the fifth point of Gulf War veterans' mission: "Never again." Even if there is no treatment for ill Gulf War veterans, there exists an absolute duty to ensure that we learn our lessons from the 1990-1991 Gulf War and try to prevent these types of illnesses in the future. He expressed his appreciation for the Committee members taking time out of their busy schedules to come together and provide their expertise to help ill Gulf War veterans.

Ms. Knox noted that the current armed forces represent less than 1% of the population, and the veterans at the table should be thanked for the freedom we have, as it really isn't free. With respect to physical conditioning, she stated that she probably would be classified as a "couch potato." One of the reasons she joined the National Guard was that it would keep her physically fit and within her weight limits. She stated that she had experienced chronic fatigue when she returned from the Gulf War. It was hard to identify because she had other ailments. She also comes from a family with immune disorders. She thought that some of the illnesses reported by Gulf War veterans may be due to "bad genetics." She noted that science had come a long way in fifteen years. She was glad that pre-and post-deployment DNA samples will be taken from the troops currently serving in Iraq. She believed the research answers may lie in this area.

Dr. Meggs stated that a key goal should be to discover an objective validated biomarker for these illnesses. He noted that early on in lupus research, patients were considered to have psychological multisymptom illness. However when the ANA test was developed, the disease became easy to study. Researchers had a method by which they could ensure the patients in their study had the disease.

Chairman Binns thanked the Committee for their comments.

Public Comment – Day 1

Chairman Binns stated that the Committee had a tradition of trying to do more than is required with respect to public participation in meetings. He then asked those in attendance whether they wished to provide a five minute statement to the Committee.

Ms. Venus-val Hammack, an Army Gulf War veteran, spoke to the Committee. She inquired about the status of the Committee's November 2004 recommendation that VA establish a Gulf War illnesses treatment research center. Chairman Binns stated that VA had been prepared to issue a Request for Applications (RFA) to develop data related to treatments. However, with the new center established in Texas and the dedication of VA effort there, the RFA had not been issued. However, it had not been

forgotten. He stated that the Committee had spent a lot of time developing the concept and was eager to see it realized.

Mr. Kirt Love, who represents the Desert Storm Battle Registry, spoke to the Committee. His summary of his comments is included with these minutes. ([See Appendix B – Public Submission 1.](#)) He presented a slide presentation which included mention of the Gulf War Registry, Veterans Health Initiative, and the VA's War-Related Illness and Injury Study Centers (WRIISCs). He made brief suggestions regarding research into: adverse drug reactions among Gulf War veterans, glutamate levels and correlation of substance abuse and/or ALS in Gulf War veterans, crystal formations reportedly found in muscle biopsies of Gulf War veterans, the possible role of aluminum adjuvant in Gulf War illness, anthrax vaccine production, nanobacteria, unusual infectious diseases, genomics, cellular/DNA methylation, chemical sensitivity in Gulf War veterans, dietary trials, electromagnetic pulse weapons used in the Gulf War, cerebral perfusion in Gulf War veterans, and the use of Transcranial Color Coded Duplex Sonography (TCCD) for Gulf War studies.

He recommend that: (1) PL 105-368 be amended to realign the assets of Institute of Medicine (IOM), WRIISC, RAC, and Gulf War research; (2) PL 102-585 be amended; (3) national monitoring/reporting of Gulf War medical conditions be improved, outside of DoD/VA control; (4) a public Gulf War program be created for all federal agencies, with one shared website; (5) the Gulf War Registry budget be reinstated until an alternative program could take over responsibility; (6) a Gulf War tissue catalog at the Armed Forces Institute of Pathology (AFIP) be pursued; (7) WRIISCs be created in Houston, TX, and Los Angeles, CA; and (8) simplified Gulf War treatment centers be started until they could be expanded for future projects.

Chairman Binns thanked Mr. Love for providing the Committee with hand-outs of his slide presentation.

Ms. Knox asked Dr. Haley if there were differences in the blood flow to the basal ganglia and whether there was compensation for these differences in some individuals. Dr. Haley stated that he hadn't seen evidence to suggest this was the case. Dr. O'Callaghan stated that the blood flow would be highly local to subparts of the basal ganglia. Ms. Knox commented that TCCD could look at the circle of Willis, and asked whether the basal ganglia were dependent on vertebral blood flow to that area. Dr. Haley stated that there were two issues: (1) total cerebral blood flow through the big vessels; and (2) local changes in blood flow in particular areas, which is primarily determined by the metabolic rate of the cells in those areas. There are probably other factors as well, e.g., cholinergic regulation of blood flow. He didn't see TCCD being utilized to identify predisposing factors, but rather for diagnostic purposes. Examination of blood flow differences is aimed at inferring how things are working. One could try to control for the "big" effects in order to see the "little" effects. He stated that he would discuss this issue in more detail during his presentation the next day.

Ms. Denise Nichols, a Gulf War veteran, spoke to the Committee. A summary of her comments is included with these minutes. ([See Appendix B – Public Submission 2.](#)) Her remarks focused on unpublished information that she had received from an anonymous source regarding the number of Gulf War veteran deaths from thyroid cancer, testicular cancer and leukemia between 1991 and 1994. She believed this information to be significant, and that something "happened" to Gulf War veterans. She stated that issues surrounding diagnosed service-connected illnesses of Gulf War veterans need to be pursued just the same as the undiagnosed and neurological diseases. She suggested that VA screen for testicular, thyroid, and brain cancers in Gulf War veterans. She stated that an individual could have a neurological disease on top of a cancer. She asked the Committee to: (1) review this information; and (2) push for more information from the VA about diagnosed illnesses and death rates. She commented that

she was “past what happened” in the Gulf War, and was now pushing the Government to provide education, training, testing and treatment to Gulf War veterans.

Ms. Alison Johnson, who represents the Chemical Sensitivity Foundation, spoke to the Committee. She distributed a selected bibliography of research articles on chemical sensitivity to the Committee, along with an email from a World Trade Center (WTC) firefighter that described his health concerns. She stated that she was in the process of finishing a documentary about chemical sensitivity following the WTC attack. She stated that Hurricane Katrina was the latest disaster that would result in an increase of chemical sensitivity and that many people hadn't thought about this aspect of the hurricane's fallout. She discussed the problems faced by individuals with chemical sensitivities, including the lack of public awareness and belief that this was not a real condition.

Kathi Krome, Director of State Advocacy Outreach for the ALS Association, spoke to the Committee. She stated that she was there on behalf of Dr. Lucie Brujin, the ALS Association's Scientific Director and Vice-President. She thanked the Committee for its leadership and hoped that it would continue to recommend research into ALS. She noted that several studies, including one by Dr. Haley, showed elevated rates of ALS in Gulf War veterans, and that these elevated ALS rates were alarming because they were occurring in relatively young veterans. She was accompanied by Jim Thew and Andy Eddowes, two veterans with ALS. She stated that they were taking time away from the ALS Association's annual advocacy conference, which was taking place on Capitol Hill that week, and wished to encourage this Committee, Congress and the administration to continue to study ALS. It is not known why Mr. Thew and Eddowes have ALS, or whether it was due to their military service, in general or specific service in the Gulf. Ms. Krome noted that there was an excess of ALS in all military veterans regardless of the time period in which they served, and it was unknown what may be causing this increase. Until these questions can be answered, the ALS Association strongly advocates the continued commitment to ALS research, including Gulf War-specific research. Through Gulf War research, insight can be gained, not only into the incidence of ALS in all military veterans, but also Gulf War-related illnesses. Moreover, this research will benefit the entire ALS community. On behalf of the ALS Association and the veterans living with ALS, she thanked the Committee for its support and expressed hope that it continue to recommend research in this disease in Gulf War veterans.

Mr. James Thew, a Navy veteran with ALS, spoke to the Committee. He expressed his deep appreciation for his wife's commitment to helping him live with this disease. He served two tours in the Gulf, starting in 1992, as an aviation machinist. He is now 34 years old, and can't run around and play with his 10-year-old son. He thanked the Committee for trying to help find some answers for the many veterans living with ALS and other neurological disorders.

Mr. Andrew Eddowes, a 46-year-old veteran who is living with ALS, spoke to the Committee. While still in the service, Mr. Eddowes manifested ALS symptoms and was ultimately diagnosed with the disease in 2003. He has since been relocated by the Navy to Northern Virginia to be closer to medical care, and has been medically retired with 100% disability.

Chairman Binns thanked the Committee members for their time and patience through the day's long proceedings. He noted that many of members of the public who were attending had been attending meetings such as these for many years, telling their stories and urging Committees and doctors to find solutions. He thanked them for their patience as well.

Chairman Binns announced that Secretary Nicholson would not be able to attend the Committee's meeting the following day, as had originally been scheduled. Former Senator Sonny Montgomery, a World War II veteran and long-time veterans' advocate, had recently passed away, and Secretary

Nicholson would be attending his funeral on the morning of May 16th. He noted that the next day's meeting would be held at VA Headquarters, in Room 230, a conference room named in honor of Mr. Montgomery. Both the Deputy Secretary and Under-Secretary of Health were traveling and would not be able to attend either. Thus, the May 16th meeting would begin with the first presentation on the agenda.

The meeting adjourned for the day at 6:15 p.m.

Day 2

The meeting reconvened on Tuesday, May 16, 2006, at 8:35 a.m. in Room 230, VA Headquarters, 810 Vermont, N.W., Washington, DC.

Chairman Binns announced that the Committee's next meeting would be held August 14-15, 2006, at VA Headquarters in Washington, DC. He reiterated for new audience members that Secretary Nicholson would not be able to join the Committee that morning.

Chairman Binns referenced two comments by veterans on the previous day with regards to special sensitivities following the Gulf War. He noted there were anecdotal stories about two Gulf War veterans, who later developed ALS, who reported major subsequent exposures to pesticides following the Gulf War. One of these veterans wrote a book about his experience, and included a plea that the VA warn veterans to avoid these types of exposures. Although there was only anecdotal evidence on which to base such a warning, Chairman Binns noted that this might be one of the easier things that could be done that could make a difference in veterans' health, and that additional research could be done in this area.

Dr. Golomb noted that there was mounting epidemiological evidence of an association of Parkinson's disease with pesticide use. Because of the issues with the striatum and illness in Gulf War veterans, she stated that many have wondered if there is an increased risk of early Parkinson's disease in Gulf War veterans. If data were available on this question, it might provide a foundation for such a hypothesis. Dr. Golomb asked if any inquiry had been made into this issue. Dr. Steele indicated that no studies of Gulf War veterans had been done, although the RAC had recommended this type of research. Dr. Golomb noted that she currently had a young Gulf War veteran patient who has advanced Parkinson's disease. Dr. Steele added that a local American Legion officer had talked with her about several Gulf War veterans with Parkinson's disease that he was assisting with disability claims.

Dr. Tilson commented that these were anecdotes and that, while they may be signals of a problem, to warn somebody about a problem that wasn't a problem was crueler than failure to warn. Dr. Golomb reiterated that there was epidemiological evidence connecting pesticides and Parkinson's disease. She stated that pesticides are known oxidative stressors, and oxidative stress has been linked to the development of these types of neurodegenerative diseases. Dr. Tilson stated that, in that case, there would be an obligation to look at the epidemiology and see if it was reported by one study or multiple studies. He wanted to clarify that there was no conclusive or scientifically-agreed-upon link between pesticides exposure and Parkinson's disease in the public health domain. Dr. Golomb noted that there were many studies regarding this issue, to which Dr. Tilson agreed. Chairman Binns stated that he understood that it wasn't something that the Committee could make a recommendation on at this time. He was only suggesting that it might be an avenue for future research.

Dr. Haley stated that VA was asked to do such an analysis a couple of years ago and asked if it had. Dr. Steele stated that it had not. Dr. Haley commented that the VA should have the data to determine whether there are elevated rates of Parkinson's disease in Gulf War veterans. Dr. Steele noted that VA had data

on how many Gulf War veterans with Parkinson's disease were seen by VA, but it did not have data on the prevalence of Parkinson's disease in Gulf War veterans. She stated that the Committee has recommended doing epidemiologic studies on this specific matter.

Dr. Meggs noted that the question of the value of chemical avoidance could best be addressed by a simple, controlled, environmental control unit experiment with individuals exposed to various agents and monitoring their health over time. He noted that besides anecdotal evidence from clinical practice, there was good solid animal research on adaptation syndrome, maladaptation to chronic exposures, shock reactions, etc, which provided a scientific basis for doing this research. He stated that this was a simple, inexpensive approach that could be done to put to rest this whole issue of whether ongoing exposures drive the continuing illness. He noted that much was already known about the pathophysiology of inhaled exposures on the respiratory system but it isn't known whether a similar pathology is occurring in the brain. He stated that the Committee should recommend this type of research.

Dr. O'Callaghan commented that the National Institute of Environmental Health Sciences had set up four or five centers several years ago to investigate the possible link between pesticides and Parkinson's disease. The results from these centers' animal studies should be published shortly. The studies were also looking at pesticide interactions with other chemicals to which one might be exposed. He indicated that this was a large program, and something on which the Committee should follow-up.

Dr. Golomb commented that there were no known health benefits associated with pesticide exposure, so a recommendation would not require the same high threshold as that needed to advocate a drug. She noted that there were several epidemiology studies, including one from England, which showed those with organophosphate exposure had 10 times the number of symptoms as non-exposed. Subsequently, Dr. Nicola Cherry's group looked at paraoxonase genotypes in individuals with chronic health problems attributed to organophosphate exposure. They found that sheep dippers with chronic health problems were more likely to have the paraoxonase variant that is a poor metabolizer of diazinon. She stated that she saw no harm in suggesting that it would be prudent to avoid future organophosphate pesticide exposures, because it is prudent for all individuals to avoid these exposures.

Mr. Adrian Atizado noted that VA had established five Centers of Excellence for Parkinson's disease. He suggested that this might be an avenue to pursue for more information.

Dr. Steele introduced Dr. Abou-Donia. She commented that his presentation would be one of two that would inform the Committee about the potential contributions of microarray technology and proteomic studies for understanding Gulf War illnesses and effects of Gulf War exposures.

Gene Expression Profiles Following Sarin Exposure

Mohamed Abou-Donia, PhD
Professor, Duke University Medical Center

Dr. Abou-Donia provided an overview of several of his team's laboratory studies related to the effects of sarin, demonstrating enzymatic, behavioral, histological, and gene expression effects of sarin exposure. This included recent findings related to delayed and long-term (10 month) effects of low-dose sarin exposures (0.1 LD50). ([See Appendix A – Presentation 8.](#)) He stated that his team started with the hypothesis that chemical exposure might be involved in Gulf War illnesses. Fourteen years later and after many studies, he was convinced that this is the case. He is also convinced that it was not one specific chemical, but rather multiple chemical exposures, that contributed to Gulf War illnesses.

Chairman Binns thanked Dr. Abou-Donia for his presentation. He also thanked DoD for funding all of these studies with reference to sarin.

Dr. Meggs commented that Dr. Abou-Donia's research was brilliant and beautiful relevant work, and was also a case of science finally catching up with 30 years of clinical observations of OPIDN in individuals sprayed with organophosphates. He went on to ask Dr. Abou-Donia, on behalf of the non-scientists in the audience, if he would briefly explain how gene array expression research was accomplished. Dr. Abou-Donia stated that the basic approach was to: (1) treat/expose the study animals to the agent in question; (2) dissect the animals' brains; (3) isolate the RNA; and (4) analyze using a variety of different gene chips. He stated that the challenge was that gene array technology generated a tremendous amount of data, and it is unclear what it means with regards to up- or down- regulation of the genes. There is software available to help analyze this data. The technology of the microarray chips is focused right now on collecting the data. They hope that the science will catch up and provide insight into what it means that some of these genes are up-regulated and others are down-regulated. He stated that his team's next step, once funds were available, would be to isolate mRNA from their test animal blood samples and see what genes are comparable to the genes in the brain that actually behave similarly, either up or down. This might provide a biomarker for organophosphate exposure.

Dr. Nettleman asked if Dr. Abou-Donia had been able to measure sarin levels in the test animals' tissue and whether the sarin persists over the three-month period of time. Dr. Abou-Donia stated that sarin doesn't persist in the body, having a half-life of a 1/2 hour. He compared sarin exposure to a bullet injury, e.g., sarin gets into and out of the body, doing its damage along the way. Dr. Golomb asked if this was equally true for the brain, i.e., was there an effective mechanism to clear sarin from the brain. Dr. Abou-Donia stated that the sarin that remained in the brain was bound to acetylcholinesterase and could stay there for months. Dr. Nettleman commented that it would seem that a small amount of the sarin would be released and become free over time. She asked whether the route of exposure, e.g., injection vs. inhalation, had an impact on the LD₅₀. Dr. Abou-Donia stated that their first step had been to determine the LD₅₀ of sarin in mice, which was 100 mg/kg. The LD₅₀ does not differ between the various routes of exposure. The LD₅₀ is the LD₅₀.

Dr. Steele noted that Dr. Abou-Donia's findings regarding the 0.1 LD₅₀ dosage findings were most interesting, considering there was no neuronal cell death seen in the animals after three months, but it was seen after 10 months. She asked if they had looked at behavioral measurements at three months, and whether there were any changes seen at that time. Dr. Abou-Donia stated that deficiencies were seen in all animals, following all treatments, at three months and even more so at six months, even though the animals looked normal. He stated that this research made a strong case that relates sarin to Gulf War illnesses, but it doesn't explain every case of Gulf War illness.

Dr. Barlow commented that microarrays were very expensive right now. She noted that samples could be stored indefinitely at -80°C until better arrays were developed. Dr. Abou-Donia stated that they had done this, i.e., frozen their samples for later use.

Dr. O'Callaghan asked whether all of the study results were dependent on the generation of seizures in the animals. Dr. Abou-Donia stated that they were not, noting that only the LD₅₀ animals had seized and there were no deaths below the LD₅₀ level. Dr. O'Callaghan noted Drs. Shih and McDonough at the U.S. Army Medical Research Institute of Chemical Defense had a microwave fixation device that preserves steady-state phosphorylation, which would allow them look at MDA receptor activation by looking at phosphoforms. He asked if this work had been completed. Dr. Abou-Donia stated that Dr. Shih's lab had done the chemistry work in this study and found up-regulation of the MDA, glutamate and APA receptors, which are involved in calcium release.

Dr. Meggs noted that AChE molecules, which are permanently bound to sarin, do not regain function. He asked what was known about these inactivated bound molecules, what happens to them, and whether they adversely affect the subject. Dr. Abou-Donia stated that it was known that the inactivated molecules were present and eventually metabolized, but it wasn't known if they caused damage.

Chairman Binns noted that Dr. Abou-Donia's findings regarding low-level sarin exposures were very striking. He asked if Dr. Abou-Donia would be repeating his gene expression study with lower exposure levels of sarin. Dr. Abou-Donia stated that he was not able to repeat the tests because his lab no longer had access to sarin. He stated that they hadn't included lower exposure levels of sarin because they wanted to make sure they saw results and didn't want to waste chips. In light of their findings, he wished now that they had done the lower exposure level tests.

Mr. Love commented that there appeared to be a certain synergism between sarin and PB. He asked if there might be a possible genetic aberration involved in certain responses to PB or PB/sarin exposures. Dr. Abou-Donia stated that he had not performed gene array studies with PB. He noted that his group had done other types of studies using sarin alone, PB alone, and sarin/PB combination, and found a synergistic effect with the sarin/PB combination. Dr. Haley noted that there was discussion on a phenomenon called promotion in the OPIDN literature. If PB is given before exposure to an organophosphate, a person's likelihood of developing OPIDN is reduced. However, if PB is administered after the exposure, a person's likelihood of developing OPIDN is actually accelerated or increased. Promotion can occur over a long time, e.g. days or weeks after the initial organophosphate exposure. One possible mechanism theory for this is that exposure to organophosphates results in negative radical production. These negative radicals may be displaced from a sequestered site when PB is subsequently administered. This could possibly increase the damage. Dr. Haley noted that there may be other possible mechanisms as well.

Chairman Binns asked what implications this might have on future treatment research, and whether this might mean that the damage has been done to these veterans and there wasn't anything that could be done to reverse it. Dr. Abou-Donia stated that there were two sarin exposure scenarios, i.e., high and low doses. He commented that it was easier to protect against sarin-induced death. Physicians can administer chemicals to hydrolyze paraoxonase-1, the enzyme that is phosphorylated with sarin. One such drug is pralidoxime (2-PAM), which must be given within the first 12 hours of exposure, i.e., before the enzyme becomes aged and negatively charged. Another way to protect against acute sarin exposure is to use muscarinic receptor antagonists, such as atropine. Atropine binds to the muscarinic receptor and prevents AChE binding and overstimulation. Valium and analogs have also been used to protect against seizures in high-dose exposure situations.

Dr. Abou-Donia stated that treating individuals who have received a low-dose of sarin was problematic and that this was new territory. He believed stress and apoptosis are related to induced problems. He stated that research should be done to study the effects of antioxidant treatment in animals as soon as possible following sarin exposure. These animals should be followed for a long period of time to see if problems ever arise. With respect to today's veterans, Dr. Abou-Donia stated that damage already done couldn't be reversed. However, the brain has a tremendous amount of plasticity and can recover some neuron function over time. He suggested that veterans would do better if they avoid future chemical exposures.

Dr. Golomb stated that a vicious cycle was created when reactive oxygen species production begins. Once the production of reactive oxygen species is initiated, mitochondria are damaged. Once mitochondrial DNA is damaged, the mitochondrial respiratory chain is affected, which leads to more

release of the reactive oxygen species in the effort to produce energy. This process can't necessarily be reversed, but may be slowed by on-going antioxidant delivery.

Dr. Clauw questioned whether giving antioxidants to someone fifteen years after injury/exposure would really work. Dr. Golomb stated that this was an empirical question, and that there was evidence that CoQ10 provided sustained benefits with regards to fatigue in a cohort of chronic fatigue syndrome patients. These benefits appeared more quickly in the higher dose group and were sustained until the CoQ10 was withdrawn. It did not lead to a permanent benefit, but did provide symptomatic improvement. There is other evidence that CoQ10 protects against many of the adverse outcomes associated with oxidative stress, and might help retard progression of problems in conditions like these. She stated that not all antioxidants were equal and discussed various ones. Mr. Graves stated that he personally tried CoQ10 for three months and found it had no effect on him. Dr. Golomb stated that there was still a question of whether it delayed progression of problems.

Dr. Barlow stated that her previous research at Merck was focused on trying to find new treatments for stroke and neurodegeneration. They did a very thorough investigation, which included looking at antioxidants. She agreed that not all antioxidants were created equal. However, in a situation like this, the purpose of taking an antioxidant would not be to try to reverse any of the processes that already exist, but rather to slow additional decline if later exposed to another stressor. She didn't think it was realistic, given any of the antioxidants available for patient ingestion, to think that there was an antioxidant that would provide reversal of these processes. Dr. Golomb agreed with this assessment, and stated that her point was the retarding of progression.

Dr. Meggs stated that, in clinical settings, Gulf War veterans' symptoms are reported as "remitting" and "waxing and waning." These are very complex temporal relationships. What is governing this is unknown, but testable. Dr. Meggs stated that antioxidants might be of benefit to those individuals who are about to flip over into a chronic progressive form of illness.

Dr. Nettleman stated that it was testable, but it would have to be a large, randomized, controlled study because it was a waxing and waning illness. There is a similar problem in Alzheimer's research. The effects would have to be significant in order to be worthwhile. She stated it may need to be done, but wouldn't be easy.

Mr. Graves discussed an intermittent vision problem that his ophthalmologist is not able to explain.

Dr. Tilson asked Dr. Abou-Donia about the dangers of doing sarin research. Dr. Abou-Donia stated that his laboratory handled lots of organophosphates. He noted that the sarin used was very diluted, and he was the only one who handled it. Dr. Tilson noted that Dr. Abou-Donia's work was very courageous and very important.

Dr. Steele introduced Dr. Baraniuk. She noted that, in other multisymptom illnesses, there have been a number of studies looking at gene expression in the blood. However, looking at proteins expressed in cerebrospinal fluid was particularly interesting and hopefully would be informative with respect to processes in the central nervous system.

“A Chronic Fatigue Syndrome Related Proteome in Cerebrospinal Fluid”

James N. Baraniuk

Associate Professor, Georgetown University Medical Center

Dr. Baraniuk presented results from his recently-published study that utilized proteomic technology to identify cerebrospinal fluid proteins that distinguished Gulf War illness and chronic fatigue syndrome patients from healthy controls. ([See Appendix A – Presentation 9.](#))

At the conclusion of his presentation, Dr. Baraniuk noted that with mRNA microarrays, there were huge increases in mRNA. However, in an activated state, some of the mRNA expressed may be inhibitory, producing an overall nil effect. He added that with proteomics, 60% of the proteins are phosphorylated and this changes their function. An advantage of using proteomics was the ability to look at oxidative damage and other changes in the protein.

Dr. Golomb stated that this was an important area of research. She personally believed that Gulf War illness probably shares several common pathways with other chronic multisymptom illnesses. However, she noted that Dr. Baraniuk’s study design didn’t allow for comparisons between different multisymptom illness cohorts, and was only capable of identifying proteins they shared. She pointed out that Cohort 1 looked at the proteins present in both chronic fatigue syndrome and Gulf War illness samples, but not the healthy control sample, while Cohort 2 was focused on the difference between chronic fatigue syndrome and healthy controls. There wasn’t a separate Gulf War group. If there were additional proteins found in the Gulf War samples but not the chronic fatigue samples, this study design would not have allowed these to be detected. Therefore, it was important to not draw conclusions about them being the same groups based upon this study design. Dr. Baraniuk agreed that was the case.

Dr. Barlow noted that Dr. Baraniuk’s group collected three sample tubes for neurotransmitters, cell count and proteomic tests. She asked if there were any differences in the cellularities. Dr. Baraniuk indicated that there were none. Dr. Barlow asked if they would go back and look to see if there were differences in the neurotransmitters measured and see if this covaries in any direction with the proteins found. Dr. Baraniuk stated that work along those lines had had been published. They had done it with: (1) opiates in collaboration with a researcher in Denmark; (2) corticotrophin-releasing factor in a fibromyalgia subset; and (3) substance P. However, in this case, they would have needed to concentrate the entire cerebrospinal fluid sample to detect the submolar concentrations present. Dr. Barlow asked if any correlations with varying levels of neurotransmitters and neuropeptides were seen in the previous studies or were they completely independent. Dr. Baraniuk stated that corticotrophin-releasing factor was decreased.

Dr. Barlow asked if any research was looking at this disease as being related to the unfolded protein response. Dr. Baraniuk stated that this was exactly the same hypothesis, because cerebrovascular amyloid angiopathies (CAA) require a protease to clip the constrained structure of the protein, which results in the potential energy being released and the protein being refolded into a beta-pleated sheet. These beta-pleated sheets will actually form stacks that can insert into membranes, forming pores, and ultimately lead to the cytoplasm pouring out of the damaged cell. It would be analogous to Complement Factor 9. It was thought that this may be one of the central nervous system’s protective mechanisms against bacterial disease because of the large number of proteins that have this capability. Dr. Barlow thought it possible that new insights into the etiology of this disease might be gained by looking at other diseases where the unfolded protein response was important. These diseases can affect the liver, brain, heart and vascular system. This might be a place to look for a new hypothesis. Dr. Baraniuk stated that his group was rechecking the protein fragment sequences to see if there was evidence of cleavage related to protease activity.

Dr. Meggs stated that he wished that he had been on the grant study section, because this work definitely needed to be extended. He asked whether it might also provide a hint at possible treatment, i.e., how would one deactivate the central nervous system's innate immune mechanism. Dr. Baraniuk stated that he had been thinking about this. Because oxidative proteins were present, this might be one area for further investigation. Dr. Baraniuk noted that, as previously discussed, it may be a question of having the proper antioxidants in the right place. He pondered whether the military's meals-ready-to-eat (MRE) have enough antioxidants in them and/or the spectrum of antioxidants required. This is another nutritional component of diet that could be investigated, along with carefully selected supplements taken at various times, e.g., before entering theater, during deployment, and upon return from theater.

Dr. Clauw asked Dr. Baraniuk to discuss the challenges of doing proteomics with plasma and cerebrospinal fluid. He noted that the protein expression profiles in serum would likely be quite different. However, lumbar puncture studies were challenging, leading to small "n"s in this study. Dr. Clauw noted that the military had collected pre- and post-deployment sera from the current troops, and this would allow for proteomic analyses of their plasma.

Dr. Baraniuk stated that both cerebrospinal fluid and plasma have twelve proteins that account for 99% of the proteins present. Therefore, when one analyzes plasma, one is looking for the 1% of less abundant proteins. These low-abundance proteins have to be concentrated so they can be detected by mass spectrometry. This is possible to do in plasma studies because a large sample (1 pint of blood) can be collected. Also, one can assess the peptide fraction of a plasma sample by a large scale peptide extraction method. They are looking into these issues at the moment.

Dr. Baraniuk said that another major issue in this type of research was the ability to determine which components produced in the brain will get into the plasma and be useful markers for CNS dysfunction. He stated that this would be difficult to tease out. A couple of known proteins can be detected in this fashion because they are not produced in the periphery. With respect to proteins produced in both the brain and periphery, Dr. Baraniuk expressed doubt at being able to detect a signal from the brain. He believed that determining whether there are other systemic manifestations that show up in the muscle, liver, etc., would be a much more productive avenue for investigation. He also believed that cerebrospinal fluid was a better source to answer these questions, but it is difficult to get samples.

Dr. Bloom asked if Dr. Baraniuk had been able to identify the cellular origin for these proteins, i.e., whether they are known to be neuronal or microglial. Dr. Baraniuk stated that there were other studies in animals and a few corroborating human studies in which histochemistry and hybridization had been done. However, changes haven't been examined in models relevant to this disorder. Most of the work had been done in Parkinson's and Alzheimer's settings or in general discovery methods.

Ms. Nichols asked whether samples from the Seabee study could be used in this type of study. She suggested that sampling of the Khamisiyah group be done as well. She stated that there needed to be a "jump start" on Gulf War research, and that she had been excited when she had read about Dr. Baraniuk's research. These same samples could be used to test for antioxidant treatments. Dr. Baraniuk stated that he hoped to do this type of research, and was looking for collaborators and research monies to do it.

Dr. O'Callaghan asked Dr. Baraniuk what the signature would look like if this study was done with Alzheimer's patients and healthy controls. Would one get a larger number of peptides? He commented that Dr. Baraniuk had nicely honed in on a difficult question with a proteomic approach versus a huge profile seen in on-going neurological diseases. Dr. Baraniuk stated that, in the supplement to this paper, they had listed all of the proteins found. In addition, in a paper in preparation, they had compiled all of

the cerebrospinal fluid proteins and all of the diseases in which they were uniquely expressed. He stated that the number found in Alzheimer's patients is not much greater than a couple score. One would expect it to be much larger at first, but one has to remember that, like every other organ, there are only a few stereotypical ways that the brain can respond to an inciting injury. All of these studies may be picking up the final common pathway. In fact, many of the proteins they found have not been previously described in cerebrospinal fluid.

Dr. Haley asked whether the protein profiles found in chronic fatigue patients were different from the protein profiles of Alzheimer's patients. Dr. Baraniuk stated that none of the proteins in their biosignature had been previously reported.

Dr. Steele stated that she thought that Dr. Baraniuk's idea of developing a multisymptom illness "signature" set of proteins was extremely useful, that is, developing a model using five of the identified proteins to predict who was a "case" and who wasn't. She asked if they had been able to look back to see if the protein expressions varied with other characteristics, e.g. gender, age, clinical characteristics, etc. Dr. Baraniuk stated that they didn't feel like they had enough subjects or power to do further analyses. With this particular biosignature, they had estimated from power calculations that they would need 13-14 subjects per group in order to find this biosignature. However, they would want to vastly increase these numbers in order to do subset analyses.

Chairman Binns asked Dr. Baraniuk for his ideas about other research opportunities coming out of this work. Dr. Baraniuk stated that it would be useful to study antioxidants in these models. Are there any amyloid deposits in these patients' brains? Is there any histology consistent with this? He mentioned that one of the proteins that can lead to this type of pattern is a prion protein, so he would ask if there was any evidence of prion disease in these patients. He also would look to see if: (1) certain people were predisposed to having the development of this syndrome; and (2) there were methods by which to turn off the glial activation. Regardless of what the inciting event was, the pathology must be actively studied so that new drugs could be developed. He stated that there was a whole host of compounds that would act on inhibitory receptors, such as the glutamanergic terminals. He stated that there was a tremendous opportunity to study muscarinic M2 agonist, GABA-B agonist, MPY-2 agonist, alpha-2C agonist, etc., that may be able to shut down any glutamanergic activation that may be part of this cascade. He stated that it is possible to measure glutamate that would be induced by an excitotoxic state, but they didn't have enough sample to do the analyses to detect it. He also mentioned that they were working towards quantifying these proteins to see if they were significantly increased in disease. This would greatly expand the proteins identified as potentially playing pathogenic roles.

Mr. Love asked, in light of the VA's proposed Gulf War veteran DNA bank, whether they might consider doing telomere research and look for telomere repeating DNA patterns. Dr. Baraniuk stated that they were looking at this in relation to another unrelated study, but agreed it would be a terrific project.

Chairman Binns thanked Dr. Baraniuk.

The meeting adjourned for a break at 10:50 a.m.

The meeting reconvened at 10:55 a.m.

Update on Research in Persian Gulf War Veterans Illnesses – May 2006

Beatrice A. Golomb, MD, PhD

Associate Professor, University of San Diego School of Medicine

Dr. Golomb provided an overview of recently-published research relating to Gulf War veterans' illnesses. ([See Appendix A – Presentation 10.](#))

The meeting adjourned for lunch at 11:30 a.m.

The meeting reconvened at 12:30 p.m.

Current Gulf War-related research at VA

William Goldberg, PhD

Gulf War Research Portfolio Manager, VA Office of Research and Development

Dr. Goldberg provided an overview of the status of VA Gulf War research, as well as the federal interagency Deployment Health Working Group's Annual Reports to Congress. He stated that the 2004 and 2005 Annual Reports to Congress had been approved and had been sent to the printer. Once they are physically delivered to Congress, the reports would be made available publicly on the VA ORD website. Copies will be distributed to Committee members.

Dr. Goldberg distributed two handouts to the Committee. This material had been incorporated into the 2004 and 2005 Annual Reports. The first document set forth criteria for how VA determined what was included in the Gulf War research portfolio. The second document outlined the revised framework for the Annual Reports to Congress. The original reports addressed a list of 21 questions that defined Gulf War research. Dr. Goldberg stated that the good news was that Gulf War research had moved beyond the boundaries of just epidemiology questions. Thus, these 21 questions have been converted into 21 topics in the 2004 and 2005 Annual Reports. This will allow the continued use of this framework without abandoning previous work. The original questions were grouped into 9 "super" topics. The reports' structure has been redesigned accordingly, and they were also categorizing VA's Gulf War portfolio in accordance with this new design.

Dr. Goldberg reviewed the list of studies and their respective categorization in the VA's Gulf War research portfolio. He noted that there were four new projects in FY2006. Two of these projects fall under the category of "pharmacogenomics on chronic multisymptom illnesses." These are projects headed by Dr. Melvin Blanchard and Dr. Nancy Klimas, respectively. The other two projects are at the Environmental Hazards Center in San Antonio, which has reconstituted itself into a different center mechanism with a focus on Parkinson's and ALS. These two projects will look at the effects of PB, permethrin and the combination on the onset and time course of illness in their animal model of ALS. Dr. Goldberg stated that these studies might provide some insights into the questions raised earlier in the meeting.

Dr. Goldberg noted an April 25, 2006, *Neurology* article by G.M. Pasinetti and colleagues entitled "Identification of potential CSF biomarkers in ALS". This is a joint VA/NIH project. These researchers found three proteins in cerebrospinal fluid that have a 90% hit rate of distinguishing ALS from neuropathic pain and controls. The next phase of this research is to take a group of Gulf War veterans with ALS and see if this group has any additional and/or similar markers. Like Dr. Baraniuk's work, this project used mass spectrometry. However the proteins identified in Dr. Pasinetti's work are not the same as those identified by Dr. Baraniuk.

Dr. Goldberg offered to answer any questions that the Committee might have. He noted that Dr. Joel Kupersmith and Dr. Timothy O'Leary, Director, VA Biomedical Laboratory Research and Development Service, and Acting Director, VA Clinical Science Research and Development Service, were present to take questions as well.

Dr. Meggs asked about the status of the VA brain/tissue banking initiative. Dr. Goldberg stated that the biorepository project was being funded through the Boston Maverick VA Center. It is in the process of being established. They were, however, able to collect the first brain sample from a Gulf War veteran sooner than expected. This veteran had made it known that he wished to donate tissue samples, and special arrangements were made to accomplish this.

Dr. Steele asked if there would be separate DNA and tissue banks. Dr. O'Leary stated that it was taking some time to establish procedures to ensure the optimal banking of central nervous system tissues. He stated that there was an ongoing educational process, in collaboration with Columbia University's brain bank, to address these concerns. He stated that both CNS and non-CNS tissues would be collected within this framework. They will also be collecting tissues from individuals who could be considered a control group. The tissue bank itself will be in Tucson, AZ, while the DNA bank will continue to be at Boston Maverick. The DNA bank is capable of accepting at least 100,000 additional specimens. Other DNA banking activities are being developed as part of the Genomic Medicine initiative. How this will play out in detail has not yet been decided and there are a lot of issues that still need to be addressed. Dr. O'Leary noted that a Genomic Medicine Advisory Committee had been established to help with some of these issues. He stated that ORD had not seen a need to expend money on additional infrastructure. Dr. Steele asked whether a broad or limited range of tissues would be collected. Dr. O'Leary stated that it would be prudent to take a broad perspective when it comes to specimen acquisition.

Ms. Nichols noted that Kirt Love had initial contact with the Gulf War veteran who had donated his tissues. She had brought this issue up in the past in relation to Jason Whitcomb, whose parents founded Desert Storm Justice Foundation. She asked about what type of publicity would be utilized to let the veteran service organizations and the veterans themselves know about this tissue/DNA bank initiative. Dr. O'Leary stated that he was interested in hearing the Committee's perspective on this question. He said that ORD would like to make sure the system was entirely up and running before they advertised the program broadly to the veteran community. This would limit frustration that could come from a patchwork system. Dr. O'Leary stated that he didn't think the program was ready at this point, but that it wouldn't take too many months to accomplish this.

Chairman Binns asked for some background information about tissue banking for the nonscientists in the audience. He asked whether tissues were simply collected and stored, or if the tissues were automatically analyzed in some way and how researchers found out that there was something significant in a particular sample. Dr. O'Leary stated that a tissue bank was a research resource. He stated that there may be some initial characterization of material. This characterization can occur in a number of different ways. Some of it may just be documentation, e.g., time lapse between time of death and specimen collection. Sometimes a tissue bank will do a preliminary assessment of DNA integrity. This will tell whether researchers will have high quality DNA samples. To some degree during initiation of this tissue bank, this level of characterization will be a moving target until they know where the demand for the tissue is. At the moment, Dr. O'Leary didn't anticipate extensive characterization of the samples. The researchers who will be generating the hypotheses are in a better position (equipment and background) to do these types of analyses.

Chairman Binns asked if ORD would do something proactive if they acquired a brain that appeared particularly worthy of study, e.g., seek out qualified researchers and encourage them to submit a proposal. Dr. O'Leary stated that ORD would certainly make the existence of this bank widely known to the research community. He expected that appropriate dialogue would develop between ORD and researchers. However, with respect to whether ORD would suggest something specific, he stated this was within the realm of possibility, but it might be premature at this point in time.

Dr. Haley asked whether they would be linking clinical information with the brain specimens. He thought this would be critical, e.g., establishing a study group of diabetics and nondiabetics. He also thought it would be important, especially with Gulf War veterans, to link information about the manifestations of a person's symptoms during life with their brain specimens. He asked if this bank would be collecting this clinical information for researchers' use. Dr. O'Leary agreed ideally that they would like to have this clinical information. However, there were a number of legal and ethical issues that may result in this being a somewhat heterogeneous collection. While they may encourage the donation of information to benefit research, it must be recognized that this is a gift on the part of the patient and/or deceased's family. This gift must be treated with reverence. Thus, the respective issues of researchers, patients and deceased's family must be balanced.

Dr. Haley posed a scenario where a researcher had a particular disease that he or she wanted to study. If this researcher actively went out and solicited the families to donate the remains to the brain bank, along with their consent to link identities with the clinical information, would the VA's brain bank honor this consent? Dr. O'Leary stated that this was a complex question and was the subject of recent litigation involving Washington University in St. Louis. He stated that he didn't think it would be prudent to shoot from the hip, but thought that it was clear that this resource needed to serve the research community while ensuring, at the level of operation of the bank, that consent was clearly and freely given. As long as these conditions are met, it seemed this would be sufficient. As to the details, these are sensitive issues and they have to balance the passionate desire to do something useful from a research position with the compassionate approach to those who are suffering.

Mr. Love asked Dr. O'Leary if there was a possibility of using a layering database for this tissue bank. The "deeper" database would contain identity and profiles, but in the "lesser" database, there is a dictionary search tool that allows keyword searches without accessing the patient's identifying information. Dr. O'Leary stated that, in theory with a large enough database, this might work. However, it is virtually impossible with a small database to maintain anonymity unless one provides a minimum of information. This is particularly true in close-knit communities. This is an issue of competing needs between what needs to be done to structure a useful database for querying and appropriately addressing HIPPA and other legal concerns. Mr. Love stated that the Armed Forces Institute of Pathology (AFIP) had a similar problem with their database. He stated that they denied access to protect anonymity. He understood this concern, but was looking for an approach that would allow access while protecting anonymity. Dr. O'Leary stated that he understood this desire. He stated that an institutional review board (IRB) was the mechanism by which these issues were adjudicated. Anyone who works with IRBs knows that they have a fierce commitment to balance scientific need against the protection of individual rights.

Ms. Nichols stated that there were identified exposure groups, e.g., Al Jubyal and Khamisiyah. She asked if the specimens could at least be identified as being from an identified exposure group. She did not believe this would fall under HIPPA, and it should be considered to identify samples as being chemically-exposed veterans. Dr. O'Leary thought this was useful input into the study design process, but the healthcare system has a series of obligations, in policy and law, that can not be disobeyed.

Dr. Meggs asked about the status of the treatment research center. Dr. O'Leary stated it would take some time to determine. He noted the new effort started at UT Southwestern and thought it was premature to ask Dr. Haley or others at UT Southwestern to flesh out the particulars of this program. Dr. O'Leary stated that VA and UT Southwestern were working through the issues necessary to implement the Congressional directive. He thought that they needed to wait and see what was developed as part of the UT Southwestern initiative so that the respective programs could complement each other. He didn't want to see an inadvertent use of the taxpayers' resources on unwise duplication.

Dr. Golomb asked Dr. O'Leary if a Gulf War veteran expressly wished their health information to be made available to researchers, would this information then be available. She stated that it would seem to dishonor the veteran not to make the full, greatest use of the tissue they were generously donating. Dr. O'Leary stated that the IRB would have an important role in issues of this type. His assumption would be that an IRB would honor veterans' wishes, but an IRB is an independent entity and not subject to external control.

Chairman Binns invited Dr. Kupersmith to share his thoughts on this issue. Dr. Kupersmith stated that he wished to clarify that it was the IRB who decided what to do on these issues. VA ORD could not provide answers about what an IRB would say. The rules are very complex. Dr. Kupersmith stated that they were currently establishing a central IRB for VA, and this would help to facilitate resolution of these concerns. It will be helpful having one central IRB, versus several local IRBs, making these decisions. The central IRB should be operational in Fall 2006. Dr. Kupersmith commented that he had been involved in litigation over the ownership of tissues in a Texas Alzheimer's tissue bank. He stated that the plaintiffs had maintained that the tissue remained the property of the individual family. They lost the case. However, this case highlights that there are a lot of unknown issues that arise when it comes to tissue banks.

Chairman Binns thanked Drs. Goldberg, O'Leary, and Kupersmith for the update on the work of VA ORD.

Plan for Gulf War Illness and Chemical Exposure Research Program

Robert Haley, MD

Professor, UT Southwestern School of Medicine

Dr. Haley provided an overview of the Gulf War research program that is being developed at UT Southwestern. ([See Appendix A- Presentation 11.](#))

After the presentation, Chairman Binns asked that the Committee's discussion begin by talking about roles, that is, roles of the Committee, roles of the differing outside agencies, etc. Dr. Haley stated that the Memorandum of Understanding (MOU) between UT Southwestern and VA requires that a merit review committee examine all proposals for research expenditures to be made through this program and that at that exact moment, Dr. Al Gilman, Dean of UT Southwestern Medical School, was meeting with prospective members of this committee. These individuals will not only review these projects for merit, but will also provide advice and good ideas. Dr. Haley stated that he expected the merit review committee, as well as his own office, to work closely with everybody interested in this research area. He hopes to establish collaborations with other investigators. Dr. Haley sees the Committee as the main source of ideas for this program. He hoped that the Committee would hold a meeting in Dallas, TX, in the near future. This would allow the Committee to hear directly from the investigators about their research, as well as provide the opportunity for discussion between the Committee and these investigators. These investigators would like recommendations from the Committee that they could

incorporate into their program. Dr. Haley also hoped to be able to call on individual members of the committee for advice as issues come up. His sincere wish is to solve Gulf War illnesses. There is funding now to do this. He hoped they would be good stewards of this funding by taking all the ideas and putting them into action. Dr. Haley stated that the lion's share of this money would probably be used at UT Southwestern. However, they also wanted to be responsive to the best minds around the country. If an outside researcher could do a project better, he would be open to having them do it. Dr. Haley stated that this was Dr. Gilman's wish as well.

With regards to Dr. Haley's own role in this process, he stated that there was a lot of money involved in this program, which created a potential for conflict of interest and malfeasance. He did not wish to have an appearance of either of these and so would be stepping down as a formal member of the Committee. He stated that he would like to continue coming to the Committee meetings and to sit at the table, if possible, so he could fully participate in the discussions. He would be doing this in his role as a UT Southwestern researcher and a liaison between the University and the Committee. However, he would not be a voting member or be involved in determining actual questions or policies. He stated that the Committee needed to conduct these activities independently. He would then be fully informed and could relay this information back to the researchers at UT Southwestern.

Dr. Golomb asked for clarification about whether funding would be available to researchers outside UT Southwestern. Dr. Haley stated that there would be, but the specifics were up to the merit review committee. It was unclear at that point as to how things would actually function, because the merit review committee was just being formed. He believed that there were smart people involved and that they would be "smart" in how they approached this. Dr. Haley stated that his organizational chart was a vision for this program. He stated that he used the Committee's 2004 report and its January 17, 2006, recommendations outlining specific research questions, to develop this organizational chart. He looked at UT Southwestern's faculty list to see who might be able to address these questions. He believed the chart reflected all but one of these questions. He noted that some of these proposed projects were being envisioned for outside researchers. However, Dr. Haley did not envision that an RFA would be issued. He envisioned that a list of specific projects would be set forth and researchers would be solicited to do the individual projects.

Chairman Binns spoke as to the Committee's role in this process. He stated that the Committee is advisory per its charter and does not do research. The Committee is officially an advisor to the Secretary, and does communicate directly with him. However, from a practical point of view, these issues are ones that the Secretary doesn't handle personally. Therefore, historically, the Committee has worked with the managers of research, e.g., Dr. Kupersmith and his predecessors and their counterparts at DoD. Now, the Committee will also be working with Dr. Haley and UT Southwestern. Chairman Binns viewed the Committee's responsibility to be a continuation of what it had been doing for four years, i.e., offering the best suggestions, whether they are positive or critical of what is happening, and helping to build a Gulf War research program that makes sense. He stated that the lack of a coherent program had been one of the most frustrating problems with Gulf War research. The funded projects have had no relationship to one another, or if they did, it was serendipitous. Chairman Binns hoped that the Committee could help UT Southwestern and other Gulf War research funding sources (VA, DoD) fill in the blanks, so that the resulting program makes sense. He noted that, as ambitious as this appears compared to the past, there was still a finite amount of money available for research. Thus, the focus must be on the mission of identifying diagnostic tests and treatments for Gulf War illnesses. Chairman Binns stated that the Committee's responsibility was to advise how this money could be spent on productive projects that could be justified to Congress and the managers of these programs.

Dr. Haley stated that a lot had changed in the twelve years since he started working on this issue. He noted that he had a good relationship with the current VA ORD administration (Drs. Kupersmith, O'Leary, and Goldberg) and hoped that it would blossom further. He stated that he also had a great relationship with DoD, which is in a good position to clearly understand this problem. UT Southwestern will be courting these relationships during the establishment of their program.

Dr. Meggs commented that he appreciated that this was the culmination of more than a decade of blood, sweat and tears. He noted, looking at the organizational chart, that Dr. Haley probably had a setup and mechanisms that could address a large number of medical problems beyond Gulf War illness. Dr. Meggs wished Dr. Haley success in this endeavor. Dr. Haley noted that the bottom of the organizational chart listed several conditions (Gulf War syndrome, PTSD, CFS, fibromyalgia, multiple chemical sensitivity (MCS), IBS, ALS, etc.). He stated that it was natural to apply the tools developed to these similar groups. He said this had been one of the major selling points to the other faculty members at UT Southwestern. He stated that it was difficult to get people to work on Gulf War syndrome. Young researchers are not likely to take a gamble on this "disreputable" disease. However, if they can make a major contribution to understanding Gulf War syndrome at the same time they are developing preliminary data for an imaging grant on depression/schizophrenia, they are more likely to express interest in this area of research. He stated that the top psychiatry and neurology assistant professors at UT Southwestern were eager to get going on these projects. Chairman Binns noted that Senator Hutchison's remarks at the press conference announcing this program specifically referred to pesticide workers as group who might be helped by this research. Dr. Haley stated that Senator Hutchison's office had recommended early on that this program be called the "Gulf War Illness and Chemical Agent Exposure Program", and that UT Southwestern had adopted this name.

Mr. Love commented that there was a need for a relational database to tie all of this information together. He stated that the lack of this type of database had paralyzed every other agency. Further, a large amount of money was spent on a database tool that failed. He stated that the database needed to provide public and private access, and must be coordinated between the agencies with mutual references. Dr. Haley stated that this was a good point and had been raised by Senator Hutchison's staff. They had noted that Dr. Haley's proposed IT budget was not large enough to address this concern.

Chairman Binns asked that each Committee member provide their thoughts as to what they believe are the most important areas that should be addressed in a Gulf War illness research program. He stated that the purpose of this discussion was to help Dr. Haley carry out his charge.

Dr. Clauw commented that, while he had not always seen eye-to-eye with Dr. Haley on the cause of Gulf War illness, he had not questioned the quality of Dr. Haley's scientific work. He stated that Dr. Haley would have a particular challenge in designing his studies and supported Dr. Haley's efforts to make the neuroimaging technology he was utilizing more sensitive. He believed, however, that the real problem would be controlling for all the nuances, e.g., possible effects of what people are thinking about in the scanner, etc. He offered to help Dr. Haley in any way he could. He stated that this was a tremendous opportunity for the entire research community and there had never been a cohesive integrated program. Dr. Clauw stated that one strength of Dr. Haley's proposed program was having the treatment center and research center side-by-side. He stated that these veterans needed care, the best possible care. He noted that the Committee had appropriately and primarily focused on the gaps in the Gulf War research portfolio, but the gaps in the clinical care portfolio were larger. Any advances that could come about by integrating research and clinical care would really help this field.

Dr. Nettleman stated that she would echo Dr. Clauw's comments supporting Dr. Haley's proposed organization of the program and was very interested in the treatment aspect of the program. She asked if

Dr. Haley would be asking questions in his group's epidemiology survey about what treatments had worked and hadn't worked for Gulf War veterans; and whether these would be specific, focused questions. Dr. Haley stated that a survey of these issues had actually been conducted by Dr. Han Kang's group, who had presented their preliminary findings at the Committee's September 2005 meeting. He noted that Drs. John Hart and Carol North were now in Dallas and would be instrumental in the process of designing a clinic with an intake screening diagnostic evaluation that would look at all the various things that might be going on with a Gulf War veteran. Dr. Haley noted that there would be a 3T magnet scanner at the Dallas VA, which would be the twin of UT Southwestern's research magnet scanner. There will also be PET scanners at both locations. They will have occupational therapists and social workers to help with adjustment reactions and other problems. He stated that at first, the clinic might be ineffective or minimally effective in treating veterans' conditions. But he hoped over time that the clinic will get better, and veterans will start seeing improvements in how they feel.

Mr. Atizado stated, as a new member, that he wanted to thank the Committee for answering several of the questions that he had. He stated that he was very appreciative of the hard work and frustration faced by the researchers addressing this very complex problem. He was also very appreciative of the audience members who were struggling, in a different role, to help Gulf War veterans as well. He wished to echo the sentiments expressed earlier, namely that Gulf War veterans were not getting younger and that this issue could become more complex as they age. He offered his support as an advocate to help in any way he could, whether it be as a Committee member or legislative director for the Disabled American Veterans.

Mr. Hardie agreed that this was a momentous time in Gulf War illness research. He wished to encourage Dr. Haley and his team to consider communication to be a significant part of their program. He hoped that they would make sure that news of their research would make it to the frontline VA clinical doctors. One of the things he has seen personally is that most of what happens in Washington, DC, never makes it out to the VA hospitals. He hoped that this would be two-way communication too. Not only a way for researchers to inform the local hospitals of their findings, but also a way for the hospitals to report back any changes that were evolving in the veteran population. Dr. Haley stated that this was a very good point. He agreed that Gulf war veterans were very frustrated with what they leave with when they go to the VA. However, he also knows that the doctors are just as frustrated because they don't know how to treat these veterans. He stated that once the researchers could present their findings and techniques, and show through scientific rigor that they work, VA doctors would be the first to jump on this issue.

Dr. Bloom congratulated Dr. Haley on his work with the statistics department at Southern Methodist University. He stated that this research really changed how one does this type of analysis. Dr. Bloom stated that he started his biotech company to do 3D comparison of gene expression patterns across mice that are more or less vulnerable to the genes that cause human diseases. His company started out doing high resolution magnetic resonance imaging to develop accurate 3D images. He encouraged Dr. Haley to take advantage of the wide range of mice strain vulnerabilities, particularly to pesticides and processes that cause neuronal degeneration. He then could take advantage of the genetic modifiers to develop medications aimed at what is causing the problem. It would be important to understand why some of the veterans exposed in the field developed a problem and some did not. He offered Dr. Haley his expertise in developing these ideas, which Dr. Haley gratefully accepted. Dr. Haley indicated they had used a rat model for several years, but switched to a mouse model a year ago for this very reason. He commented that they planned also to take advantage of a particular mouse strain suggested by Dr. Barlow when she spoke to the Committee several years earlier.

Dr. Melling commented that Dr. Haley was creating an incredibly powerful diagnostic evaluative engine that could have application across a range of diseases, and that the drug development industry should take

note of this work. Dr. Haley stated that he had not anticipated this, but it brought up an interesting point. He stated that, in the imaging field, there is competition between clinical and research use of the technology resources. It was important that they were careful in maintaining the autonomy of their center and their resources for research.

Dr. Golomb stated that Dr. Haley was already familiar with her recommendations, many of which were implemented in his organizational chart. These included: (1) looking at different cholinergic challenges in different brain areas; (2) looking for objective markers and specific mechanisms; (3) identifying exposure illness clusters; and (4) ultimately identifying groups that may be targeted for different treatments so that they would be less likely to miss an effective treatment that may be good for a particular subset. Dr. Haley stated that he would need further advice about mitochondrial function and CoQ10 influence on this.

Dr. Barlow commented that it seemed that a lot of research progress had been made and it was time to make the leap into clever, strategic clinical trial designs, utilizing this very motivated group of patients to test therapies in a very rapid way. She stated that there are a number of people who have moved from research into industry and are now doing translational medicine. These researchers are coming up with many novel and fast trial designs with patients with psychiatric disorders. She commented that it may be a cerebrospinal fluid marker, imaging technique, or urinary or other biochemical markers that allow researchers to cycle through many options. Once this is identified, trials can be appropriately designed. Dr. Haley stated that their main initial effort was to develop an intake screening protocol with a battery of tests, allowing them to classify individuals into groups. This would then allow them to conduct clinical trials in the homogenous groups.

Dr. Tilson stated that he was pleased with Dr. Haley's depiction of the epidemiology opportunities identified in his organization chart. However, he was not clear how the lines on the left-hand side of the chart connected with the right-hand side. He expected that this was to be explored and offered to help Dr. Haley with this issue, because the disconnect between population-based research and individual research is even worse than the disconnect between bench and bedside. He also wished to reinforce the earlier comments that informatics and particularly population-based databases would play a large role in the ability to bridge this gap, allowing for the establishment of dialogue between DoD, VA and some of the civilian-based population databases. Dr. Tilson then brought up the issue of public and private collaborations. He commented that he was the chairman for the National Steering Committee for the Center for Education and Research on Therapeutics, and they are currently pursuing public/private partnerships and bringing the opportunity for private funding to address some of these public policy questions.

Dr. O'Callaghan commented that Dr. Haley had the opportunity to establish a very high profile and in-depth center of excellence. Taking Dr. Bloom's comments further, Dr. O'Callaghan suggested that Dr. Haley look at regional gene expression profiles that are directed by the imaging findings. This would allow them to possibly get to the genetic basis of patient subclasses.

Mr. Graves stated that he did not have anything to add to his previous comments.

Dr. Meggs expressed his hope that Dr. Haley's program would investigate the role of ongoing exposures to chemicals in relation to the waxing and waning of the disease. He noted a study by Dr. Mike Hodgkins, which found painters' attention, concentration, confusion and other parameters improved when they were laid-off from their jobs. Dr. Meggs stated that Dr. Haley had a wonderful opportunity to use imaging techniques to evaluate individuals who are exposed to low levels of solvents and organophosphates over time, and see if avoidance of these compounds might be a treatment that would

see them improve with time. Dr. Haley added that individuals with post-bypass and post-chemotherapy syndrome, as well as 9/11 and anthrax survivors, also have developed cognitive problems. There are several of these mysterious brain syndromes, and the question is whether they are the same or different.

Dr. Steele referenced the new Committee members' comments on the need to find treatments. She noted that the Committee had been saying this from the beginning, but little progress had been made in this direction. She commented that a lot of data had already been collected but often these data hadn't been analyzed thoroughly or compared with other data. These resources would be informative about etiology or meaningful subgroups, and should be explored more thoroughly. She also commented that Dr. Haley had a unique opportunity to bring clinical information into a systematic research paradigm. She noted that he would have a group of people that had been fully evaluated clinically, and whose progress could be followed through time. Dr. Steele commented that one of the shortcomings of VA's War-Related Illness and Injury Study Centers (WRIISCs) was that no one had followed these veterans to see what treatments were effective and for which subsets. She pointed out that Dr. Haley's team would have baseline information on patients and would be able to conduct outcomes research through the clinical aspects of the program.

Mr. Smithson extended the resources of his organization, the American Legion, to assist in Dr. Haley's efforts. Dr. Haley stated that the American Legion had already been helpful in finding Gulf War veterans for their pilot survey. He stated that they would need additional help when this survey was ready for full implementation. He noted that a good response rate in epidemiological studies is 70%. Obtaining a good response rate is a real challenge, and they are hoping to achieve 90% on their upcoming survey. He stated that many veterans, especially healthy veterans, are losing interest in participating in these studies. He said that they needed to figure out how to inspire veteran participation. Dr. Haley suggested that he meet and discuss this issue with some of the veteran service organizations.

Chairman Binns commented that Dr. Baraniuk's work was very intriguing to him as a nonscientist. He asked if the neuroscience experts at the table felt this type of research is heading in the right direction. Several members indicated they agreed it was a worthwhile direction. Dr. Bloom stated this was especially true, combined with the ALS proteomic research highlighted by Dr. Goldberg earlier in the meeting. Dr. Nettleman stated this approach would allow independent testing.

Chairman Binns stated that he appreciated all of the comments and good wishes for Dr. Haley. He would like to see the Committee focus on more detailed recommendations. He encouraged Committee members to write down the top research recommendations that they would make, being as specific as they could be. He understood that there was always an issue in research that: "Hey, it is my idea. I would like to do the research." He noted that UT Southwestern wasn't the only group with research money, but DoD and VA would also be funding Gulf War research. He would like the Committee to develop a very detailed research agenda that would accomplish its mission of identifying research that would improve the health of ill veterans.

Dr. Golomb asked for more information about the money available at DoD. Chairman Binns stated that the language included in the Congressional record was that 20% of the 5 million dollars must be spent on existing therapies, including pilot trials. Some of the projects must be competed (25%), while the rest could be assigned to various researchers. He stated that there were new program managers at DoD, and that Dr. Steele and he would be meeting with them to discuss issues. He noted that several DoD representatives had attended the Committee's meeting on the previous day, and that their Program Officer was currently present.

The meeting adjourned for a break at 2:40 p.m.

The meeting reconvened at 2:45 p.m.

Federal Advisory Committee Ethics Training

Susan Bond

VA General Counsel Office

Ms. Bond gave a presentation to the Committee on the Federal Advisory Committee Act (FACA) and the ethical rules which pertained to them as Committee members. She covered five of the fourteen principles of ethical conduct which Committee members are to observe, including that an individual: (1) can not hold co-mutual interests that would conflict with their official duties on the Committee; (2) can not use non-public information to further private interests; (3) can not solicit or accept gifts given on the basis of their being a Committee member; (4) can not use public office for private gain; and (5) is prohibited from violating any ethical standard. She added that one also has to avoid the appearance of violating any ethical standards. She covered the rules and exceptions pertaining to gifts from outside sources and misuse of government resources.

Ms. Bond and Committee members discussed aspects of the criminal conflict of interest statute (18 USC § 208), such as: (1) one can not participate personally and substantially in a matter that will have a direct and predictable effect on a Committee member's financial interests, as well as that of a spouse, minor children, general partner, employer or prospective employer; and (2) one can not "switch sides." In other words, one can not represent or accept compensation for representing a different party on a matter that the Committee member worked on personally and substantially as a member of the advisory committee.

Chairman Binns encouraged Committee members to contact the VA General Counsel Office with any detailed ethical questions that they may have. Ms. Bond provided her email and telephone number so that Committee members could contact her with any questions. She indicated that they may also discuss issues with Mr. Jonathan Gurland or Mr. Jim Adams. Chairman Binns encouraged Committee members to submit their questions in writing so that they would have documentation of their requests for advice.

RAC Committee Business

Lea Steele, PhD

Scientific Director, Research Advisory Committee on Gulf War Veterans' Illnesses

Dr. Steele discussed two issues pertaining to Committee activities. First, she stated that staff was working on the Committee's 2006 report. The plan was to have a draft available for Committee review and comments by mid-to-late summer. Chairman Binns noted that this report would reflect the work of the Committee in 2004 and 2005. He stated that those individuals who served on the Committee during those years would need to pay special attention to the draft report, because their names would be on it. He stated that any comments by new Committee members would certainly be appreciated.

Dr. Steele stated that future Committee meetings would be focused on specific pathophysiological mechanisms of interest, along with specific studies that could be done. She stated that she had preliminary ideas from several Committee members concerning specific topics that could be covered in meetings. She encouraged all members to contact her if there was something that they would like to see addressed.

Public Comment – Day 2

Chairman Binns opened the meeting to public comment and asked all speakers to limit their comments to 5 minutes or less.

Mr. Love spoke to the Committee about the need for recruitment and retention of veterans. He stated that there was no longer a large base of veterans to pull from for research studies. He suggested encouraging veterans' participation through the use of the Gulf War Registry. He noted that it was a free exam and one doesn't need VA benefits to get it. The veteran can walk in, have an exam and this information could be entered into a database. He stated that this needed to be done on a nationwide scale. He expressed concern that small studies would result in finite results and that larger studies needed to be done, e.g., 4,000 participating veterans. Mr. Love stated that there was very little media attention on Gulf War illnesses today, and therefore the veterans think there is no interest in their problems. He stated that the VA would have to win the veterans' trust back before they would participate in these studies again. He noted, from a technical standpoint, the agencies needed to coordinate their relational databases.

Ms. Venus-Val Hammack spoke to the Committee. She stated that it was very important that the veterans know that the Committee and VA are working on this issue. She asked that the proposed treatment center be listed more prominently on the Internet. She stated that this would help her convince other veterans that the Committee is working on treatments. She hoped that this would be in the Committee's next advisory report.

Ms. Alison Johnson spoke to the Committee. She reviewed her experience with MCS and her search for treatments that work. She conducted her own survey in 1996, and found that the only thing that really worked was avoidance therapy. She stated that, unfortunately, avoidance takes money. She expressed her desire to see Dr. Haley establish an environmental medical unit as part of his new program. She stated this was necessary to show scientifically how low levels of chemical can affect people that are sensitive to them and that there were several good scientists who could collaborate on this. She stated that there was probably not a "magic bullet" for MCS, and there probably wasn't one for Gulf War illness. She saw potential in gene modification therapy but didn't see promise in any of these other medications or therapies. She stated that the goal must be to get people back to work and make the public understand that this is a real condition. Chairman Binns asked if Ms. Johnson could discuss the benefit of provocation neutralization for MCS patients. She stated that one of her three daughters who suffered from MCS had benefited from it. She stated that it wasn't a placebo cure, and that only a handful of physicians were doing it correctly. Chairman Binns stated that it was important to note that some things do work in isolated individuals. Mr. Hardie asked whether Ms. Johnson's survey had looked at chelation therapy, and if so, what the results were. Ms. Johnson stated that she believed that chelation therapy might work in some cases with heavy metals, but not necessarily with MCS.

Mr. Cheyne Worley, who is a Gulf War veteran and is on the board of the National Gulf War Resource Center (NGWRC), spoke to the Committee. He thanked the Committee members for their hard work and service, and welcomed the new members to the Committee. He thought it was an exciting time for Gulf War veterans with what the researchers were trying to accomplish, i.e., identify a diagnostic tool for Gulf War illness and then develop a treatment program. He stated that he isn't sure he will ever know exactly what caused his symptoms, but he is definitely interested in whatever treatment he could use to help in his everyday life. He commented that he had been following Drs. Haley's, Clauw's and Golomb's work for awhile. Mr. Worley stated that Dr. Haley was stigmatized initially because of the direction he was going with his research, and that it was ironic considering his position today. Mr. Worley noted that VA doctors were just as frustrated as Gulf War veterans. He stated that the VA clinical guidelines for Gulf War veterans were very out-dated and needed to be reexamined. Dr. Golomb noted that many VA

physicians don't read these anyway. Mr. Worley noted that many VA physicians still did not believe that there is a Gulf War illness, and attention needs to be paid to this. He agreed with Mr. Love that the Gulf War Registry is a valuable tool to be used. He noted that the WRIISC centers are located on the East Coast and not accessible to most veterans. Mr. Worley visited the East Orange, NJ, WRIISC. While the VA paid for the travel expenses, it still cost him a few hundred dollars to make the trip. He would like to see a WRIISC located on the West Coast.

Ms. Nichols spoke to the Committee. She stated that communication with VA physicians was important, and video conferencing was needed to educate them. She asked that there be a press plan for future announcements. She stated that the press release about the MOU between VA and UT Southwestern was picked up, but was lost quickly in the mainstream media. She stated that there needed to be a plan to grab back the mainstream media and their attention on this issue. She believed that, if approached, Larry King would have devoted part of his show to this issue. She stated that she and others like Kirt Love were trying to pull more veterans into the fold, and there were people who would come if there was better coordination. She commented on CoQ10 research possibilities. She stated that the VA's raw Gulf War veteran data, by year and age, should be made public. She stated that there were no privacy issues with this data, and researchers should have it readily available. She noted the need to assess anthrax and secondary exposures in nondeployed control groups. She wished that there would be another center location similar to Dr. Haley's, and that Khamisiyah veterans would be studied there. She stated that the veteran service organizations were doing a good job, and noted that Mr. Hardie represents the veterans' board of a state government. She urged that VA physicians seeing Gulf War veterans with cancer be notified about the tissue bank.

Mr. Smithson noted that the location of the WRIISCs had been raised at earlier meetings, i.e., only being located on the East Coast. He asked if VA had considered establishing WRIISCs in other parts of the country. Dr. Goldberg noted that WRIISCs were clinical centers, and clinical appropriations were separate from research appropriations so VA ORD couldn't speak as to future WRIISC developments. He noted that the Committee, however, could make recommendations to the Secretary about this matter as he oversees both clinical care and research.

Chairman Binns thanked the Committee for their service over the two long days of meetings. He stated that he was optimistic that progress and results would come from the input of the Committee, both old and new members, and strongly encouraged all members to write down their ideas for future research.

The meeting adjourned at 3:37 p.m.

Appendix A

Presentation 1 – Joel Graves

Gulf War Illness

~ A Review & A Proposal ~

Joel Graves

Captain, Army, Retired

Member 1st Battalion, 67th Armored Regiment,
1st “Tiger” Brigade (Independent Task Force)
camped vicinity Al Jahrah after Desert Storm

Low-Level Sarin Gas Exposure

- Low-level exposures to certain organophosphorous compounds including sarin nerve agents to which our troops may have been exposed, can cause delayed, chronic neurotoxic effects. In addition, German personnel who were exposed to nerve agents during World War II displayed signs and symptoms of neurological problems even 5 to 10 years after their last exposure (GAO Report on Gulf War Illnesses, Feb 24, 1998, p6).
- “Previously, we reported that approximately 4% of a battalion of US Gulf War veterans developed a subtle neurologic syndrome that was strongly associated epidemiologically with perceived wartime exposure to low-level nerve agent (Haley and Kurt, 1997)(Journal of Psychopharmacology 14(1)(2000: 87-88).
- Laboratory research prompted by soldier’s reports compiled during the Gulf War suggest that the psychological stress experienced in wartime may enhance the penetration of AChE inhibitors (i.e. sarin) to the brain potentially exaggerating the effects of either alone (Soreq, H, The Health Impact of Chemical Exposures During the Gulf War, Feb28, 1999: ref Friedman, A. et al. Nat Med 2, 1382-1385 – 1996).

Low Dose Sarin Gas Exposure

- Gulf War veterans have complained of a variety of symptoms, including headaches, joint pain, fatigue, diarrhea, skin rashes, and dizziness. Evidence suggests they might have suffered neurological damage from some combination of stress and exposure... non-lethal doses of sarin. A recent Government Accountability Office report confirmed that exposure to low-level sarin during the Gulf War was more frequent and widespread than previously acknowledged (Suits, R. Vital Signs, Spring 2005, p10). This report is based on the results of the four-year, DOD funded study at Wright State, directed by Marianna Morris, PhD, professor and chair of pharmacology and toxicology and Daniel Organisciak, PhD, professor and chair of biochemistry and molecular biology.
- Even subclinical doses of sarin cause subtle changes in the brain; subclinical exposure to sarin has been proposed as an etiology to the Gulf War Syndrome (Sopori, Mohan L. Neuroimmune Effects of Inhaling Low Dose Sarin, Lovelace Institutes, Albuquerque, NM, Feb 2005).

Arms Control Today Jan/Feb 2006

Report Confirms Iraq Used Sarin in 1991

- U.S. investigators have confirmed that Iraq used chemical weapons to quash a Shiite uprising after the 1991 Persian Gulf War.
- The report marked the first outside confirmation that the regime had used chemical weapons to quell a growing 1991 insurgency.
- The report said the use of chemical weapons was an example of the “dire nature of the situation” and the regime’s “faith in ‘special weapons’” that it would consider using chemical weapons *while coalition forces were still in Iraq.*



Early Warning

By William M. Arkin

Excerpt from Washington Post Blog
November 28, 2005

http://blogs.washingtonpost.com/earlywarning/2005/11/another_saddam_1.html

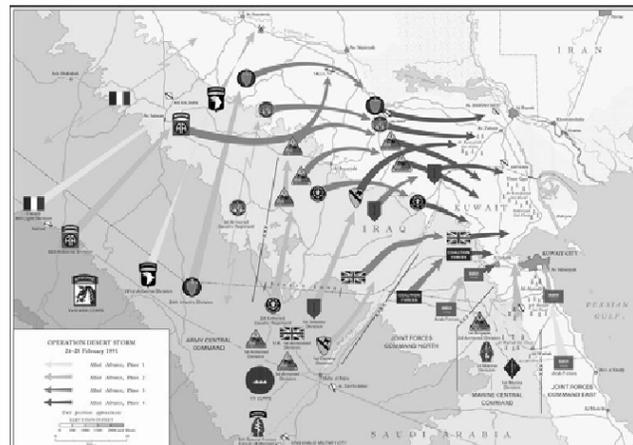
William M. Arkin on National and Homeland Security

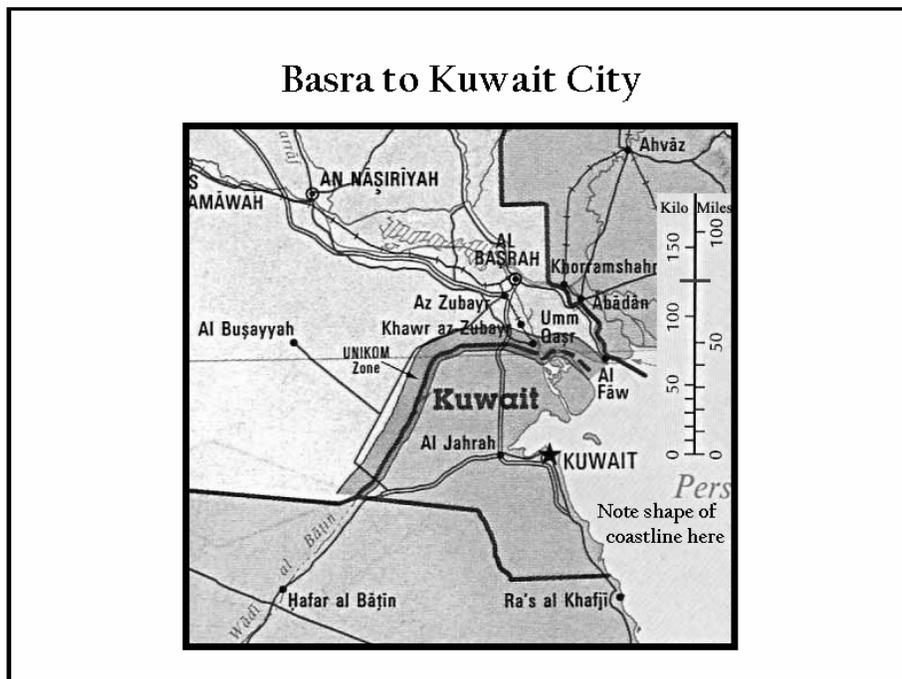
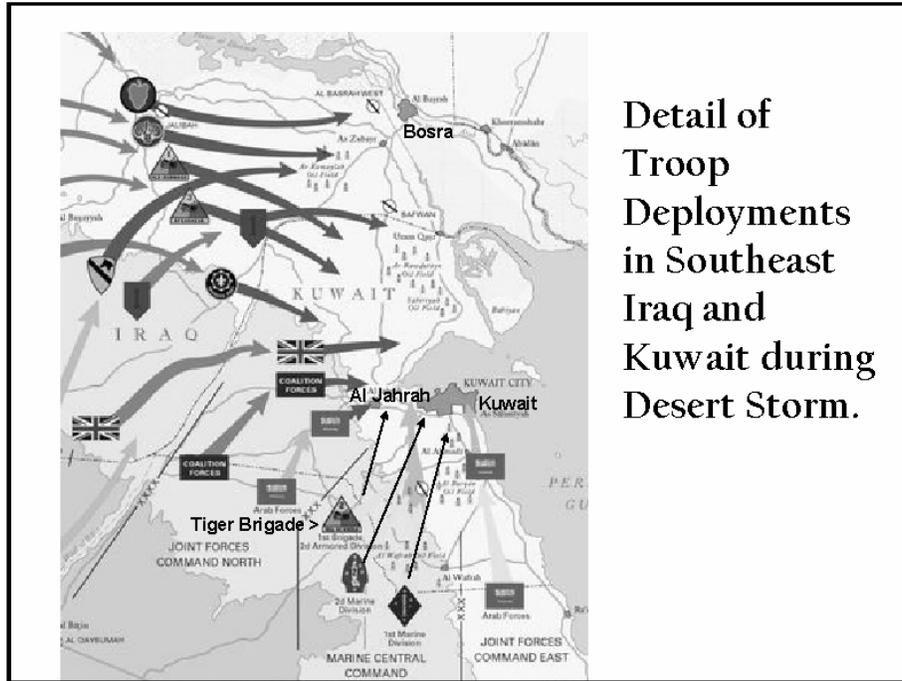
- I have a suggestion for another massacre, one that was unleashed in response to the worst instance of civil unrest since the beginning of President Saddam Hussein's rule.

What happened in this massacre bears heavily on the current health of American veterans, on our view of the competence of the U.S. intelligence community and the current weapons of mass destruction debate.

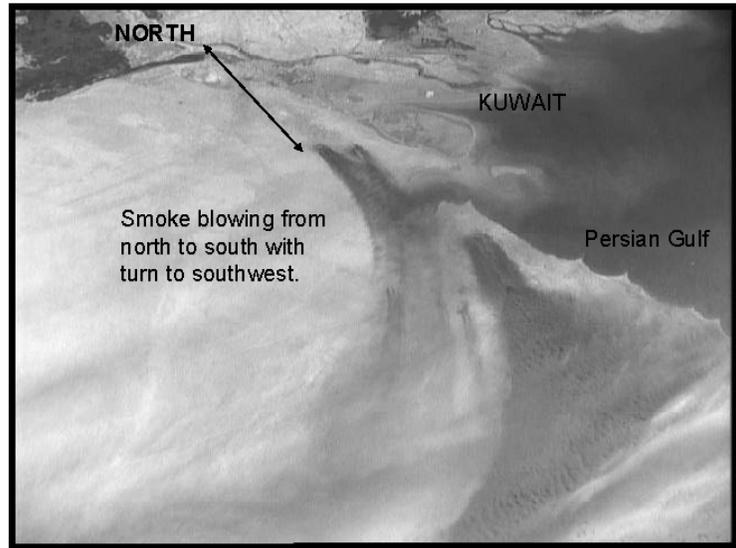
- In a little noticed discovery, the Iraq Survey Group investigating Iraq's WMD concluded last year that the former regime dropped chemical weapons on Shi'ite rebel groups during their post-Desert Storm revolt in *March 1991*. This finding directly contradicts the Pentagon review of potential causes of Gulf War Syndrome as well as the earlier conclusions of the intelligence community which had looked into the matter.

Troop Deployments During Desert Storm





Oil Well Fire Plume View from Space



On March 15th, after the evening meal, everyone got sick. Some were very sick and went to bed. I was nauseated and dizzy for several hours. We thought it was food poisoning, but we heard a rumor that chemical alarms had gone off in some units around us. Our own chemical alarms had been put away a month before after the war.

Unusually strong north winds blew down on us for a few days. At that time, Bosra, 75 miles to the north, was gassed by Saddam Hussein to put down the Shiite uprising, and the nerve agent blew down on us. I acknowledge that it was a small dose, but it was enough to set off alarms and make people sick. Even if a small dose, it was a significant amount.

Picture taken out of Humvee window at midday during attack. It was so dark, we could barely see road. Picture is of oil well fire to west blowing down on us as we attacked north to Kuwait City, 2nd day.

People were exposed to many different toxins and environmental contaminants, and many synergistic effects are possible. But I believe sarin gas, perhaps along with combat stress, is most likely responsible for most Gulf War illness.



Soldier on top of captured Iraqi T-72, taken with camera flash at noon time. It really was that dark. It was so dark, you could not read a book outside. All of these pictures are of the 1st Battalion, 67th Armor Regiment, 2nd Armor Division.

My Proposal

I propose that Gulf War Illness in most veterans of that era is due primarily to sarin gas exposure, whether from Khamasiya and other bunker demolition events or from the Bosra uprising.

I am sure that the sarin gas exposures have been exasperated by other exposures, combat stress, and their synergistic effects; and therefore, has manifested itself differently in different people.

But if sarin gas exposure is the one common thread to the overall illness, then it is possible to create a case definition and propose more exact research guidelines and treatments for veterans.

Presentation 2 – Lea Steele

The Gulf War and Gulf War Illnesses:

**An Overview of Research
Reviewed by the RAC-GWVI**

**Lea Steele, Ph.D.
May 16, 2006**

☆☆☆ **RAC-GWVI**
Research Advisory Committee on Gulf War Veterans' Illnesses

**The Gulf War and Gulf War Illnesses:
Overview of the Research Review Process So Far**

- **The Gulf War and Gulf War Illnesses**
- **The work of the RAC**
- **Highlights of research findings and conclusions**

☆☆☆ **RAC-GWVI**
Research Advisory Committee on Gulf War Veterans' Illnesses

The Gulf War and Gulf War Illnesses: Overview of the Research Review Process So Far

- **The Gulf War and Gulf War Illnesses**

- The work of the RAC
- Highlights of research findings and conclusions



RAC - GWVI
Research Advisory Committee on Gulf War Veterans' Illnesses

1990-1991 Gulf War: Operations Desert Shield/Desert Storm



Aug 2, 1990 - Iraq invaded Kuwait

Jan 16, 1991 - Air strikes began

Feb 24, 1991 - Ground combat began

Feb 28, 1991 - Cease fire declared



RAC - GWVI
Research Advisory Committee on Gulf War Veterans' Illnesses

**1990-1991 Gulf War: Operations Desert Shield/Desert Storm
Circumstances Very Different from Current Iraq War**

- ~700,000 U.S. troops deployed
- War ended after 6 weeks of heavy air strikes, 4-day ground war
- Decisive victory, few casualties (< 150 battle-related deaths)



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

**Gulf War Illnesses:
Chronic Symptoms in the Wake of Desert Shield/Desert Storm**

- After the 1991 war, widespread reports of unexplained health problems in Gulf War veterans, typically included:
 - *Chronic headaches*
 - *Joint pain, muscle pain*
 - *Dizziness, memory problems*
 - *Mood problems, cognitive difficulties*
 - *Unexplained fatigue*
 - *Persistent diarrhea*
 - *Respiratory problems*
 - *Unusual skin rashes*



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

Gulf War Illnesses (GWI)

- Little progress in understanding or treating GWI for many years; in 1998, Congress directed Committee be appointed
- RAC appointed in 2002, charged with reviewing Gulf War research and federal research programs, making recommendations



RAC-GWVI

Research Advisory Committee on Gulf War Veterans' Illnesses

Research Advisory Committee on Gulf War Veterans' Illnesses

- RAC charter identifies central objective of all GWI research:

to improve the health of ill veterans



RAC-GWVI

Research Advisory Committee on Gulf War Veterans' Illnesses

The Gulf War and Gulf War Illnesses: Overview of the Research Review Process So Far

- **Big picture: The Gulf War and Gulf War Illnesses**
- **The work of the RAC**
- **Highlights of research findings and conclusions**



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

**Comprehensive review of scientific and other
information relevant to the health of Gulf War
veterans**

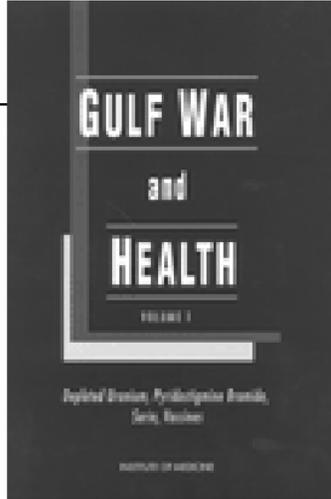
***The Committee has considered evidence
from diverse sources***



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

- Reports from DOD, VA, CDC, NIH
- Series of reports commissioned by DOD, VA (RAND, IOM)
- Congressional reports
- Special panels (e.g. PAC, PSOB)
- Reports from foreign governments, special panels



☆☆☆ **RAC-GWVI**
Research Agency Committee On Gulf War Veterans' Health

April 2003 Report from DOD Special Assistant for Gulf War Illnesses

Environmental Exposure Report

Pesticides

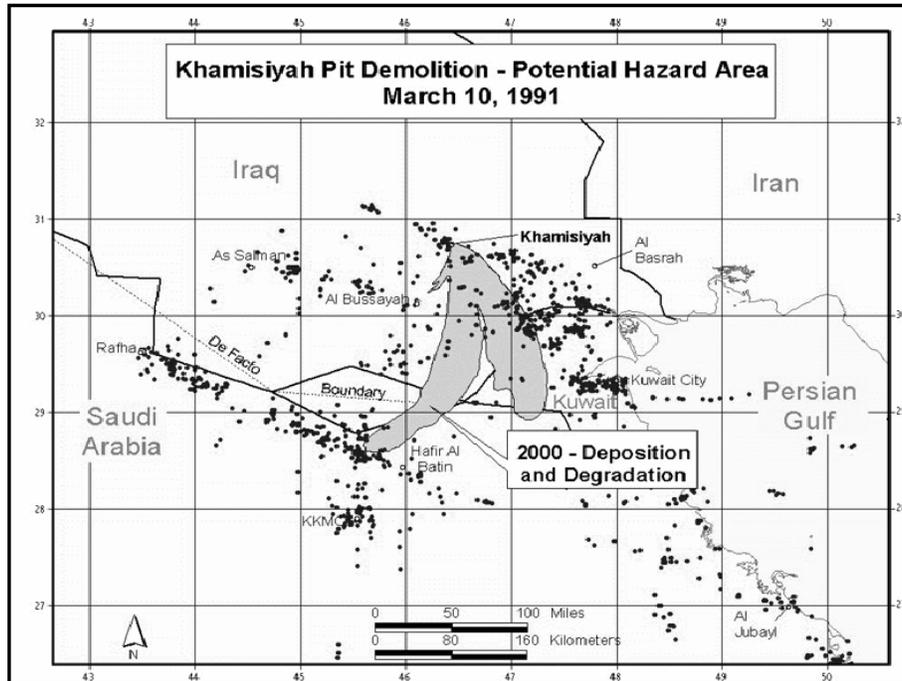
Environmental Exposure Reports are reports of what we know today about certain events of the 1990-1991 Gulf War. This particular environmental exposure report focuses on the use of pesticides by US military personnel and the resulting exposures to these compounds. Our goal is, to the extent possible, to determine if the pesticides used during the Gulf War contributed to unexplained illnesses reported by some Gulf War veterans. This is an interim, not a final, report. We hope that you will read this and contact us with any information that would help us better understand the events reported here. With your help, we will be able to report more accurately on the events surrounding pesticide use and exposures. Please contact my office to report any new information by calling:

1-800-497-6261

Dale A. Vesser
Acting Special Assistant for Gulf War Illnesses, Medical Readiness, and Military Deployment
Department of Defense

2001023-0000014
Ver 1.1

☆☆☆ **RAC-GWVI**
Research Agency Committee On Gulf War Veterans' Health



Primary focus has been on the now large body of relevant scientific research

The Committee has considered evidence related to a wide variety of Gulf War-related experiences and exposures



Large amount of laboratory, clinical, and epidemiologic research related to Gulf War exposures of potential interest

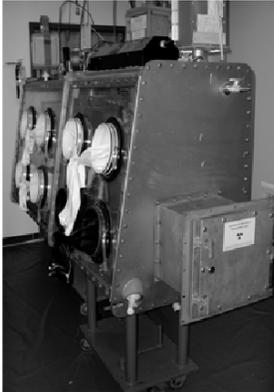
- Deployment-related stressors
- Chemical weapons
- Pesticides/repellants
- PB
- Vaccines
- Depleted uranium
- Oil well fires
- Infectious diseases
- Tent heaters
- Particulates
- Fuel exposures
- Solvents, CARC paint



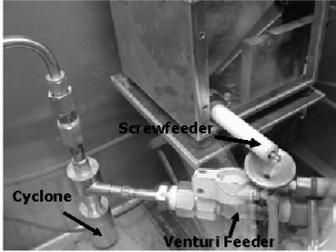
Table 7. Studies of Chronic Effects of Low-Dose Sarin Exposure in Animals

Study	Year	Animal Model	Major Finding
Burchfiel ¹⁴	1976	monkey	Persistent effects on electroencephalograph readings
Husain ¹²⁸	1993	mouse	Delayed development of spinal cord lesions
Jones ¹⁴⁰	2000	rat	Chronic reduction in nicotinic ACh receptor binding in cerebral cortex
Kassa ¹⁶⁵	2000	rat	Chronic alteration in immune function (lymphocyte proliferation, bactericidal activity of macrophages)
Kassa ¹⁶⁷	2000	rat	Persistent changes in DNA and protein metabolism in liver tissues
Kassa ¹⁶⁶	2001	rat	Subtle chronic signs of neurotoxicity and immunotoxicity with repeated exposures
Kassa ¹⁶³	2001	rat	Impaired spatial memory
Conn ⁵⁷	2002	rat	No persistent effects on reported indices of temperature regulation and motor activity
Henderson ¹¹³	2002	rat	Delayed, persistent changes in cholinergic receptors in brain areas associated with memory loss and cognitive changes
Hulet ¹²⁶	2002	guinea pig	Persistent failure to habituate on functional test battery
Scremin ²⁶³	2002	rat	Persistent increase in cerebral blood flow in specific areas
Kalra ¹⁵¹	2002	rat	Suppression of immune response (antibody-forming cells and T cell responses) mediated by the autonomic nervous system
Roberson ¹⁶⁴	2002	guinea pig	Chronic depression of AChE activity, persistent behavioral changes (disordered activity, increased rearing behavior)
Husain ¹²⁷	2003	mouse	Persistent reductions in respiratory exchange, blood AChE activity and BChE activity, NTE activity in various tissues
Scremin ²⁶²	2003	rat	Down-regulation of muscarinic receptors in hippocampus, decreased habituation
Kassa ¹⁶²⁻¹⁶⁴	2003 2004 2004	mouse	Chronic alteration in immune function (increase in CD19 cells, decrease in CD4 cells, decrease in mitogen-induced lymphoproliferation, increased NK cell activity)

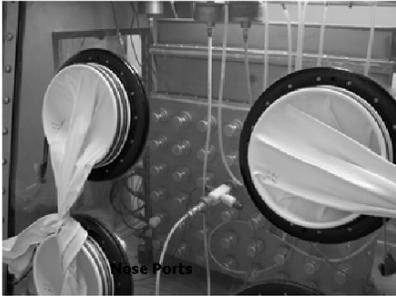
Glove Box Enclosure System



Aerosol Generation System

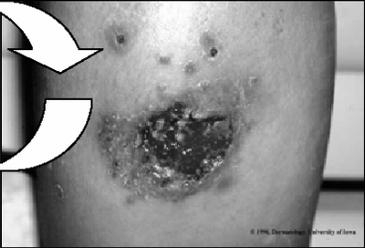
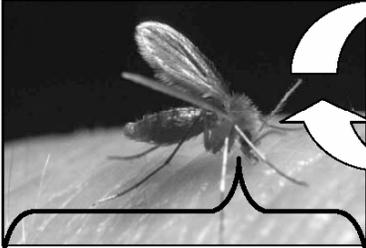
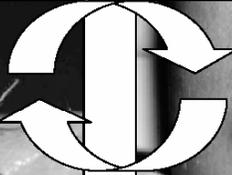


96-Port Nose-Only Exposure Chamber

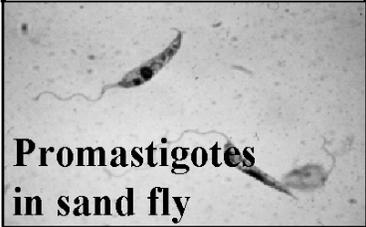


Slide adapted from: Lewis J. Inhalation of Uranium Oxides to Mimic Gulf War Exposures: Deposition and toxicity in brain, lung, and kidney. Presentation at: Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses; February 24, 2004; Washington, DC.

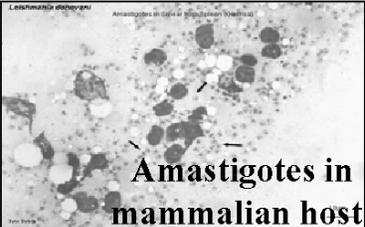
***Leishmania* Parasite Life Cycle**



Promastigotes in sand fly



Amastigotes in mammalian host



Slide adapted from: Magill AJ. Leishmaniasis in Veterans of Desert Storm & Iraqi Freedom. Presentation at: Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses; February 23, 2004; Washington, DC.

Table 10. Population Studies Assessing Relationships of Multiple Exposures in Theater to Gulf War Veterans' Illnesses

Population Studied	Sample Size	Health Measure	Association with Self-Reported Exposures		
			Chemical Weapons	PB	Pesticide Use
*Air Guard veterans ²²²	1,002	severe CMI	+	+	+
		mild/moderate CMI	+	+	+
*Army veterans from New England, New Orleans ²³⁴	291	neurological and musculoskeletal symptoms	+	--	+
Australian veterans ²⁴	1,456	functional impairment	+	+	+
Iowa veterans ¹³⁸	1,896	cognitive dysfunction	+	+	+
*Navy Seabees ²⁶	11,868	CMI (modified)	+	+	+
*Navy construction battalion ¹⁶⁷	249	1 or more of 3 defined syndromes	+	+	+
*New England Army veterans ²¹¹	1,290	CMI (modified)	na	+	na
*Pacific Northwest veterans ²⁰¹	354	unexplained illness	--	+	+
UK male veterans ³⁴⁰	2,735	CMI (modified)	+	+	+
*UK veterans ⁵²	7,871	symptom severity	na	+	+

CMI: chronic multisymptom illness as defined by Fukuda et al.⁹⁵
 + : statistically significant association; -- : association not statistically significant; na: association not assessed
 * Indicates analyses controlled for possible confounding due to concurrent exposures

Diverse types of scientific studies considered

- **Published scientific research**
 - > Epidemiologic studies of Gulf War-era veterans
 - > Clinical studies of Gulf War veterans
 - > Occupational health studies related to exposures
 - > Animal studies
 - > In vitro studies

- **Research-in-progress**



Information Synthesis/Analysis

- > *Compare findings from different studies: how are they similar? how are they different?*
- > *Evaluate strength of evidence related to each health finding and exposure of interest*

What does all this information tell us about the nature of Gulf War illnesses?



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

The Gulf War and Gulf War Illnesses: Overview of the Research Review Process So Far

- **Big picture: The Gulf War and Gulf War Illnesses**
- **The work of the RAC**
- **Highlights of research findings**
 - *Overview, emphasis on epidemiologic findings*
 - *Dr. Abou-Donia: Toxicological studies*
 - *Dr. Haley: Neurological findings*



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

Epidemiologic Studies: General Findings

- **Mortality:** no overall increase in disease-related mortality; higher rate of brain cancer mortality in relation to Khamisiyah
- **Hospitalizations:** no overall increase; some differences with nondeployed
- **Diagnosed medical conditions**
 - Excess rate of ALS
 - Excess rates of skin conditions, “dyspepsia”
 - Excess rates of chronic fatigue syndrome, fibromyalgia
 - Possible excess rate of asthma for oil-fire exposed
 - Cancers?
- **Psychiatric conditions**
 - Higher rates of PTSD, depression than in non-deployed veterans
 - Overall rates of psych conditions are low (e.g. PTSD: 2 – 10%)
 - Higher PTSD rates associated with combat, other psych stressors during deployment



RAC-GWVI

Research Advisory Committee on Gulf War Veterans' Illnesses

Epi findings:

Symptoms, symptom complexes significantly elevated in Gulf War veterans compared to nondeployed era veterans

Gulf War Illnesses

- Symptoms in multiple domains
GW veterans have more symptoms, more types of symptoms, more severe symptoms
- Nonrandom distribution
Rates vary by branch of service, deployment location
- Prevalence of GW depends on definition



RAC-GWVI

Research Advisory Committee on Gulf War Veterans' Illnesses

Table 3. Prevalence Estimates of Multisymptom Illness in Gulf and Non-Gulf Veterans

Group Studied	Case Definition Used	Prevalence in Gulf War Veterans	Prevalence in Non-Gulf Veterans	Excess Illness in Gulf Veterans
Pennsylvania Air Guard ⁸⁵	CMI	45%	15%	30%
U.K. male veterans ³⁴⁹	CMI (modified)	62%	36%	26%
Kansas veterans ²⁸⁵	KS Gulf War Illness	34%	8%	26%
Kansas veterans ²⁸⁵	CMI (modified)	47%	20%	27%
New England Army veterans ²⁴³	CMI (modified)	65%	33%	32%

CMI: chronic multisymptom illness, as defined by Fukuda et al.⁸⁵

Gulf War Illnesses

Gulf War Illnesses

Epidemiologic studies also find GWI rates significantly associated with veteran-reported exposures

☆☆☆ **RAC-GWVI**
Research Activity Concerning Gulf War Veterans' Illnesses

Epidemiologic Studies: General Findings

- **Epidemiologic studies typically identify significant associations between GWI and most self-reported exposures**
- **Has led to observations that:**
 - *Veterans with GWI more likely to overreport every exposure*
 - *All epidemiologic findings are result of reporting bias*
 - *Epidemiologic results not informative; no exposure clearly implicated in GWI*



RAC - GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

Basic Problem with This Assessment (Epidemiology 101)

- **Complex exposure scenario: multiple exposures in theater; exposures highly correlated**
- **When data analyses do not account for this, expect results to be highly confounded, even nonsensical**
- **Relatively few Gulf War epidemiologic studies have been analyzed to address this**



RAC - GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

Weighing the Evidence from Epidemiologic Studies

- **When analyses control for effects of multiple exposures, studies typically identify only a limited number of “risk factors” for GWI**
- **With an eye to this and other study strengths/weaknesses, epidemiologic information can be a valuable resource in understanding GWI**
- **Important to consider consistency of epidemiologic findings across different studies**



RAC - GWVI

Research Advisory Committee on Gulf War Veterans' Illnesses

RAC Analysis: Comparing the Weight of Evidence for GWI in Relation to Different Wartime Exposures

- **Big picture** extent and patterns of exposure during deployment
- **Known toxic effects** info from toxicological, occupational health studies
- **Epidemiologic studies of Gulf War veterans**



RAC - GWVI

Research Advisory Committee on Gulf War Veterans' Illnesses

Psychological Stressors During Deployment



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Health

Psychological Stressors Associated with Gulf War Deployment

- Big picture: exposures
- Known toxic effects
- GW epidemiologic studies



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Health

→Big Picture Psychological Stressors

- ◆ Many types reported, from less severe to extremely traumatic
- ◆ How common?
 - Chemical alerts 66 %
 - SCUD exploded nearby 43 %
 - Participation in combat 27 %
 - Witnessed deaths 26 %
 - Family problem 7 %
 - Sexual assault 1 %
- ◆ Some more common among ground troops
- ◆ Many of these were not unique to 1990-91 Gulf War



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Health

→Known Toxic/Adverse Effects Psychological Stressors

- ◆ Severe trauma associated with PTSD, other psychiatric conditions
- ◆ PTSD, other psych conditions associated with higher levels of somatic symptoms
- ◆ Less is known re:
 - Persistence of somatic symptoms many years after lower-level stressors?
 - Somatic symptoms after trauma in the absence of psych illness?



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Health

**→Known Toxic/Adverse Effects
 Psychological Stressors**

- ◆ Animal studies have shown that stress can alter effects of other Gulf War-related exposures
 - Can increase adverse effects of PB, DEET, permethrin combinations
 - Effects on blood brain barrier?
 - May modulate neurotoxic effects of DU



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

**→Epidemiologic Findings in Gulf War Veterans
 Psychological Stressors in relation to GWI**

	<u>Unadj OR</u>	<u>Adj OR</u>	<i>Ref</i>
Chemical alerts	2.6*	1.2	GG
	2.2*		CU
	1.9*, 2.7*	ns	JW
SCUD exploded nearby	1.6*		CU
Participated in combat	2.6*	1.3	GG
High combat stress		(2.5)	PS
Witnessed deaths	3.1*	1.3	GG
	1.6*		CU
Family problem	1.7*	1.6	RN
Sexual assault	8.3*		HK
"Combat stress index"	p = 0.02	ns	RH, syn 1



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

→Epidemiologic Findings Psychological Stressors

- ◆ All significantly associated with multisymptom illness in unadjusted analyses, with ORs ~ 1.6 – 3.1
- ◆ High crude OR (8.3) for sexual assault in Kang study
- ◆ None significant in studies adjusting for other wartime exposures



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

Vaccines



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

→Big Picture Vaccines

◆ Self-reported exposures:

- Anthrax 41%
- Typhoid 44%
- Botulinum 3%
- Plague 15%
- Meningococcus 6%
- 10 shots or more 34%

◆ Combat troops reported most likely to have received anthrax, botulinum toxoid

☆☆☆ **RAC-GWVI**
Research Advisory Committee On Gulf War Veterans' Health

→Epidemiologic Findings in Gulf War Veterans Individual Vaccines

	<u>Unadj</u>	<u>Adj</u>	<i>Ref</i>
Botulinum	1.8*		KB
	4.9*	1.4	GG
Meningococcus	1.6		
	3.0*	1.3*	GG
Anthrax	1.5*, 1.9*	1.5*	JW
	1.7*		KB
	3.7*	1.0	GG
	1.3		MH(post)
	1.5*	0.9	CU
Plague	1.3		KB
	3.2*	0.9	GG
	0.9		MH(post)
	1.3*		CU

☆☆☆ **RAC-GWVI**
Research Advisory Committee On Gulf War Veterans' Health

**→Epidemiologic Findings in Gulf War Veterans
Number of Vaccines**

	<u>Unadj</u>	<u>Adj</u>	<i>Ref</i>
Post deploy:			
0-1	1.0		
2	2.2*		<i>MH</i>
3	2.4*		
4	2.2*		
5+	5.0*		
Symptom score/# vaccines		p<.001	<i>NC</i>
0	1.0		<i>Austr</i>
1-4	0.9		
5-9	1.3*		
10+	1.2*		



**NAPP Pills
(Pyridostigmine
Bromide)**



→Big Picture PB Exposures

- ◆ Self-reported exposures:
 - Used PB 49 - 60%
 - Seabees study 32%
 - Used NAPS > 14 days 60% (UK)
- ◆ More commonly reported by ground troops; Guard use may be higher than active
- ◆ Widespread use of PB unique to 1990-91 Gulf War



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

→Known Toxic Effects PB

- ◆ Used for many years to treat myasthenia gravis, considered safe in clinical use
- ◆ Acute side effects (mostly GI) reported to have affected about 1/3 with PB use during the Gulf War
- ◆ Animal studies indicate synergism with DEET, permethrin
- ◆ Preliminary evidence of PB causing severe difficulty for individuals with low BChE activity



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

**→Epidemiologic Findings in Gulf War Veterans
 PB use in relation to GWI**

	<u>Unadj</u>	<u>Adj</u>	<i>Ref</i>
Took PB tablets	3.0*	1.5*	<i>GG</i>
	1.4*		<i>Aust</i>
	1.4*, 3.0*	1.6*, 2.9*	<i>RN</i>
	2.6*		<i>CU</i>
	ns	ns	<i>SP</i>
Took 1-21 PB pills 22 + PB pills	1.9*, 2.3*	1.4	<i>JW</i>
Took > 21 PB tablets No. of days took NAPs Side effects from NAPs	4.44*	2.2*	<i>PS</i>
Advanced PB side effects		p<.001*	<i>NC</i>
Used PB	p<.001*	p<.001*	<i>RH syn2,3</i>
			<i>Iowa</i>

☆☆☆ **RAC-GWVI**
Research Advisory Committee On Gulf War Veterans' Illnesses

- Epidemiologic Findings
 PB**
-
- ◆ PB variables sign. associated with GWI in studies that adjust for other wartime exposures, ORs ~ 1.5 – 2.9 (not in Ft. Devens study or at lower level in 2nd Ft. Devens study)
 - ◆ 3 studies indicate a dose/response effect
 - ◆ 2 studies support association with acute side effects of PB
- ☆☆☆ **RAC-GWVI**
Research Advisory Committee On Gulf War Veterans' Illnesses

Putting It All Together

Weight of Evidence Relating Individual Wartime Exposures to GWI

☆☆☆ **RAC-GWVI**
Research Advisory Committee On Gulf War Veterans' Illnesses

Gulf War Exposures in relation to GWI: Summary of Epidemiologic Evidence

	<u>Unadj</u>	<u>Adj</u>	<u>Adj Results Consist</u>	<u>Dose/ resp</u>
Psychological stressors	1.6-3.1	ns	yes	-
Pesticides	1.9-3.8	1.7-8.7	yes	yes
NAPP/PB pills	1.4-4.4	1.5-2.9	yes	yes
Chemical weapons	1.9-6.3	2.3-7.8	~	-
DU	4.5*	no studies	-	-
Oil well fires	1.8-4.5	2.1	no	yes
Vaccines: anthrax meningococcus	1.5-3.7 3.0	1.5 1.3	little info	-
Number of vaccines	3 sign	1 sign	little info	yes

☆☆☆ **RAC-GWVI**
Research Advisory Committee On Gulf War Veterans' Illnesses

Exposures and GWI: *Evidence Supports Association*

- > **Strongest evidence from epidemiologic studies supports pesticides and PB as causal factors in GWI**
 - **Animal studies support plausibility, especially when PB combined with other exposures**
 - **Overall pattern of exposures consistent with association**

- > **Two studies support positive associations with s/r exposure to chemical weapons, but s/r exposure questionable**
 - **Unknown if exposures extensive enough to explain large proportion of cases**



RAC - GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

Exposures and GWI: Little/Poor Evidence

- > **Very little useful information concerning associations between vaccines and GWI**
 - **Significant associations generally modest**
 - **Little animal or human research informs plausibility**

- > **Almost no epi information concerning associations between DU and GWI**
 - **Animal studies suggest possible neuro effects**
 - **Unknown if similar conditions seen in other deployments with comparable DU exposures**



RAC - GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

Exposures and GWI

- > Oil well fires, overall, unlikely to be primary cause of GWI
 - 2 studies suggest higher exposure levels may be problematic
 - May be associated with diagnosed asthma

- > Consistent epi findings that psych stressors are not associated with GWI
 - Animal studies suggest possible synergism w/exposures
 - Epi studies consistently identify association of psych stressors with PTSD, other psych diagnoses



RAC-GWVI

Research Advisory Committee on Gulf War Veterans' Illnesses

Gulf War Illnesses: General Observations

- Studies have identified consistent patterns of excess symptomatology in Gulf War veterans; not associated with objective signs, tests
- Substantial proportion of veterans affected
- A number of dx conditions elevated, but affect far fewer veterans. CFS, FM account for small % of veterans with GWI.



RAC-GWVI

Research Advisory Committee on Gulf War Veterans' Illnesses

Gulf War Illnesses: General Observations

- Epidemiologic findings indicate consistent associations with neurotoxic exposures, little/no association with psychological stressors
- Clinical studies have identified a variety of indicators of CNS impairment in veterans with GWI; other findings limited
- Toxicological studies indicate synergism between some Gulf War-related exposures



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses

Broad Spectrum of Evidence Considered by the Committee Thus Far

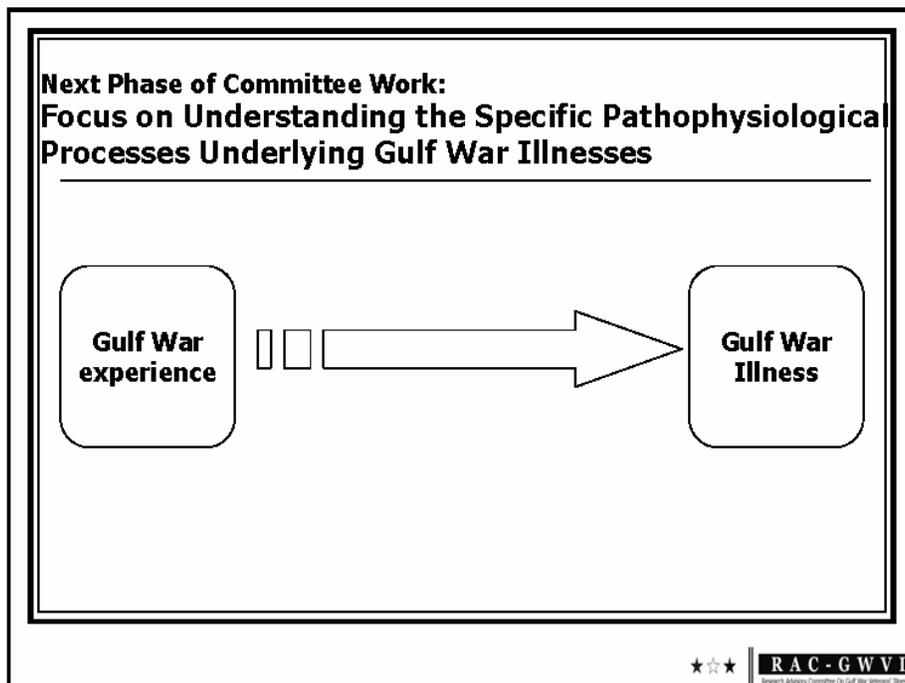
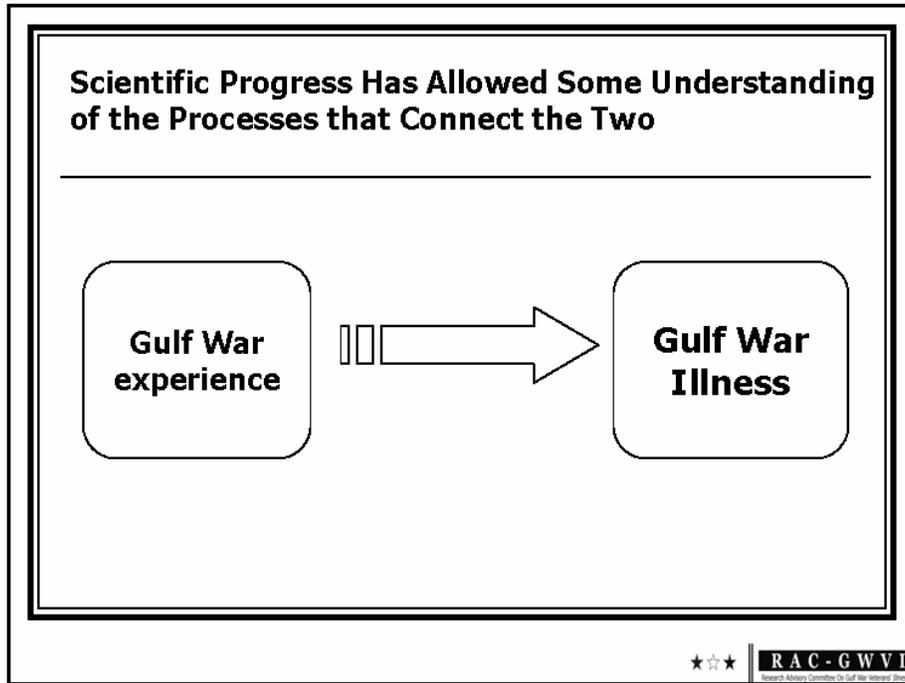
**Gulf War
experiences
and
exposures**

**Gulf War
Illnesses**



RAC-GWVI

Research Advisory Committee On Gulf War Veterans' Illnesses



Presentation 3 - Mohamed Abou-Donia

**Toxicological Studies Evaluating
Synergism between Gulf-War
Exposures**

Mohamed B. Abou-Donia
Duke University Medical Center
Durham, North Carolina
donia@duke.edu

Introduction

Many Persian Gulf War Veterans complained of symptoms including:

chronic fatigue,
muscle and joint pain,
ataxia,
inability to concentrate,
forgetfulness, and
behavioral abnormalities.

Exposures

1. Between the invasion of Kuwait by Iraq on August 2, 1990 and March 1991, the U.S. Had 697,000 military personnel in the Persian Gulf Region (IOM, 1995).
2. Women accounted for 7% and the Reserve/National Guard were 17%.
3. Exposure was to a combination of psychological, biological and chemical environments.

Hypothesis

Gulf War illnesses were caused by combined exposure to:

Pyridostigmine bromide (PB)
DEET, and
Permethrin

Combined Chemical Exposure

This hypothesis was prompted by

1. Failure to identify bacterial, viral, or parasitic as a source of veterans' complaints.
2. Our previous studies that exposure to multiple chemicals increased toxicity of single compounds,

Concurrent Chemical Exposure Increases Neurotoxicity of single Compounds

1. Methyl *iso*-butyl ketone (Non-neurotoxic) increases the neurotoxicity of the weak neurotoxicant, *n*-hexane.^a
2. Methyl *iso*-butyl ketone Increases OPIDN induced by the OP insecticide EPN.^b
3. The OP insecticide, Safrotin increases OPIDN induced by the OP, chlorpyrifos.^c

^aBiochem. Pharmacol. 41:877-883 (1991)

^bJ. Pharmacol. Exp. Therap. 257:282-289 (1991)

^cToxicologist 15:205 (1995)

Mechanisms of Synergism

1. Pharmacokinetics

Increased neurotoxicity results from increased “effective concentration” of the neurotoxic chemical at the neurotoxicity target

- a. Activation
- b. Increased Bioavailability

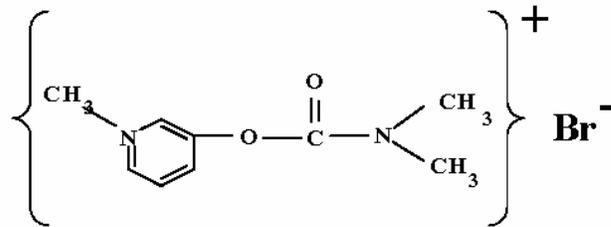
2. Pharmacodynamics

Alterations of the neurotoxic target, e.g., receptor up- or down-regulation

Neurotoxicity of Pyridostigmine Bromide (PB), DEET, and Permethrin

1. All U.S. Military personnel were given PB to protect against the nerve agent, sarin.
2. Military personnel were given the insect repellent, DEET, 70% in ethanol.
3. Many military personnel used uniforms impregnated with the insecticide permethrin.

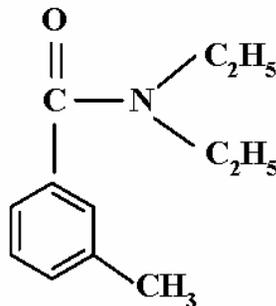
Pyridostigmine Bromide



1. A quaternary dimethyl carbamate
2. Does not cross the BBB
3. Reversibly shields peripheral ChE
(30 – 40 % inhibition)

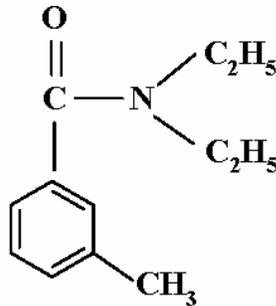
Dosage: 30 mg, 3times/day

DEET



1. A personal insect repellent (1946).
2. Used by 30% of the population.
3. Prolonged use causes brain neuronal degeneration.

DEET



1. A personal insect repellent (1946).
2. Used by 30% of the population.
3. Prolonged use causes brain neuronal degeneration.

DEET

1. Developed by U.S. Army 1946; Registered for public use 1957; Every year used by 1/3 of US Population (75 million)
2. DEET products range from 10% to 100%, as liquids, gels, sticks, impregnated materials; more than 30 million packages are sold annually
3. Approximately 230 products containing DEET are registered with U.S. EPA by about 70 different companies

Neurotoxicity of PB, DEET, and/or Permethrin in Hens

1. Exposure to a large dose of a single compound resulted in minimal toxicity.
2. Combination of two compounds produced greater neurotoxicity than by individual chemicals.
3. Neurotoxicity was further enhanced after concurrent administration of the three compounds

J. Toxicol. Environ. Health 48:35-56, (1996)

Neurotoxicity of PB, DEET, and/or Chlorpyrifos in Hens

1. Co-exposure to binary compounds caused greater neurotoxicity than single treatments.
2. Concurrent administration of the three compounds further increased neurotoxicity
3. This was indicated by: AChE activity, NTE activity, Neurological dysfunction, histopathological alterations

Fund. Appl. Toxicol. 34:201-222, (1996)

Locomotor and Sensorimotor Performance Deficit in Rats exposed to PB, DEET, and/or Permethrin in Rats

Male, Sprague-Dawley rats were treated:

1. **Control:** 70% ethanol dermal, water oral, 1 ml/kg.
2. **PB:** 1.39 mg/kg in water/d, Oral, 15 days.
3. **DEET:** 40 mg/kg/d dermal in 70% ethanol, 45 days.
4. **Permethrin:** 0.13 mg/kg/d dermal in 70% ethanol, 45 days.
5. DEET + Permethrin, 45 days.
6. DEET, 45 days + PB, last 15 days.
7. Permethrin 45 days + PB, last 15 days.
8. DEET, 45 days + Permethrin 45 days + PB, last 15 days.

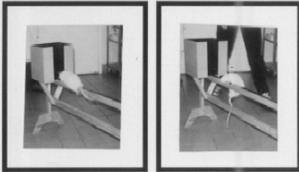
Toxicol Sci. 60:305-314 (2001)

Sensorimotor Performance

A battery of behavioral test included:

- a. *Beam-Walk Score and Beam-Walk Time*
- b. *Incline Plane Performance*
- c. *Forepaw Grip*

Beam Walk



The apparatus consists of an elevated wooden beam, a goal box with an opening located at the end of the beam, and a light source.

BW Time: The time until the animal's nose entered the box (up to 90 sec.).

BW Score: A 7-point scoring system for the use of the hind paw to aid locomotion.

Beam-Walk Time and Beam-Walk Score

At 45 days:

1. PB alone or in combination with DEET and/or permethrin caused significant deficit in beam-walk time and beam-walk score.
2. DEET and or permethrin did not have significant effect on beam walk-time or beam-walk score

Incline Plane



Description
Rats are placed on a flat plane in the horizontal position, with the head facing the side of the board to be raised.
The angle at which the rat begins to slip is recorded.

Incline Plane

All Chemicals, alone or in combination, resulting in significant impairment in incline plane testing.

Forepaw GRIP TIME

PURPOSE: To assess forepaw grip strength

PROCEDURE:

1. Have the rats grip a 5-mm diameter wood dowel
2. Time to release grip is recorded in seconds.

Forepaw Grip Time

All Chemicals, alone or in combination, resulting in significant impairment in forepaw grip time testing.

Sensorimotor Performance: Dose-Response

Dose (x, mg/kg/day)

PB, 1.39, oral in water;

DEET, 40 in 70% ethanol

Permethrin, 0.13 in 70% ethanol

Single-compound treatments

PB, DEET, or permethrin: 0.1 x, 1 x and 10 x

Two-compound treatments

PB + DEET, PB + Permethrin, or DEET + Permethrin: 0.1 x, 1 x and 10 x

Three-compound treatments

PB + DEET + Permethrin: 0.1 x, 1 x and 10 x

J. Toxicol. Environ. Health 62:523-541 (2001)

Pharmacol. Biochem. Behav. 77:253-262 (2004)

Plasma BChE Brain AChE, and Brain AChR: Dose-Response

1. Plasma BChE:

PB alone slightly inhibited plasma BChE, PB in combination with DEET and/or permethrin increased its activity.

2. PB alone or in combination increased brainstem AChE activity. This leads to decreased ACh and slow down of cholinergic functions, e.g., memory deficit

3. PB alone or in combination increased ligand binding to cortex m2AChR and nAChR.

Sensorimotor Deficit: Summary

1. Exposure to PB, DEET and permethrin, alone and in combination, causes significant sensorimotor deficits.
2. Sensorimotor deficit is associated with cortical injury.
3. Beam-walk performance involves consciousness, memory, sensorimotor, and cortical functions. An injury to the cortex is reflected by a deficit in beam-walk task.

Brain Neuronal Cell Death Caused by DEET and/or Permethrin

Experimental

1. Adult, male, Sprague-Dawley rats were treated with a daily dermal dose, for 60 days with:
DEET, 40 mg/kg and Permethrin, 0.13 mg/kg
2. Twenty four hours after last dose, the animals were anesthetized and perfused via the heart with saline followed by 4% paraformaldehyde and 0.1% gluteraldehyde in Tris buffer.

Neuropathological Studies

The following brain areas were altered:

1. Motor cerebral cortex
2. Hippocampal formation
 - a) CA1 subfields
 - b) CA 3 subfields
 - c) Dentate Gyrus
3. Cerebellum

Histopathological Assessment: Hematoxylin and eosin stain

Immunohistochemical Studies: Monoclonal antibodies (SMI 52) against MAP-2 and polyclonal antibodies against GFAP

TYPES OF NEURONS IN CEREBRAL COTRTEX

- I. Molecular layer
- II. External granular layer
- III. External Pyramidal layer
- IV. Internal granular layer
- V. Internal Pyramidal layer
- VI. Multiform

Alterations in the cerebral cortex

1. Density of dying neurons was greater in deeper layer (V) and in larger pyramidal neurons of the motor cortex layer
2. Axons of these neurons form the corticospinal descending (motor) tracts, controlling the movement of muscles
3. Significant death of these neurons results in muscular weakness and loss of strength

Neuronal degeneration of the Hippocampus

1. Hippocampus is involved in learning, memory, and emotional expression.
2. A loss of significant amount of neurons in different subfields may lead to a progressive loss of memory and results in learning disabilities.

NEURONAL DEGENERATION OF THE CEREBELLUM

Widespread of Purkinje cell death was the hallmark lesion in the cerebellum. Since cerebellar cortex modulates cortical motor commands, its lesions may cause:

1. Delays in initiating and terminating movements.
2. Terminal tremor at the end of movement.
3. Disorders in the spatial coordination of hand and finger muscle.

CONCLUSIONS

**Daily dermal dose of 40 mg/kg DEET and/or
0.13 mg/kg permethrin for 60 days in rats:**

1. **No change in body weight or clinical condition.**
2. **Impairment of sensorimotor performance .**
3. **Neuronal cell death in: cerebral cortex, hippocampal formation, and cerebellum.**
4. **Consequences: *Motor deficits; learning and memory dysfunction.***

Mechanisms of Neuronal Cell Death

The results demonstrate that although DEET, an insect repellent and permethrin, an insecticide, are chemically unrelated, with different biological actions, they both produced similar histopathological lesions, both in morphology and distribution.

Conclusion: Both compounds have a common mechanism pathway leading to neuropathological lesions.

Susceptibility of the Brain to Free Radical-Mediated Injury

Free Radical-Induced Injury

1. Brain is rich in polyunsaturated fatty acids .
2. Some brain regions, e.g., substantia nigra and striatum, have high concentration of iron.
3. Mitochondrial respiratory activity is higher in brain tissue, that may risk free radical “leak” from mitochondria.

The result is increased susceptibility of brain cell membrane damage and to lipid peroxidation.

NEURONAL VULNERABILITY TO ROS

Neurons that are selectively vulnerable to reactive oxygen species (ROS) include:

1. Cortical pyramidal neurons
2. Hippocampal CA1 pyranidal neurons
3. Cerebral Purkinje cells
4. Subpopulations in amygdala, striatum, thalamus and brainstem nuclei

Oxidation Reaction

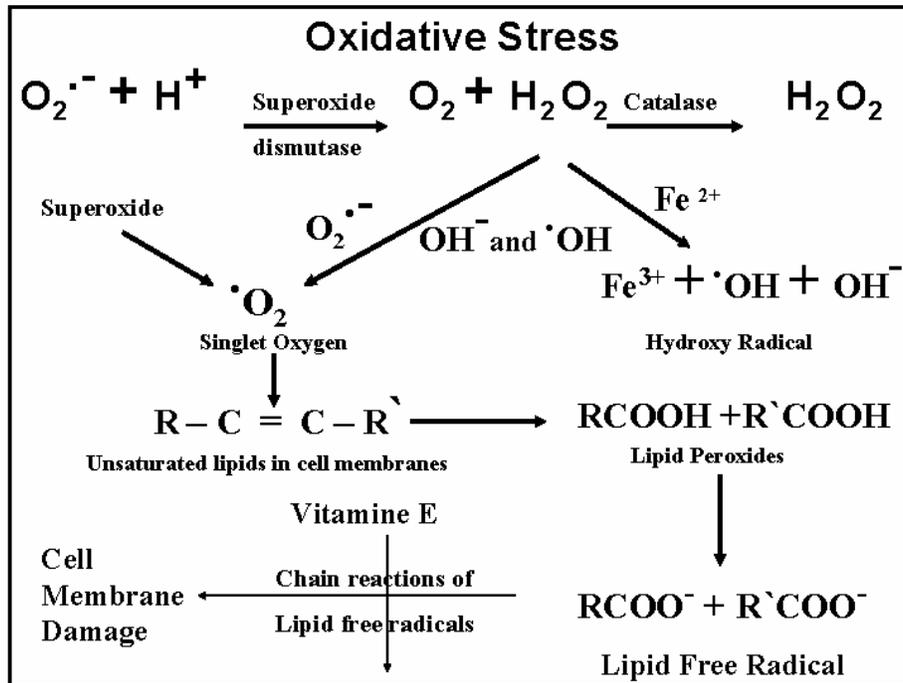
1. Oxidation of small molecules by CYP450 enzymes , requires activation of molecular oxygen (O_2) to atomic oxygen (O^*).
2. Most oxidative enzymes bind a metal ion, e.g., Fe, Cu, Co, or Se, which destabilizes the O_2 molecule .

Oxygen Free Radicals

- A. Oxygen free radicals are intermediates in many biological reactions, but may damage macromolecules during oxidative stress.
- B. Free radicals are molecules that possess a single unpaired electron in outer electron orbital, such as:
1. Hydrogen atom, H^\bullet
 2. Oxygen molecule, O_2 ; possesses 2 unpaired electrons
 3. NO^\bullet
 4. Superoxide, $\text{O}_2^{\bullet -}$; one unpaired electron
 5. Hydroxyl radical, $^\bullet\text{OH}$
 6. Transition metals, e.g., **Cu** and **Fe**

Oxygen Free Radicals

- A. Oxygen free radicals are intermediates in many biological reactions, but may damage macromolecules during oxidative stress.
- B. Free radicals are molecules that possess a single unpaired electron in outer electron orbital, such as:
1. Hydrogen atom, H^\bullet
 2. Oxygen molecule, O_2 ; possesses 2 unpaired electrons
 3. NO^\bullet
 4. Superoxide, $\text{O}_2^{\bullet -}$; one unpaired electron
 5. Hydroxyl radical, $^\bullet\text{OH}$
 6. Transition metals, e.g., **Cu** and **Fe**



Brain Antioxidant Defenses

- Superoxide dismutase:** (SOD1 represents as much as 1% SOD of total protein in the brain)

$$O_2^{\cdot-} \xrightarrow{\text{SOD}} H_2O_2$$

Superoxide \rightarrow Hydrogen Peroxide

- Catalase:** More abundant in astrocytes and white matter than neurons and gray matter.

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O$$

- Glutathione (GSH) Peroxidase:** GSH peroxidase uses glutathione as a co-factor to convert H_2O_2 to water.

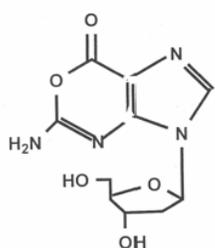
Consequences of Oxidative Stress

1. Damage to cellular macromolecules
2. Fragmentation of lipids
3. Addition of peroxy or hydroxyl groups to unsaturated fatty acids
4. Cleavage of fatty acid carbon chain to fatty aldehyde
5. Reaction of fatty aldehydes with free thiol groups to produce thioesters , affecting protein structures and stability
6. Free radicals may cause cross-linking, carbonyl formation, and protein denaturation
7. DNA may be modified, resulting in single-and double strand breaks or miss-parings of purine and pyrimidine during DNA replication.

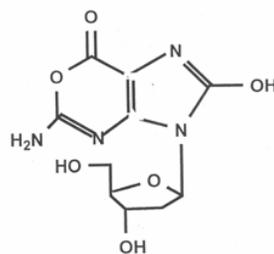
Mechanisms of Neuronal Death

- Combined exposure to PB, DEET, and permethrin caused brain cell death via:
1. Impeding the body's ability to metabolize and eliminate test compounds.
 2. Increasing the level of reactive oxygen species (ROS), by enhancing their production or reducing the brain's defenses against them.

A BIOMARKER FOR OXIDATIVE DNA DAMAGE



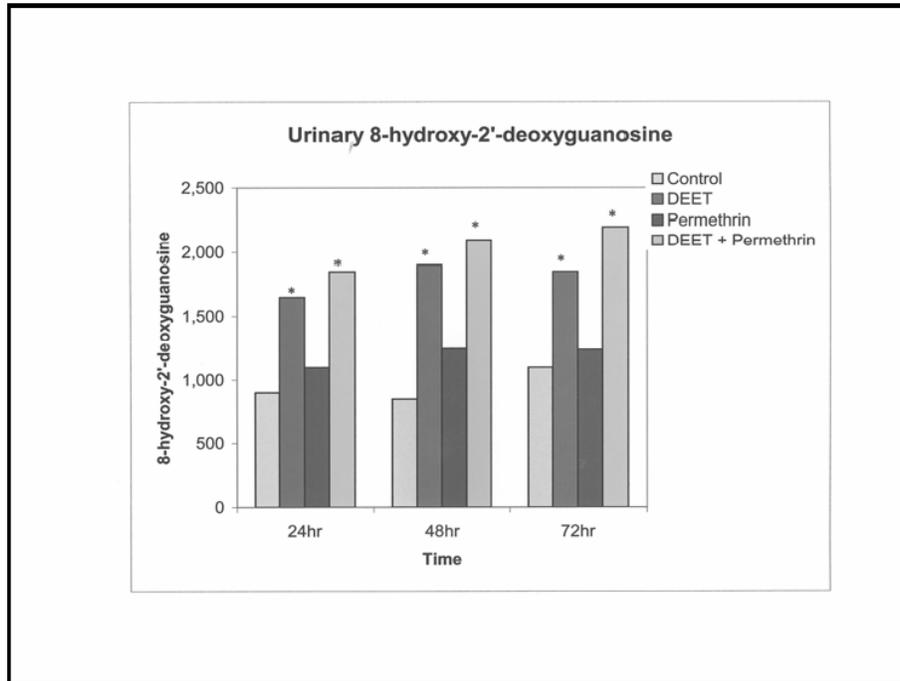
2'-deoxyguanosine



8-Hydroxy-2'-deoxyguanosine

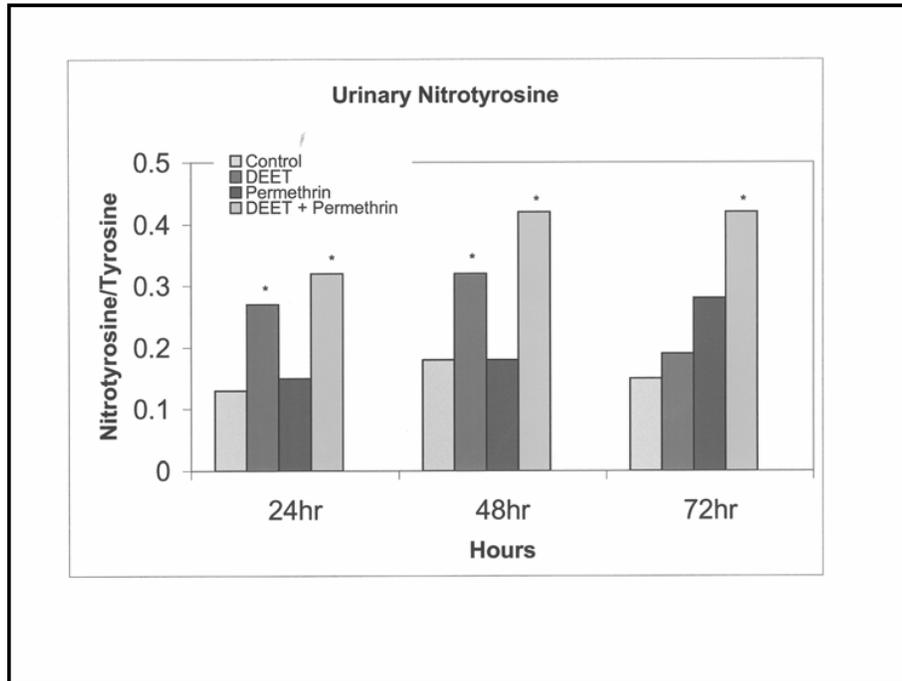
8-hydroxy-2'-deoxyguanosine, a Biomaker for Oxidative DNA Damage

1. DEET alone or in combination with permethrin induced excretion of 8-OHdG in rat urine.
2. Dermal applications of DEET could generate free radical species, causing DNA damage in treated rats.



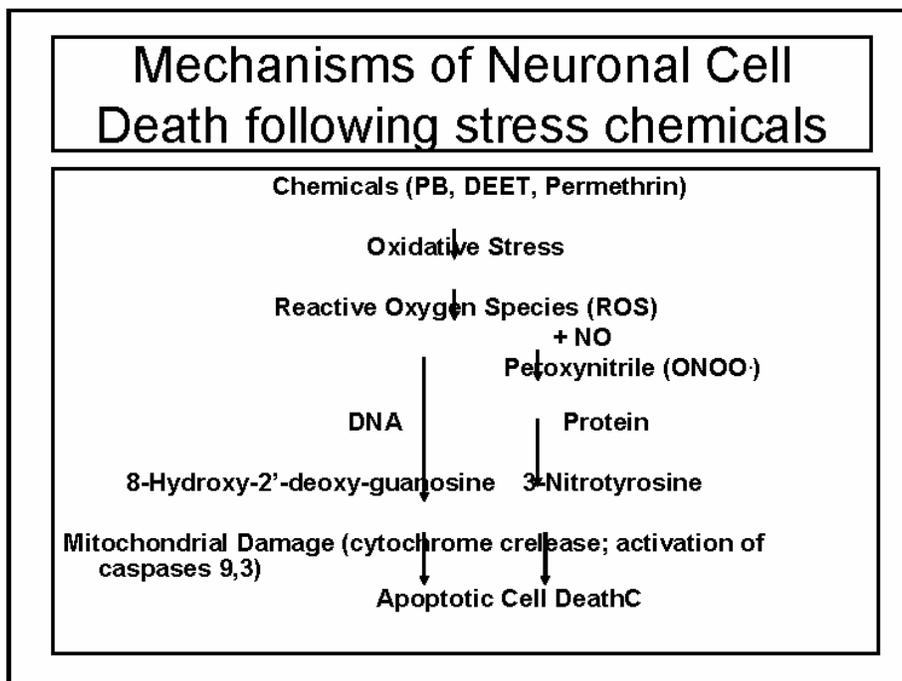
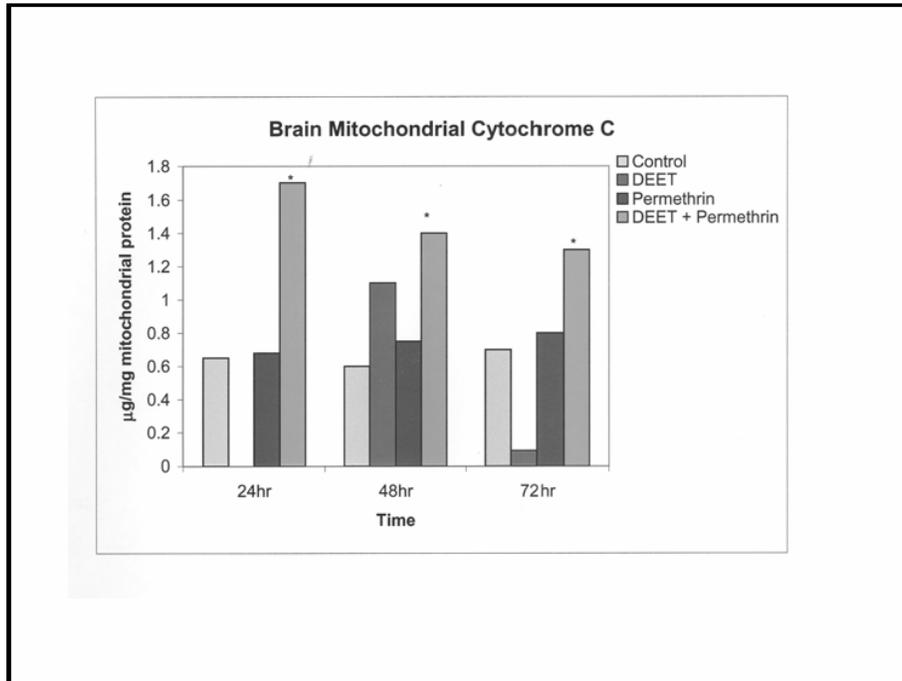
3-nitrotyrosine, a Biomaker for Oxidative Stress

1. A single oral dose of PB or a dermal dose of DEET, alone or in combination significantly increased levels of 3-nitrotyrosine in rat urine.
2. These treatments could generate free radical species, increasing levels of excreted 3-nitrotyrosine.



Brain Mitochondrial Cytochrome c, a Biomarker for Oxidative Stress and Apoptosis

1. A single dermal dose of a combination of DEET and permethrin, significantly increased the release of brain mitochondrial cytochrome c.
2. Neither DEET nor permethrin alone had any effect on mitochondrial cytochrome c of rat brains.
3. Combined exposure to DEET and permethrin could generate reactive oxygen species, leading to an early release of mitochondrial cytochrome c that is involved in apoptotic processes by activating caspases 9 and 3.



Neuronal cell Death Consequences

1. **Significant death of cerebral cortex neurons results in muscular weakness and loss of strength.**
2. **A loss of significant amount of hippocampal neurons leads to progressive loss of memory and results in learning disabilities.**
3. **Loss of Purkinje cells in the cerebellum may cause:**
 - a. **Delays in initiating and terminating movements.**
 - b. **Terminal tremor at the end of the movement.**
 - c. **Disorders in the spatial coordination of hand and finger muscle.**

“THESE SYMPTOMS ARE SOME OF THE GULF WAR VETERANS’ COMPLAINTS”

ACKNOWLEDGEMENTS

Ali A. Abdel-Rahman, Ph.D.
Sarah L. Bullman, B.S.
Tirupapuliyur V. Damodaran, Ph.D.
Anjelika M. Dechkovskaia, M.D.
Larry B. Goldstein, M.D., Ph.D.
Xiangrong Guan, M.D.
Elizabeth A. Herrick, B.S.
Katherine H. Jones, Ph.D.
Wasiuddin A. Khan, Ph.D.
Ashok K. Shetty, D.V.M., Ph.D.
Eman M. El Masry, Ph.D.

Presentation 4 – Robert Haley

UT Southwestern Research on Gulf War Syndrome



**Robert W. Haley, M.D.
Department of Internal Medicine
University of Texas
Southwestern Medical Center
Dallas, Texas**

Typical Symptoms of Gulf War Syndrome

- **Chronic fatigue**
- **Cognitive problems (attention, concentration, confusion)**
- **Personality change**
- **Constant body pain, paresthesias and hypesthesias (without arthritis)**
- **Balance disturbances, vertigo attacks**
- **Unrefreshing sleep and insomnia**
- **Hot flashes and night sweats**
- **Watery diarrhea alternating with constipation**

Mostly subjective symptoms without objective signs.

Environmental Exposures in the 1991 Gulf War*

- OP chemical warfare agents (sarin, cyclosarin)**
- OP pesticide spraying
- OP pesticides on uniforms
- DEET insect repellants
- Pyridostigmine bromide
- Ciprofloxacin
- Chloroquine
- Multiple immunization including anthrax vaccine
- Smoke from oil well fires
- Fumes from jet fuel sprayed on roads
- Fumes from burning jet fuel in tent stoves
- Petroleum in drinking water
- Depleted uranium
- CARC pain
- Combat stress/PTSD

*Defense Science Board 1994; NIH Consensus Conference 1994; etc.

**Pentagon officially denied that chemical weapons were in theater.

The Epidemiologist's Rule Number 1

*The first step in investigating
a new disease is:
Establish a Case Definition **

*Gregg et al. *Field Epidemiology*. Oxford University Press:1996

The Epidemiologist's Rule Number 1

*The first step in investigating
a new disease is:
Establish a Case Definition**

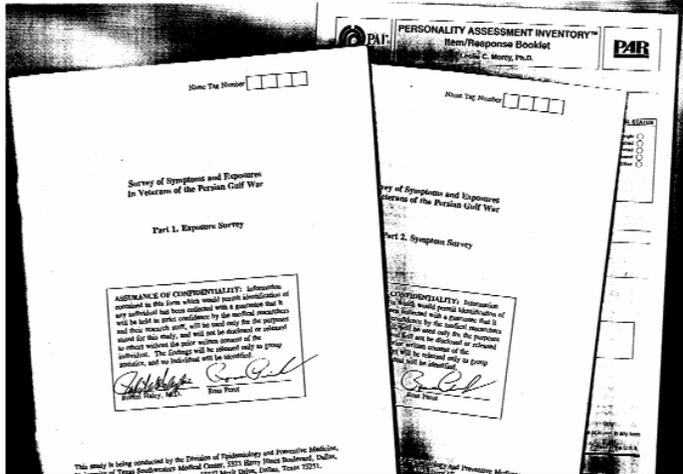
*And if you can't:
then
Establish a Case Definition!*

*Gregg et al. *Field Epidemiology*. Oxford University Press:1996

Development of a Case Definition

- One of Mr. Perot's aides had developed a "registry" of over 2,000 ill Gulf War veterans.
- I requested medical records of the 60 "sickest veterans."
- Two weeks later I received 60 medical records.
- Review of the records identified the "10 sickest veterans" with no other possible explanation.
- The Perot Foundation paid for full evaluation of these 10 at Mayo Clinic.
- The Mayo evaluation ruled out all known diseases.
- I built their symptoms into a survey questionnaire.

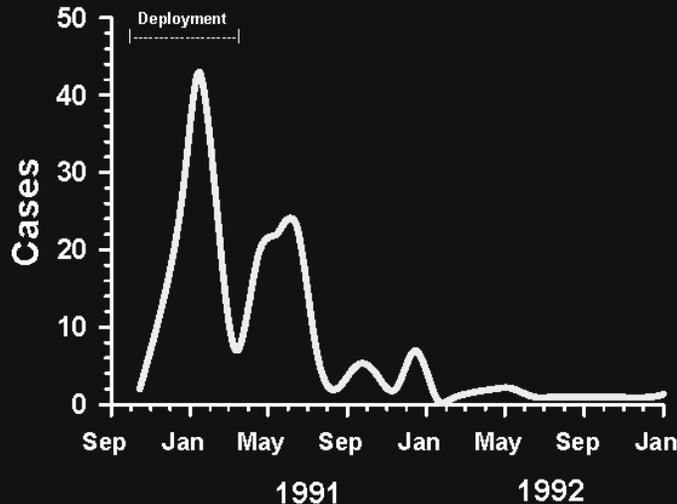
Conducted a Survey in a Reserve Seabees Battalion 24th Reserve Naval Mobile Construction Battalion* December 1994 – February 1995



Knoxville
Birmingham
Winston-Salem
Charlotte
Atlanta

*Seabees uniquely go all over the theater, and this was the only Reserve seabees battalion.

Epidemic Curve of Gulf War Syndrome Date of First Symptom, RNMCB-24



Haley Symptom Questionnaire: Example Question on Paresthesias

2-stage factor analysis
Symptom factors
Syndrome factors

4. In the past 5 years, have you experienced *(tingling, burning or stinging pain)* in any part of your body *lasting all day and continuing for at least a month?* (Do not count feelings that come and go quickly and are not present continuously.)

CIRCLE ONE

Yes..... 1

No..... 2

If you answered "Yes," answer Question #4A.
 If you answered "No," skip to Question #5.

4A. Please indicate what part of your body was affected by this pain, in what month and year it began, and whether it is still a problem for you.

	Was this area involved?		If yes, in what month/year did it begin?		Is it still a problem?	
	YES	NO	MONTH	YEAR	YES	NO
Scalp.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Face.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Lips.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Tongue.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Chest.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Back.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Hands.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Arms.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Abdomen.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Groin.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Genital area.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Rectal area.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Thighs.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Calves.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Feet.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>
Other.....	<input type="checkbox"/>	<input type="checkbox"/>		/		<input type="checkbox"/>

→ specify _____

-6-

Stage 1 Factor Analysis of 7 Anatomical Sites of Symptom “Tingling/Numbness” (249 veterans)

Rotated Factor

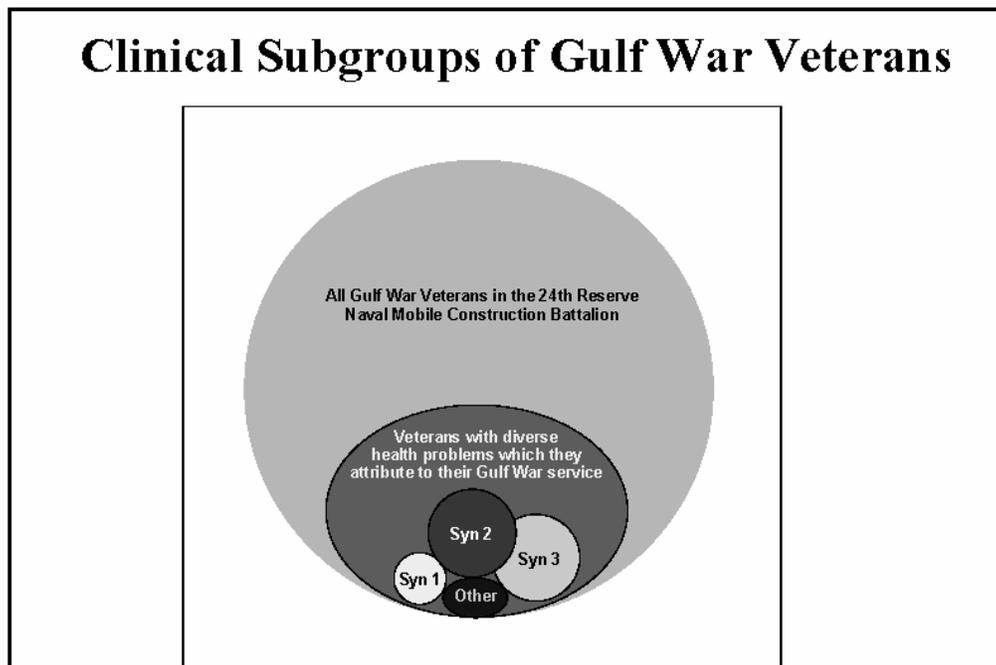
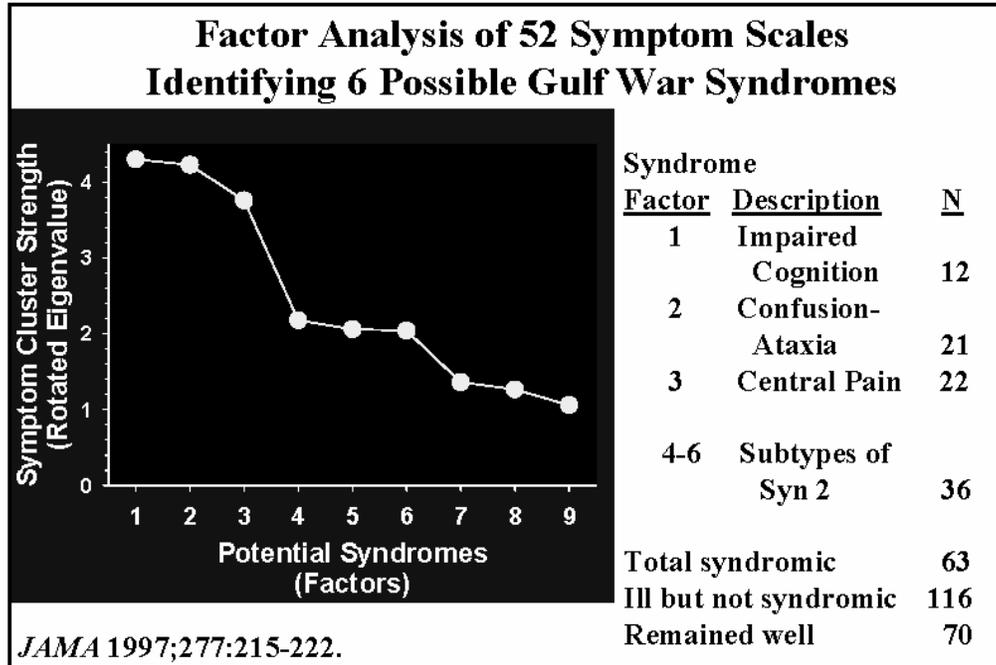
Factor 1

Factor 2

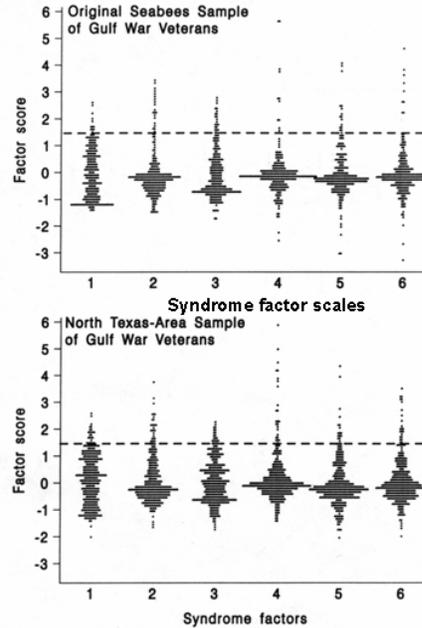
Anatomic location of paresthesias	Correlation with: Symptom Factor 1	Symptom Factor 2
A. Arms	.84*	.19
B. Feet	.82*	.17
C. Hands	.79*	.21
D. Calves	.78*	.19
E. Face	.21	.74*
F. Tongue	.18	.69*
G. Lips	.14	.67*

Separate factor analyses of 21 ambiguous symptoms yielded 52 unambiguous symptom factors.

JAMA 1997;277:215-222.

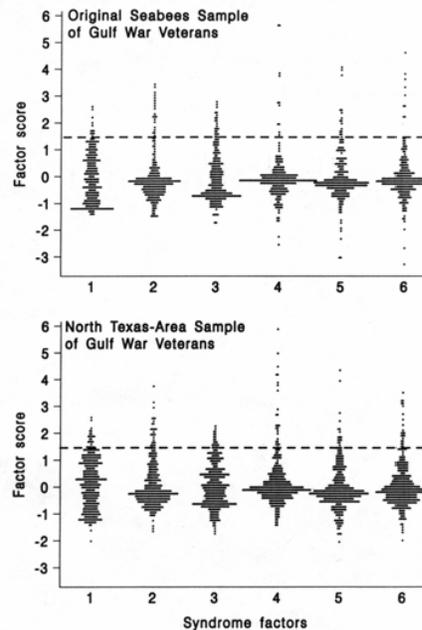


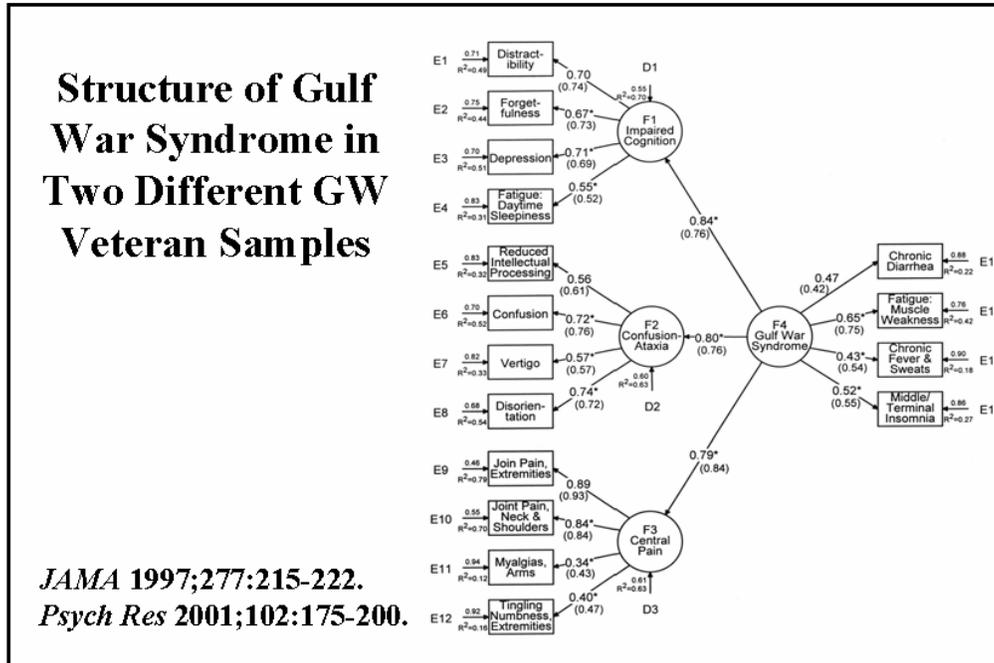
**Distribution of
the 249 Naval
Reservists on
each of 6
Syndrome
Factor Scales**



**Validation of the
Case Definition on
335 Army Veterans
Surveyed at the
Dallas VA
1997- 1998**

**Distribution of 2 Independent
Samples of GW Veterans on 6
Factor Scales of GW
Syndrome**





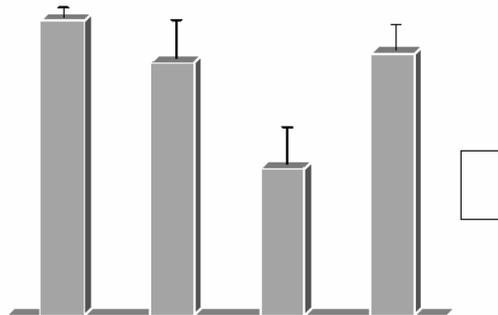
Comparison of Factor Models from Symptom Surveys of Gulf War Veterans

Year	Author	Cognitive	Neurologic	Pain/sensory
1997	Haley	F1	F2	F3
1998	Fukuda	F1	---*	F2
1999	Ismail	F1	---*	F3
2001	Cherry	F1	F3	F2
2002	Kang	F1	F2	F3

*Did not measure the symptoms of the neurologic factor.

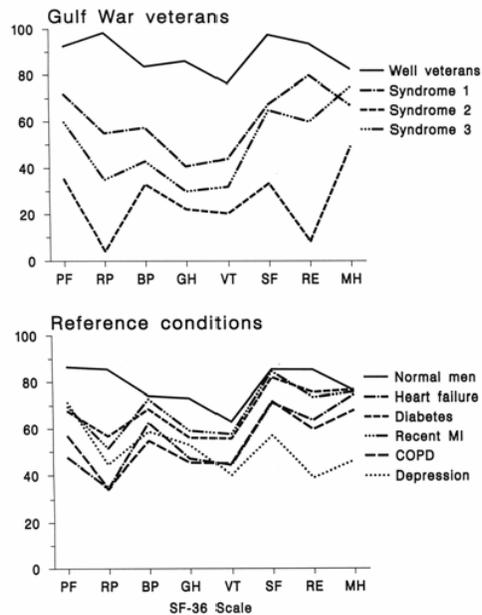
The surveys of Knoke (2000) and Doebbeling (2000) measured symptoms of standard psychiatric conditions and thus found none of the syndrome factors found by the other groups.

Comparison of Syndromes On Percentage Employed in 1995 (N=249)



JAMA 1997;277:215-222.

Functional Status (MOS SF-36) of 22 Ill GW Veterans vs 16 Well Veterans (Top) and 6 Reference Medical Conditions (Bottom)



Am J Public Health 2001;45:121-2.

Epidemiologic Study of Risk Factors for Haley Gulf War Syndromes (N=249)

<u>Syndrome</u>	<u>Exposure</u>	<u>RR</u>	<u>P value</u>
1 Impaired cognition	Wore flea collar (chlorpyrifos)	8.2	.001
	Military security	6.4	.007
2 Confusion-ataxia	Chemical nerve agent exposure	7.8	<.0001
	Many advanced side effects of PB	32.4	<.0001
	N.E. Saudi on 4 th day of Air War*	4.3	.004
3 Central pain	Many advanced side effects of PB	5.1	<.0001
	Index of DEET insect repellent use	7.8	<.0001

*Paths crossed near Khafji on Jan. 19-20, 1991.

JAMA 1997;277:215-222.

Soldiers who were near Khafji on 19-20 Jan. had the highest rate of Gulf War illness (Syndrome 2).



The 4 main U.S. troop concentrations during the Air War

Hypothesis Regarding The Nature of Gulf War Syndrome

- **There is a Gulf War *syndrome* with 3 variants, or subgroups.**
- **It is due to brain cell damage or destruction in deep brain structures (basal ganglia and brainstem).**
- **The symptoms resemble those of well understood diseases of these deep brain structures (early Parkinson's, Huntington's).**

Hypothesis Regarding The Cause of Gulf War Syndrome

- **The most likely causes include low-level sarin, possibly in combination with OP pesticides, pyridostigmine tablets, pesticides, DEET, etc., caused cellular damage in deep brain structures**
- **Probably more pronounced in those soldiers with low natural resistance to OP effects (blood esterase activity).**

Undertook a Series of Clinical Case-Control Studies

**Purpose: To attempt to validate the
case definition**

**Research Question: Do the syndromes
differ from controls and among
themselves on objective biological
parameters?**

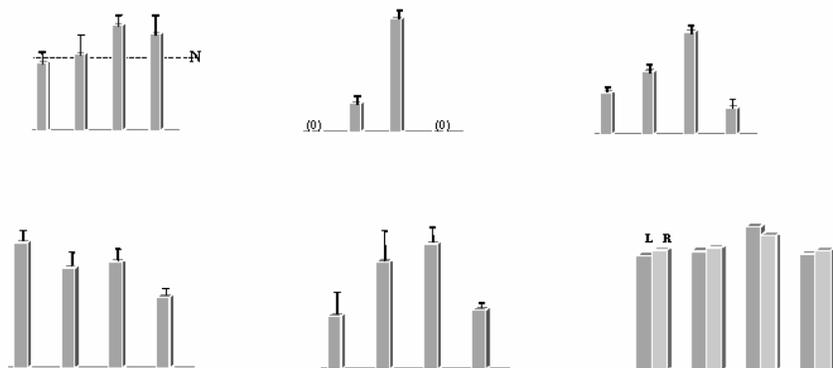
From the 249 Surveyed Veterans Selected Smaller Samples for Case-Control Studies of Brain Function and Serologic Markers

- **23 ill veterans (“cases”)**
 - 5 Syndrome 1
 - 13 Syndrome 2
 - 5 Syndrome 3
- **20 well veterans (“controls”)**
(from the same battalion and
age-sex-education-matched to cases)

Negative Results No Significant Group Differences

- Clinical neurologic examination
- Clinical interpretation of brain MRI and resting HMPAO-SPECT scans (read blindly by 3 radiologists)
- Routine blood work (CBC, chemistries, glucose, ESR)
- Creatine kinase
- Serum protein electrophoresis
- Serum cholinesterase levels and variant phenotypes
- ANA, RF, immunoglobulins, C3/C4
- Anti-double stranded DNA, acetylcholine receptor antibodies

Positive Results On Neurophysiologic Tests



JAMA 1997;277:2223-230.

Jan. 15, 1997 Issue of JAMA

Reprinted from JAMA © The Journal of the American Medical Association January 15, 1997 Volume 277 Copyright 1997, American Medical Association

Original Contributions

Is There a Gulf War Syndrome?

Searching for Syndromes by Factor Analysis of Symptoms

Robert W. Haley, MD; Thomas L. Kurt, MD, MPH; Jim Horn, PhD

Evaluation of Neurologic Function in Gulf War Veterans

A Blinded Case-Control Study

Robert W. Haley, MD; Jim Horn, PhD; Peter S. Roland, MD; Wilson W. Bryan, MD; Paul C. Van Ness, MD;
Frederick J. Bonte, MD; Michael D. Devous, Sr., PhD; Dana Mathews, PhD, MD; James L. Fleckenstein, MD;
Frank H. Wians, Jr., PhD; Gil I. Wolfe, MD; Thomas L. Kurt, MD, MPH

Self-reported Exposure to Neurotoxic Chemical Combinations in the Gulf War

A Cross-sectional Epidemiologic Study

Robert W. Haley, MD; Thomas L. Kurt, MD, MPH

Pilot Study with Col. Bill Davis



U. S. Army Special Forces
Army Ranger
HALO/Scuba

Commander, 5th Special Forces
Group in the 1991 Gulf War
Commanded border salient
Rescued downed fliers
Let Coalition forces in
assault on Kuwait City

Developed Gulf War neurological
illness soon after returning from the
Gulf War.

Col. Bill Davis had a twin!



Clinical Evaluation of the Davis Twins

- **Identical twins are ideal because they normally perform identically on most medical tests; any differences would suggest pathology underlying Gulf War illness.**
- **They visited UT Southwestern Medical Center 4 times.**
- **First performed genetic tests to confirm monozygosity.**
- **Then performed many sophisticated medical tests sensitive to subtle abnormalities in brain function.**
- **Spent over 20 hours in MRI, SPECT and PET scanners to develop brain scanning approaches that showed differences in brain function.**
- **Tests showing differences were then run on 23 cases and 20 controls selected from the earlier study.**

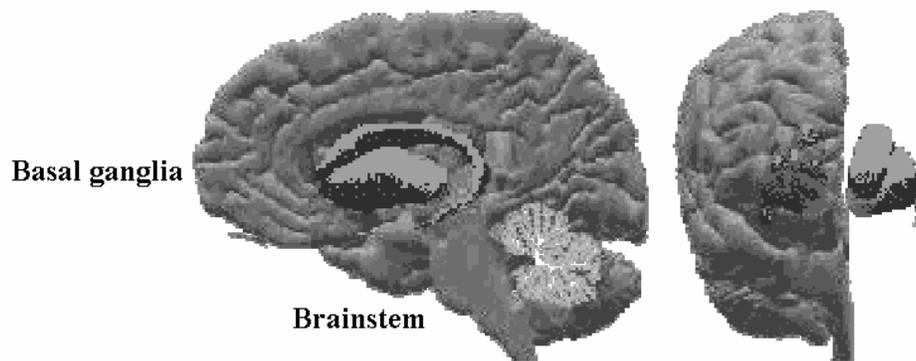
January-June 1998

Second Clinical Case-Control Study with More Advanced Tests of Brain Function and Genetic Predisposition

- **23 ill veterans (“cases”)**
 - 5 Syndrome 1
 - 13 Syndrome 2
 - 5 Syndrome 3
- **20 well veterans (“controls”)**
(from the same battalion and
age-sex-education-matched to cases)

Hypothesis to Be Addressed in the Second Clinical Case-Control Study

Gulf War syndrome variants represent neurotoxic injury to brain cells in deep brain structures involved in well understood diseases that present with similar symptoms: Huntington's, Wilson's, Farr's.

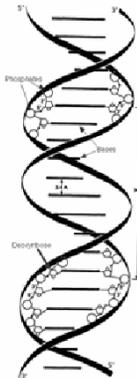


May 29 - June 5 -- Veteran A					Revised 6/1/98			
Time	Friday 29	Saturday 30	Sunday 31	Monday 1	Tuesday 2	Wednesday 3	Thursday 4	Friday 5
6:00		Wake at 6:00 Breakfast 6:30	Wake at 6:00 Breakfast 6:30 Void at 7:00	Wake at 6:00 Breakfast in Sleep Lab Void at 7:00	Wake at 6:00 Breakfast 6:30 Void at 7:00	Wake at 6:00 No Breakfast Void at 7:00	Wake at 6:00 Breakfast 6:30	Wake 6:00 Breakfast 6:30
7:00		↑ MR Spectroscopy (Rogers)	start 1 st urine	start 2 nd urine	start 3 rd urine			
8:00				Waking EEG (JA)		Blood Drawing (Aston 5) SEP Neurophys. (PMH 6)		Joint X-Ray (Aston 6)
9:00				Neuro-Psychology (CS4)			Audio-Vestibular (Aston 7)	
10:00						↑ Ingestion		
11:00			Psychiatric Interview (GCRC)		Neuro-Muscular (Aston 4)	(MPAL) 9705 happy rines		End 4hBP
12:00	Lunch Tu ES 120	Lunch	Metabolic Wt Liquid Lunch	Pat Brings Lunch	Lunch	Liquid Lunch To ES 120	Lunch	Lunch
1:00	HMPAO Inj							
2:00			Micro-Neurography (GCRC)		Neuro-Psychology (CS4)			Plane 2:10
3:00								
4:00	SPECT (1 st)						SPECT (2 nd)	
5:00					Dinner			
6:00	Dinner	Dinner	Dinner	Cab 5:15	Dinner	Dinner	Dinner	
7:00			End Holter Monitor		Joint MRI (Rogers)	Start 48 hr BP		
8:00	Plane 8:45pm	Start Holter Monitor	SEP Breakfast to Sleep Lab					
9:00	GCRC	Sleep Lab 1 (A)	Sleep Lab 2 (A)	Sleep Lab 3 (A)	Sleep Lab 4 (A)	GCRC	GCRC	

GCRC Protocol for Cases and Controls

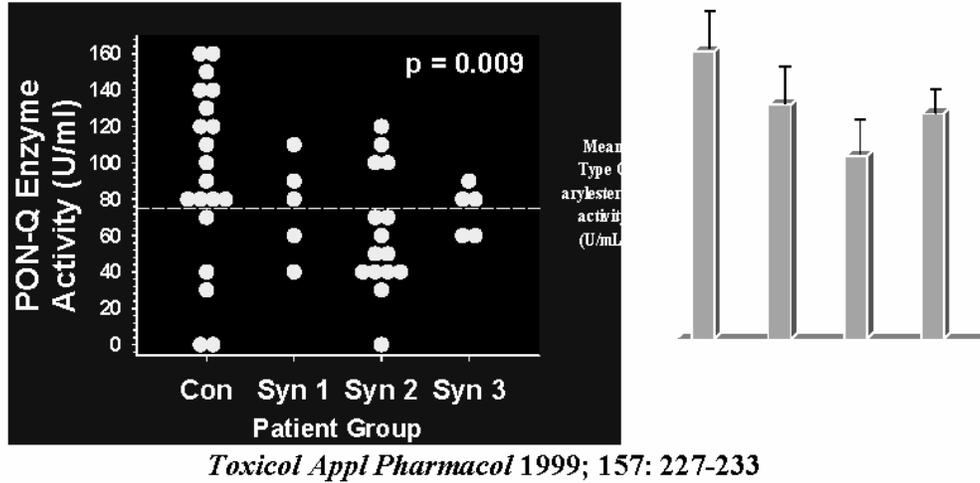
- Low tyrosine diet
- Brain MR spectroscopy scans
- Autonomic evaluation
- Neurophysiologic tests
- Quantitative sensory tests
- Psychiatric/neuropsychological evaluation
- Sleep studies over 4 nights
- Blood tests for dopamine metabolites
- Brain SPECT scans with cholinergic challenge
- Etc.

Genetic Predisposition: Paraoxonase (PON1) Enzyme Assay



Dr. Bert La Du
 U. of Michigan

Lower PON1 Type Q Allozyme Levels in Blood of Ill Gulf War Veterans than Controls



Brain Scanning with Nuclear Magnetic Resonance Spectroscopy (MRS Scan)

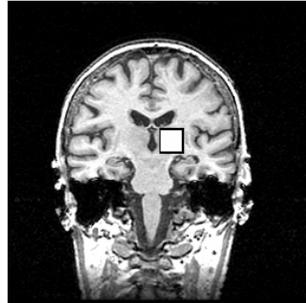
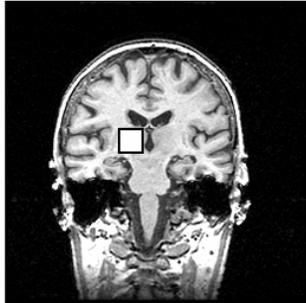


3 Brain Regions Scanned by MRS

Left BG

Right BG

Brain stem

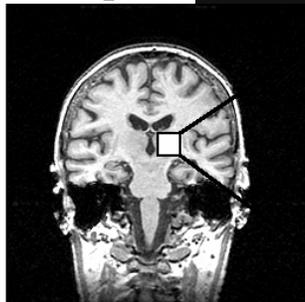


Rear view

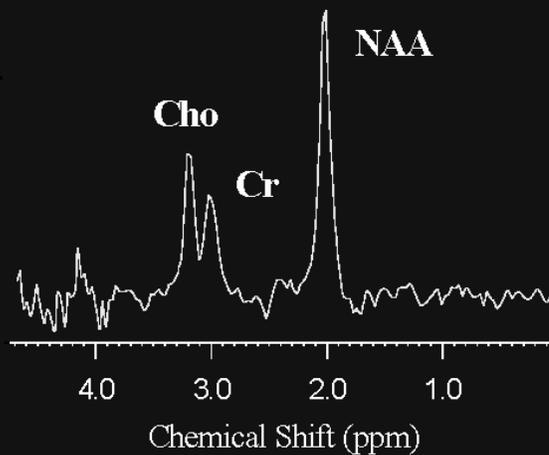
Side view

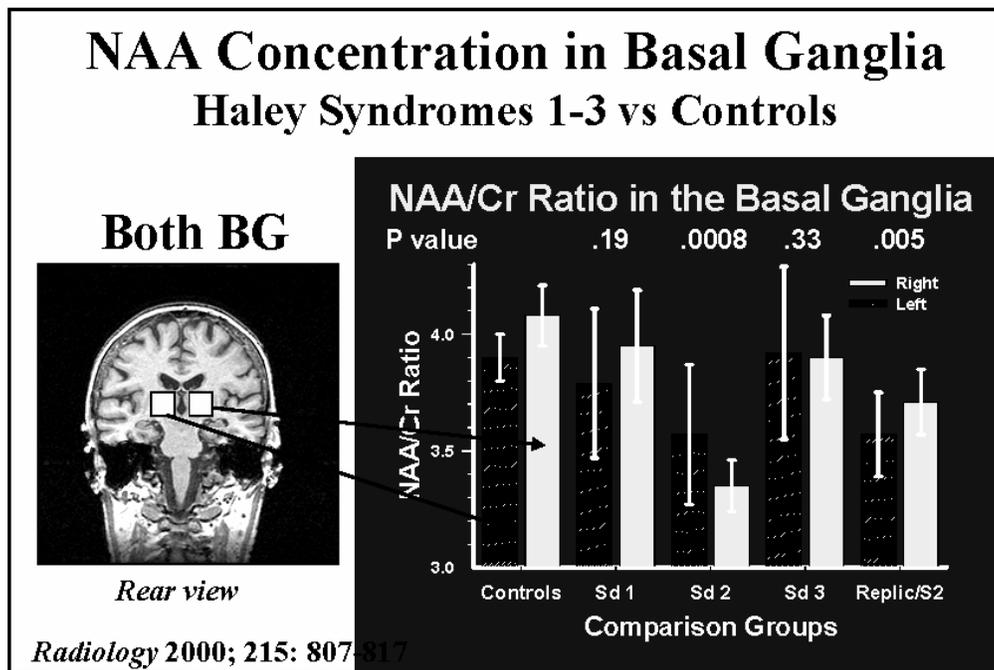
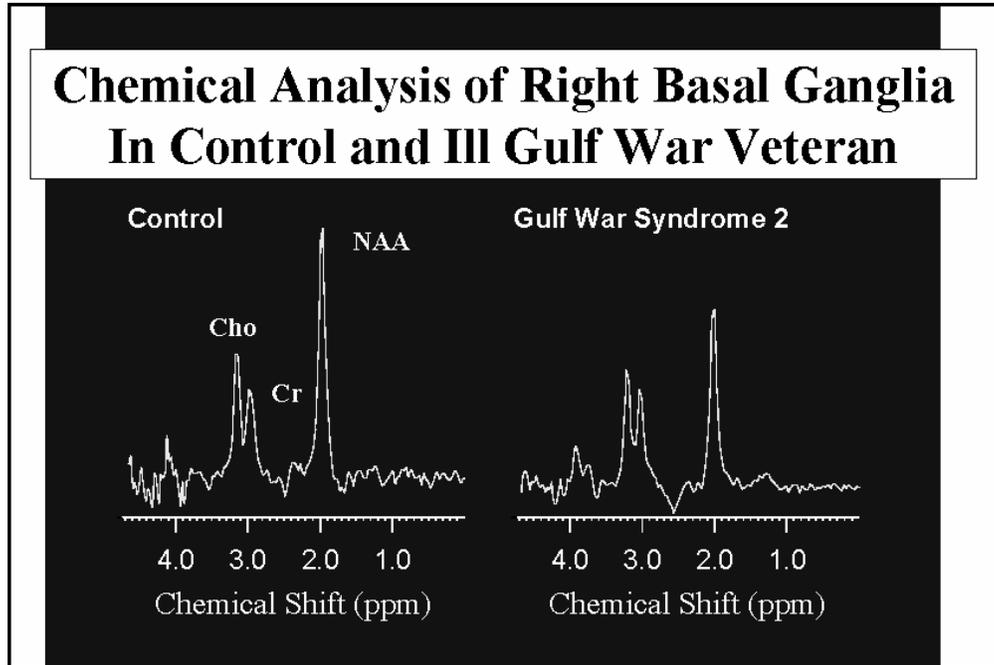
Chemical Analysis from an MRS Scan Of Normal Brain

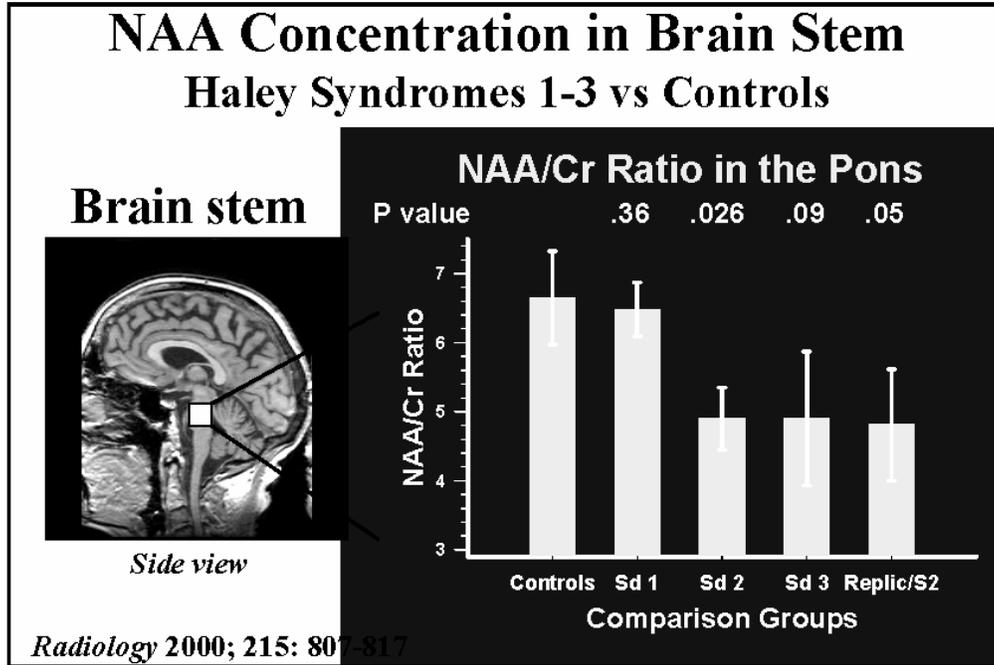
Right BG



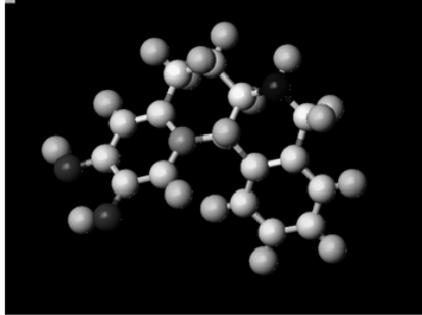
Rear view



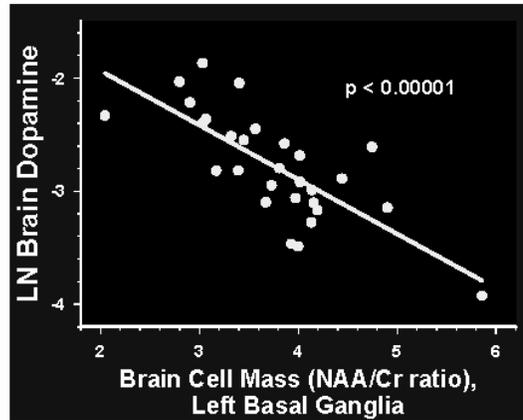




Brain Chemistry: Dopamine Production Rate (Homovanillic Acid / MHPG)

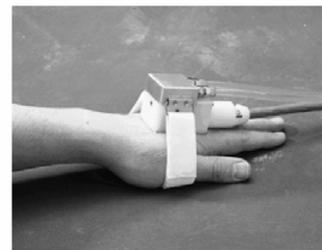
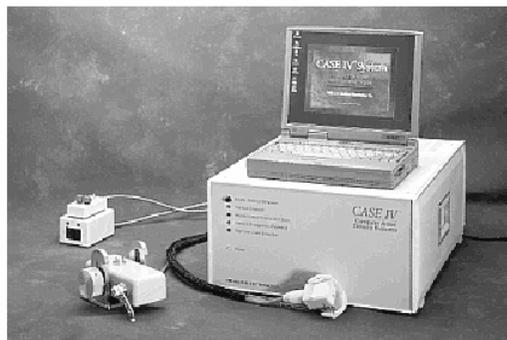


Increased Brain Dopamine Production with Brain Cell Damage

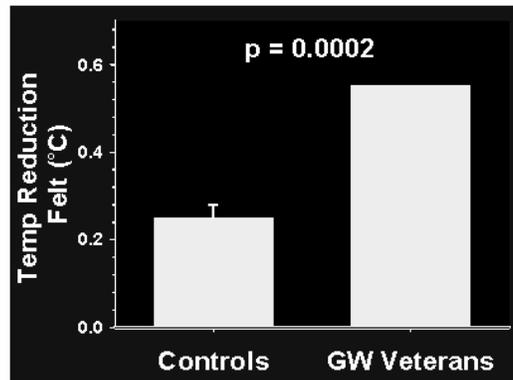


Archives of Neurology 2000; 57: 1280-1285

Quantitative Sensory Testing: Cooling Threshold



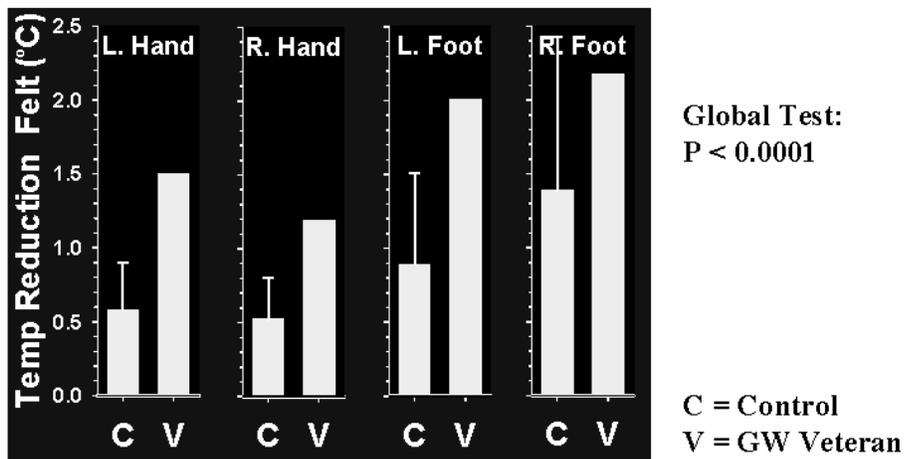
Jamal et al. 1996 Study: Abnormal Ability to Sense Changes in Temperature



J Neurol, Neurosurg, & Psychiatr 1996; 60: 499-451

Impaired Cooling Perception Threshold*

R. Haley, G. Wolfe, MD, W. Bryan et al.



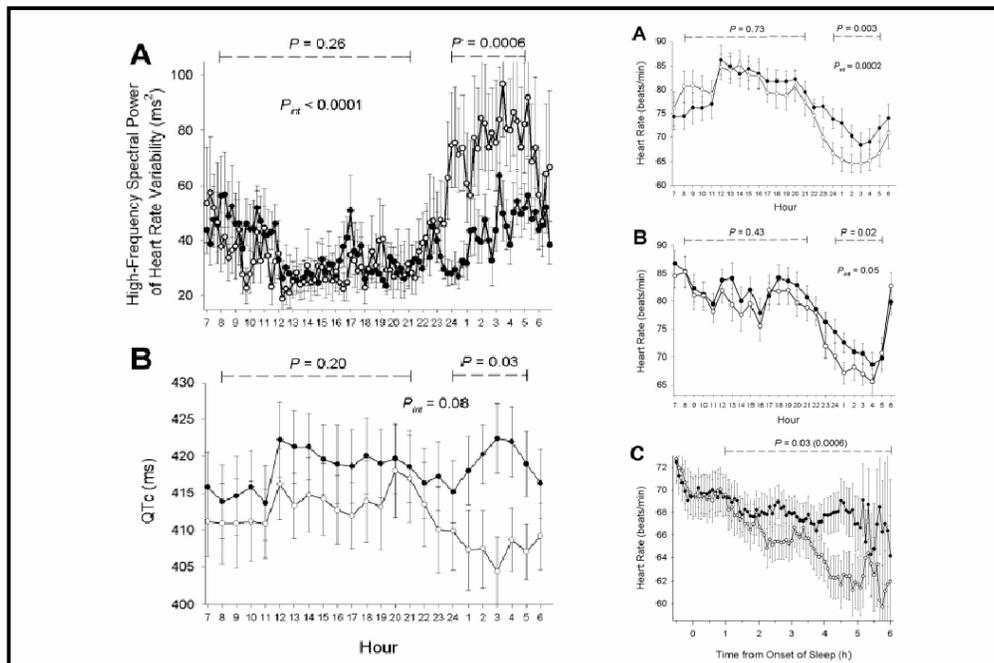
*Similar findings for warming threshold but not vibratory

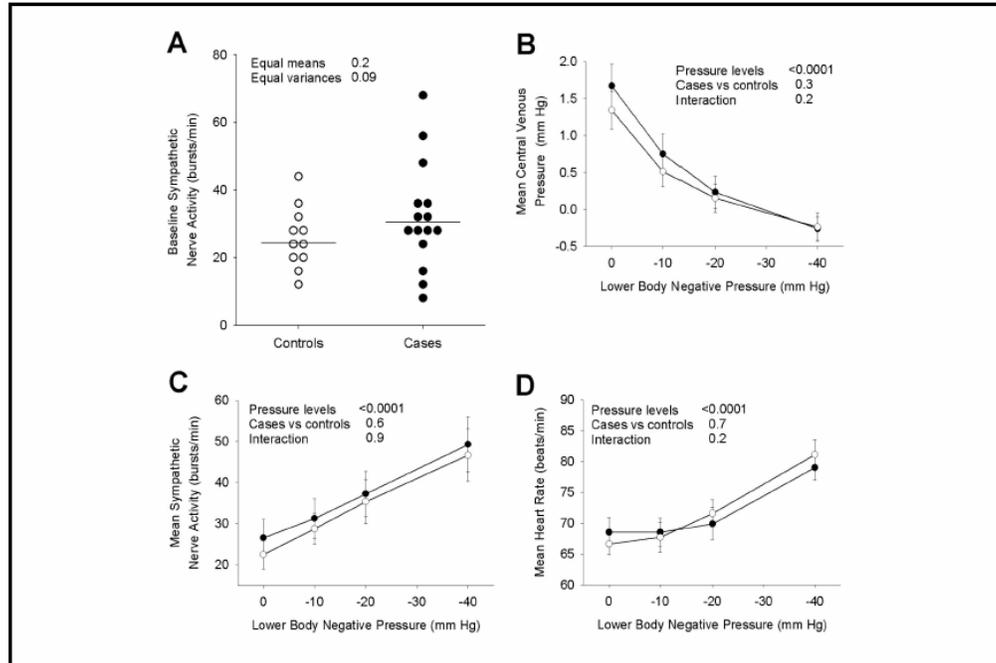
Tests of Autonomic Function

Embargo Date: September 27, 2004, 5:00 a.m. EST October 1, 2004, The American Journal of Medicine, Volume 117, No. 7

Blunted Circadian Variation in Autonomic Regulation of Sinus Node Function in Veterans with Gulf War Syndrome

Robert W. Haley, MD, Wanpen Vongpatanasin, MD, Gil I. Wolfe, MD, Wilson W. Bryan, MD, Roseanne Armitage, PhD, Robert F. Hoffmann, PhD, Frederick Petty, PhD, MD, Timothy S. Callahan, PhD, Elizabeth Charuvastra, RN, William E. Shell, MD, W. Wesley Marshall, MD, Ronald G. Victor, MD





Neuropsychological Findings

- Psychological scales of the *MMPI (1, 2 and 4)* and *PAI* are elevated in ill veterans but not in the well veterans.
- *SCID* interviews show high rate of depression, little PTSD.
- A few sicker veterans have mild psychotic features.
- Inability to perform skills easily done before the war.
- *WAIS Verbal IQ*, but not *Performance IQ*, is significantly lower, as are *Arithmetic* and *Vocabulary* subtests.
- *The Halstead Impairment Index*, *Connors Continuous Performance Test (CPT)*, and other tests of *attention-concentration* best distinguish ill from well veterans.
- In general, many tests of subcortical function are slightly skewed in the abnormal direction in ill veterans but not significantly.

**April 22, 2001 ISMRM Meeting in Scotland
Replication of Our MR Spectroscopy Finding
Michael W. Weiner, M.D.**



Michael W. Weiner, M.D.

**Professor of Medicine,
Radiology, Psychiatry, and
Neurology**

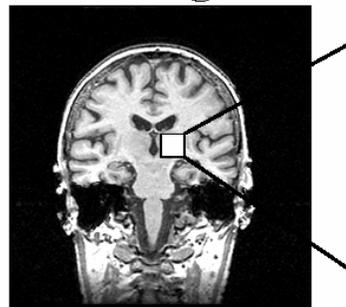
**University of California San
Francisco**

Director, MRS Unit

**VA Medical Center San
Francisco**

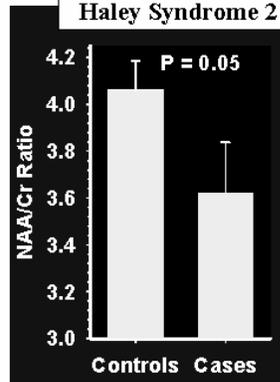
**First Replication by Weiner et al. :
NAA Concentration in Right BG**

MRS of Right BG



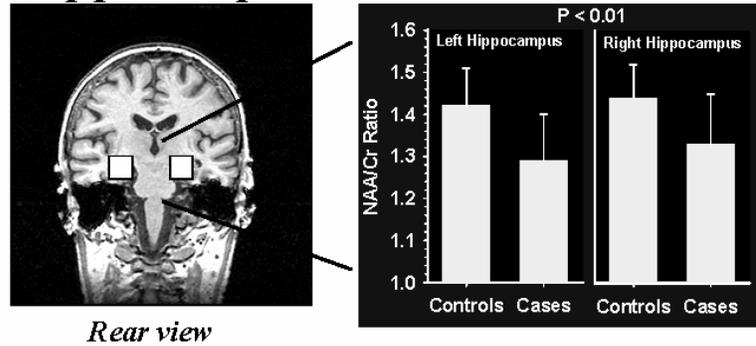
Rear view

California Gulf War
Veterans with
Haley Syndrome 2



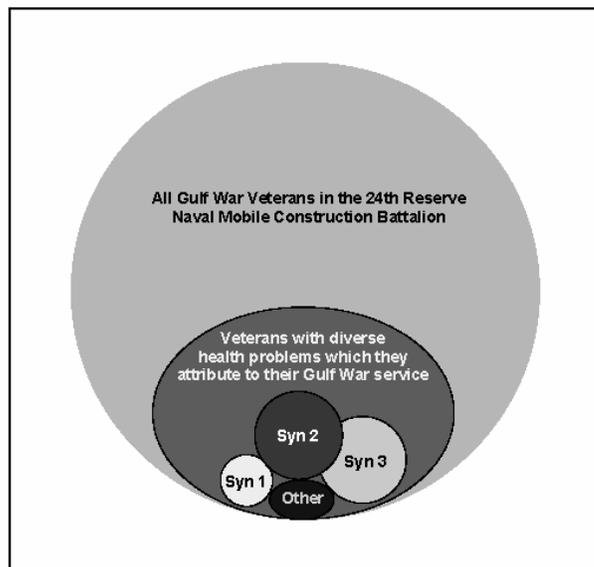
Proc Intl Soc Mag Reson Med 2001; 9: 994.

Second Replication by Menon et al. : NAA Concentration in the Hippocampus Left and Right Hippocampus



Brain Research 2004; 1009: 189-194.

Clinical Subgroups of Gulf War Veterans



October 2002

Alteration of Cholinergic Receptors in Rats by Low-Level Sarin

Rogene Henderson
Senior Scientist
Lovelace Respiratory
Research Institute
Albuquerque, NM



Funded by the U.S. Army Medical Research Institute for Chemical Defense
Henderson et al. *Toxicology Applied Pharmacology* 2002;184:67-87

Toxicology and Applied Pharmacology **184**, 67–76 (2002)
doi:10.1006/taap.2002.9495

Response of Rats to Low Levels of Sarin

Rogene F. Henderson,* Edward B. Barr,* Walter B. Blackwell,* Connie R. Clark,† Carole A. Conn,* Roma Kalra,*
Thomas H. March,* Mohan L. Sopori,* Yohannes Tesfaigzi,* Margaret G. Ménache,*¹ and Deborah C. Mash‡

*Lovelace Respiratory Research Institute, Albuquerque, New Mexico 87108; †U.S. Army Medical Research Institute of Chemical Defense,
Aberdeen, Maryland 21010; and ‡University of Miami, Miami, Florida 33101

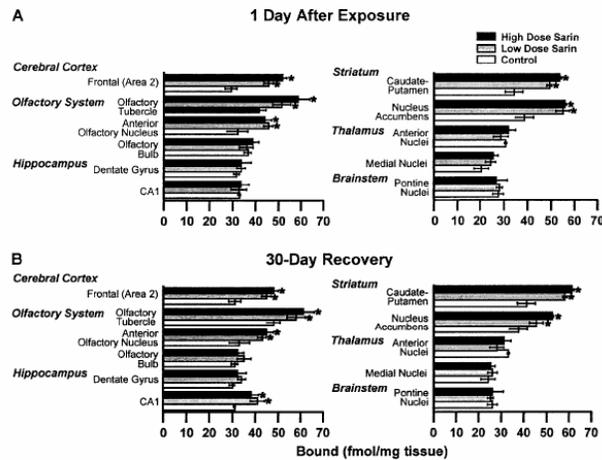
Toxicology and Applied Pharmacology **184**, 82–87 (2002)
doi:10.1006/taap.2002.9497

Subclinical Doses of the Nerve Gas Sarin Impair T Cell Responses through the Autonomic Nervous System

Roma Kalra, Shashi P. Singh, Seddigheh Razani-Boroujerdi, Raymond J. Langley, Walter B. Blackwell,
Rogene F. Henderson, and Mohan L. Sopori

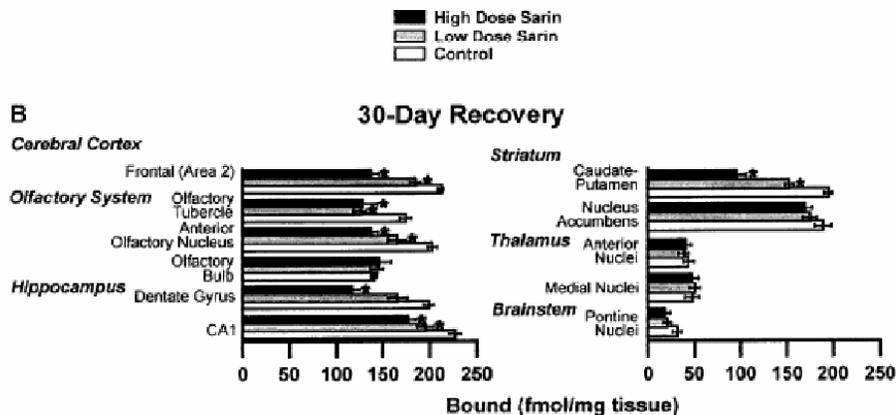
Lovelace Respiratory Research Institute, Albuquerque, New Mexico 87108

Up-regulation of M3 Receptors at 1 and 30 Days After Inhalation of Sarin for 5 Days



Henderson et al. *Toxicology Applied Pharmacology* 2002;184:67-87

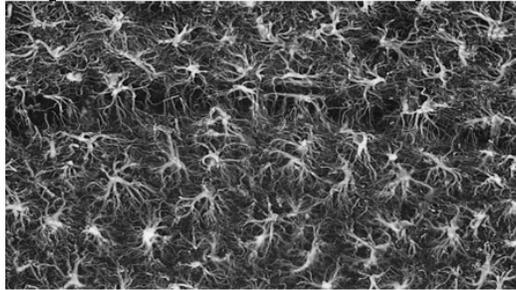
Down-regulation of M1 Receptors 30 Days After Inhalation of Sarin for 5 Days



Henderson et al. *Toxicology Applied Pharmacology* 2002;184:67-87

Presentation 5 – Jim O’Callaghan

**Biological Mechanisms Potentially
Associated with GWI:
Neuroinflammation/Cytokine Activation
in Response to Toxic Exposures**



James P. O’Callaghan, Ph.D.

Molecular Neurotoxicology Laboratory

Centers for Disease Control and Prevention-NIOSH



Outline

- GWI & Neuroinflammation: links/definitions
- Neuroimmune vs. Immune-neuro
 - “Hostage Brain” (McEwen)
 - Autonomic nervous system (Tracey)
- Glia as targets/mediators/modulators
 - Role of TNF- α
- Modulation of “inflammatory” signaling as therapy

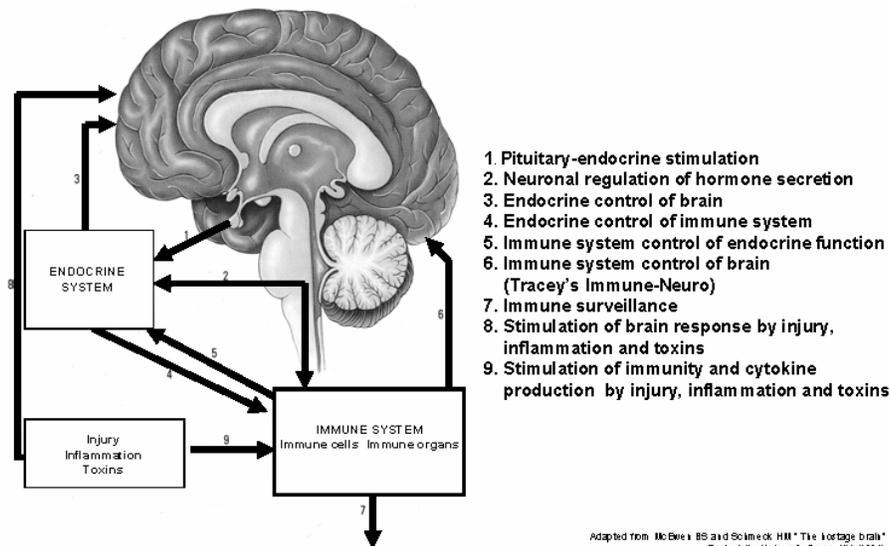


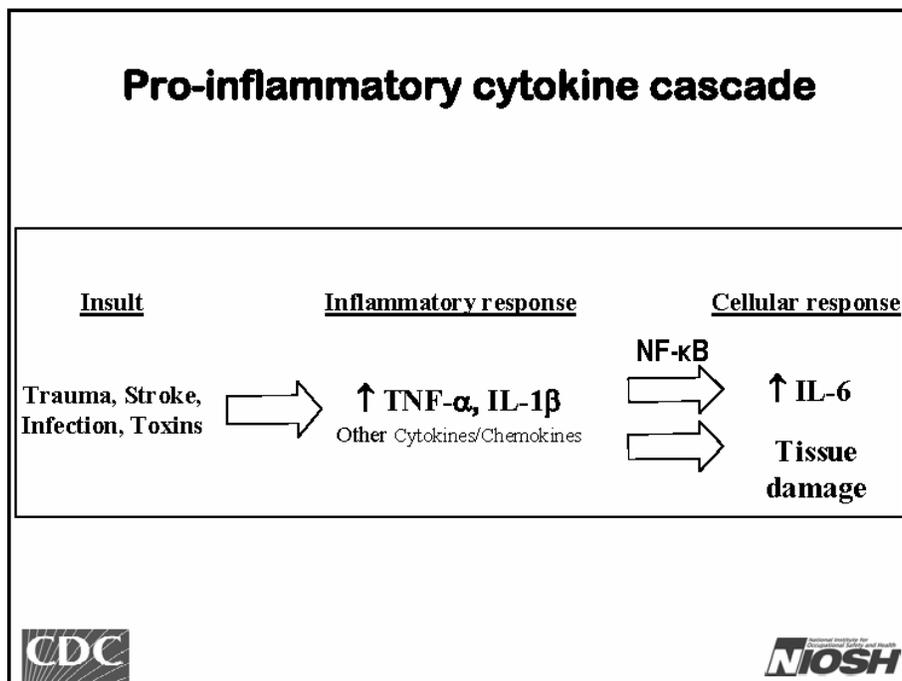
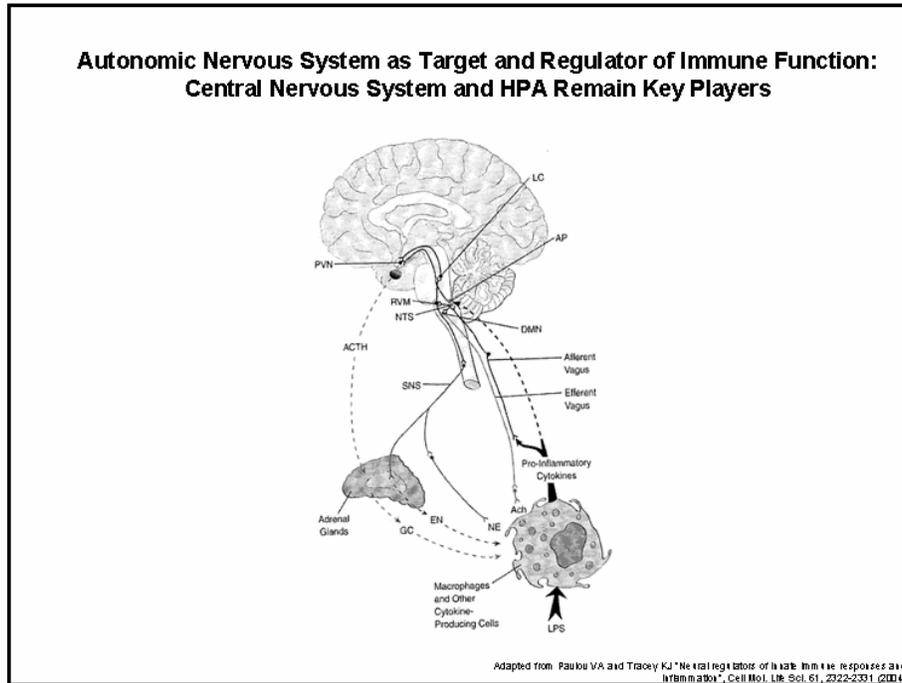
Definitions/Links

- **Neuroinflammation: hard to define**
 - Synthesis/release of proinflammatory mediators (cytokines/chemokines)
 - Monocytes/Neutrophils/Macrophages in the periphery/microglia in the CNS
- **Links to GWI symptoms????**
 - Chronic fatigue
 - Neurocognitive effects/depression
 - Chemical sensitivity
 - PTSD-like symptoms
 - Pain
 - Persistence after initiating event (“memory”)
- **Answer: yes...implicates cytokines and glia**
 - altered cytokine profiles already reported in GW vets



Neuro-Immune/Immune-Neuro Relationships





Cytokine Theory of Disease

- Immune and Nervous System Communicate Via Cytokine Signals
- Cytokines are “Proinflammatory” in nature (but some anti-inflammatory)
- Dysregulated Cytokine Signaling (usually viewed as an increase) leads to debilitating immune related disease
 - Rheumatoid arthritis as extreme example
 - Depression as a potentially more subtle example (sickness behavior)
- Regulation and termination of cytokine signaling is mediated via HPA
 - Glucocorticoids suppress cytokines (clinically and experimentally)
 - Dexamethasone suppression test used to test HPA axis



Cytokine Theory of Disease and GWI

- Is there a GWI cytokine “phenotype”?
- There Are Some Supporting Data:
 - Elevated IL-2, IL-10, TNF- α and IFN- γ (Th1 phenotype)
 - Th2 phenotype not prevalent (glucocorticoid responsive)
- Is it PTSD?
 - No, not associated with elevated serum IL-6



Cytokine Theory of Disease and GWI

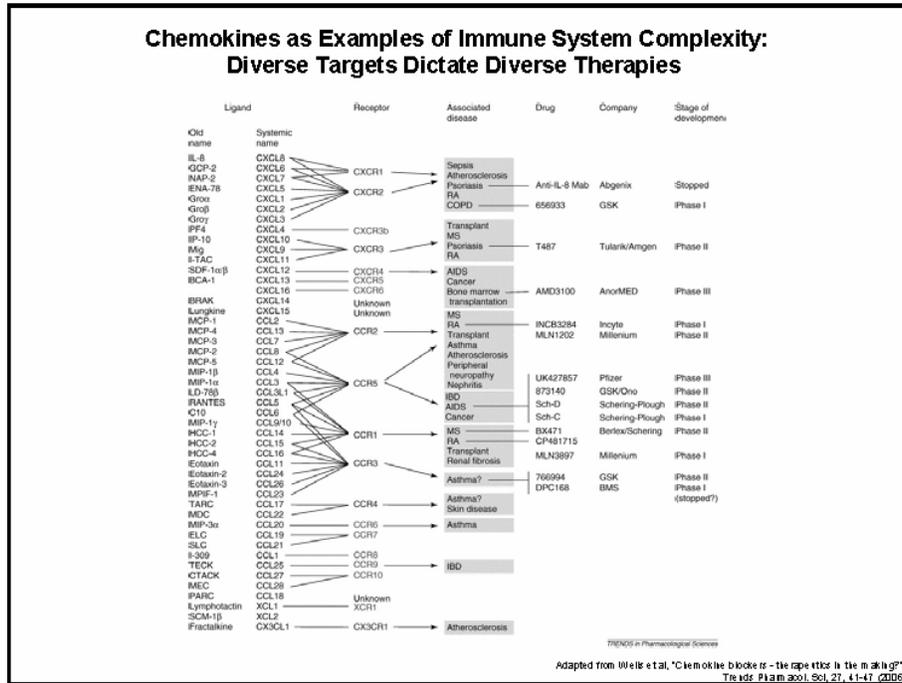
- **Data Gaps:**
 - **Complete serum cytokine profiles**
 - **Glucocorticoid Responsiveness
(Dexamethasone Suppression Test)**



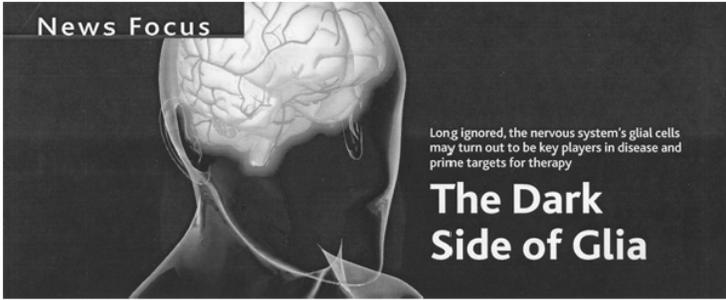
Cytokine/Chemokine Blockers as Therapeutics

1. **Original Hypothesis: One disease, one (“bad”) cell, one cytokine**
2. **Reality: many cells, many cytokines, many therapeutic targets**





Cytokine/Chemokines, Glia and Neurotoxicity



Long ignored, the nervous system's glial cells may turn out to be key players in disease and prime targets for therapy

The Dark Side of Glia

Science 308: 778-781, 2005

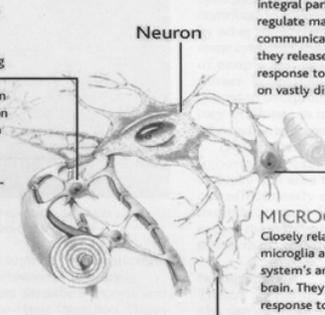



More Background on the “Dark Side”

Meet the Glia

OLIGODENDROCYTES

These cells provide the fatty myelin sheaths that insulate axons, the long extensions that convey signals from one end of a neuron to the other. When they die off, as in multiple sclerosis, neural communication breaks down.



Neuron

ASTROCYTES

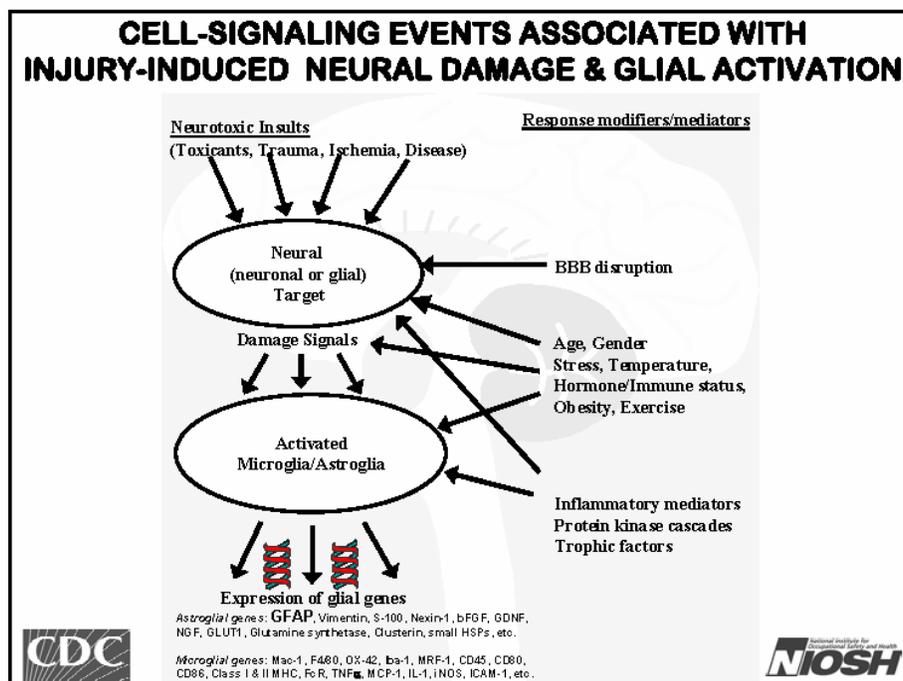
The most mysterious glia, astrocytes have many roles in the brain. They are integral parts of synapses, where they regulate many molecules important for communication between neurons, and they release neural growth factors. In response to injury, however, they take on vastly different personae.

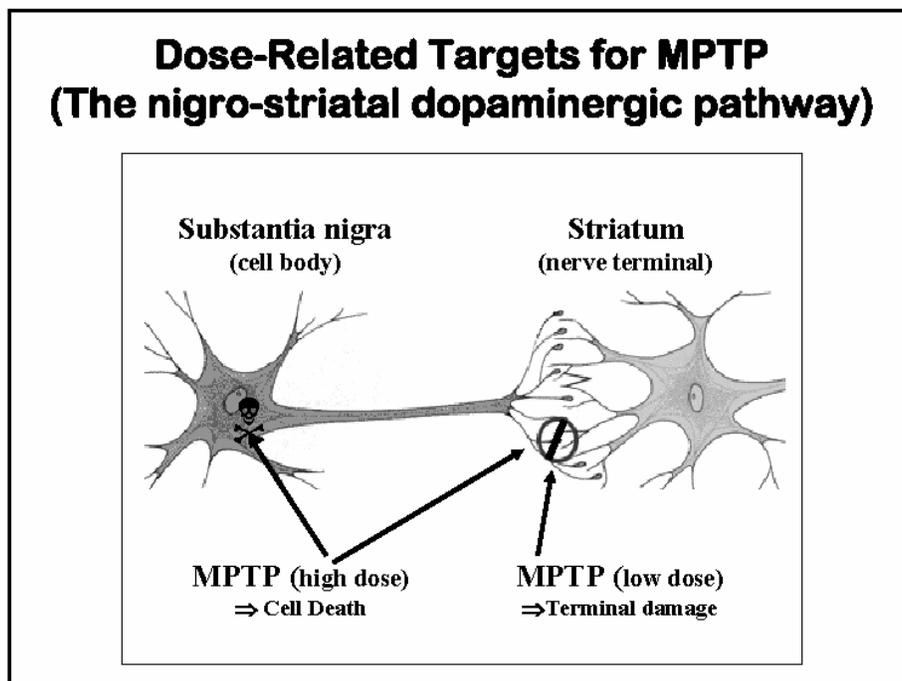
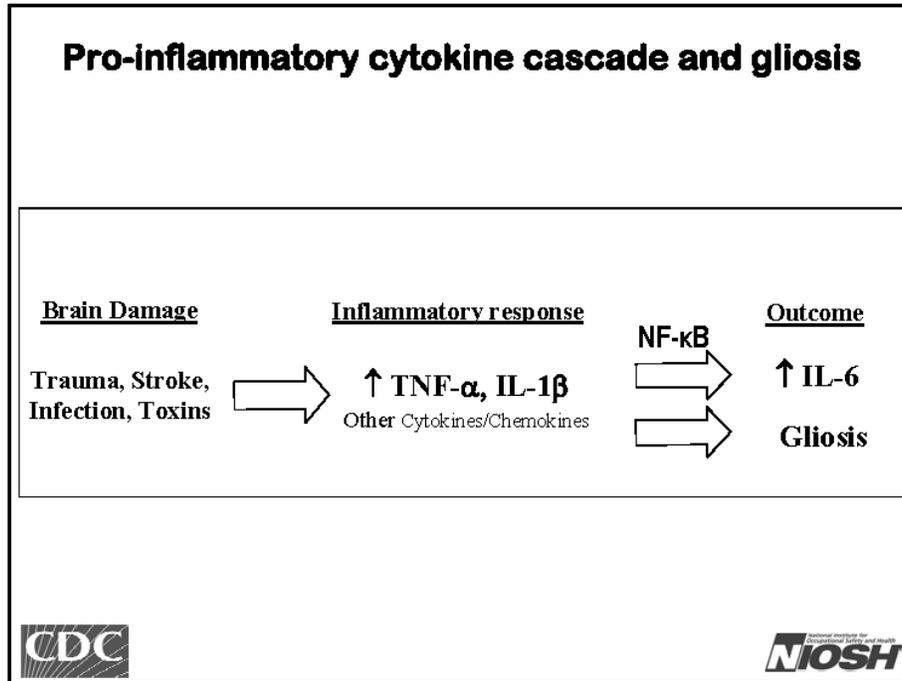
MICROGLIA

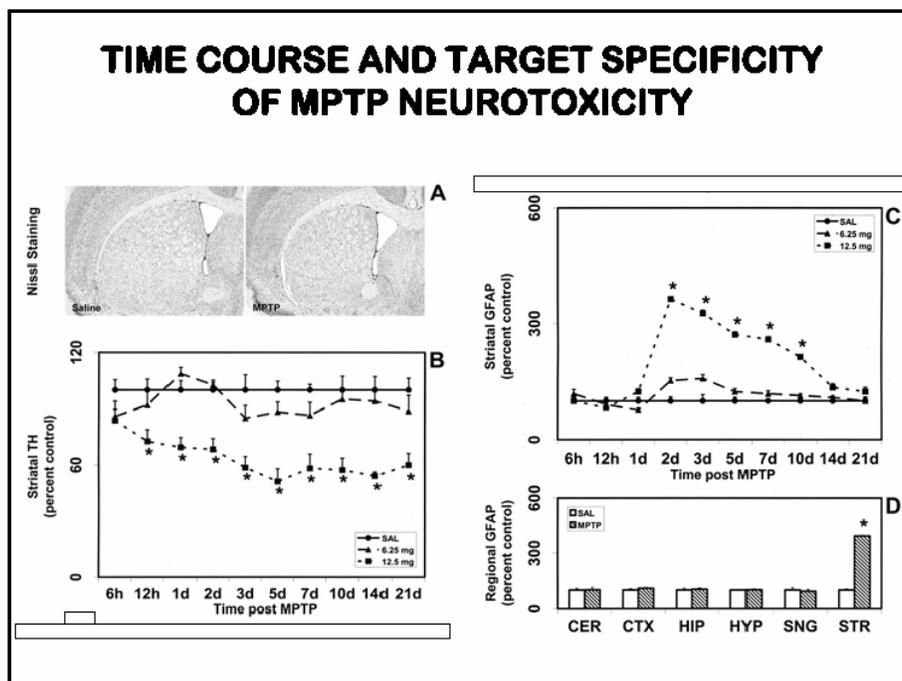
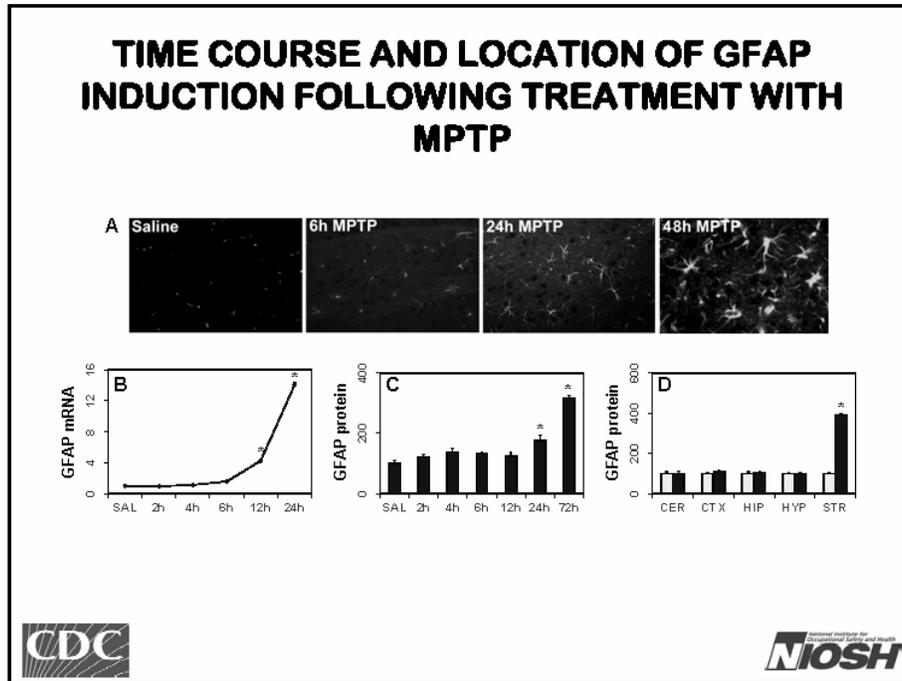
Closely related to macrophages, microglia are the immune system’s ambassadors to the brain. They fight infections, but in response to injury, they release a slew of compounds that may damage neurons.

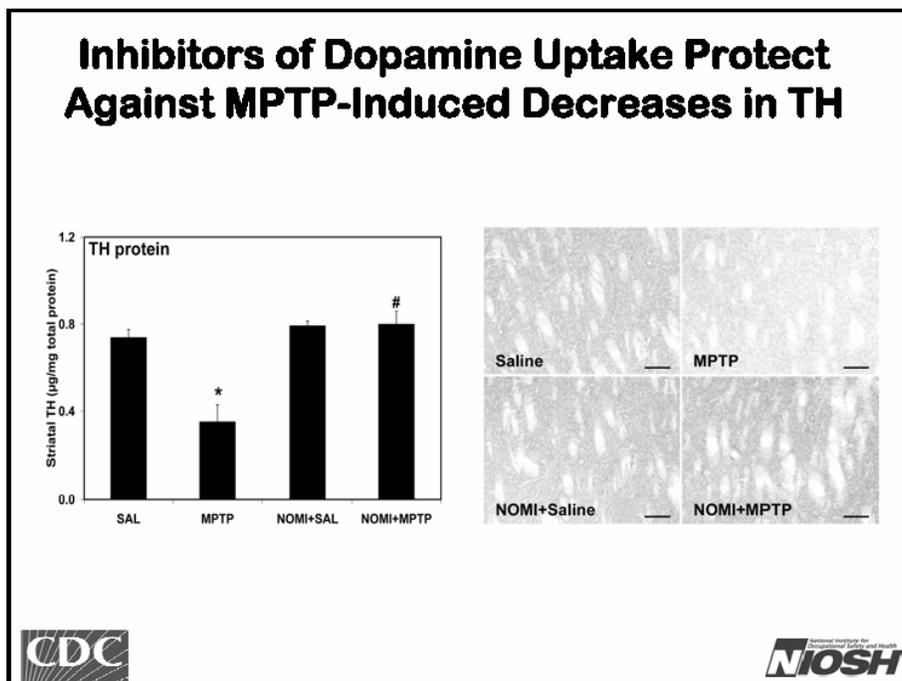
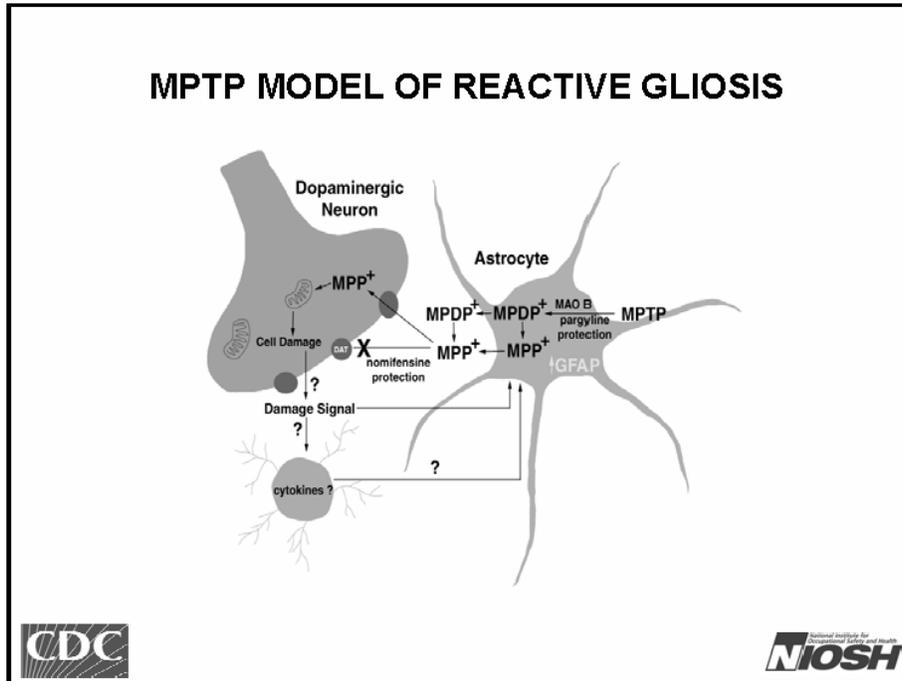
Science 308: 778, 2005

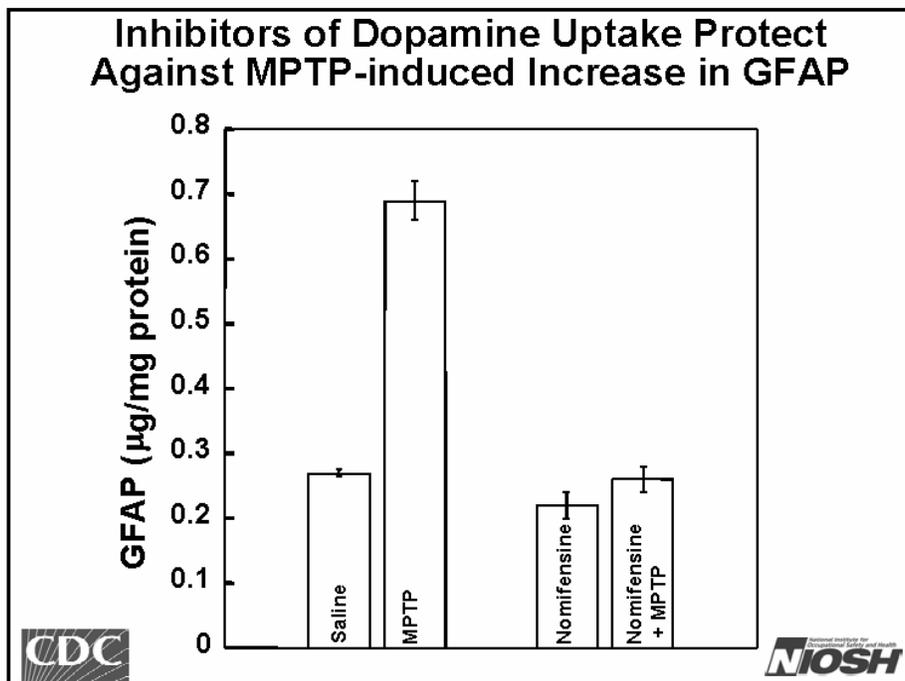
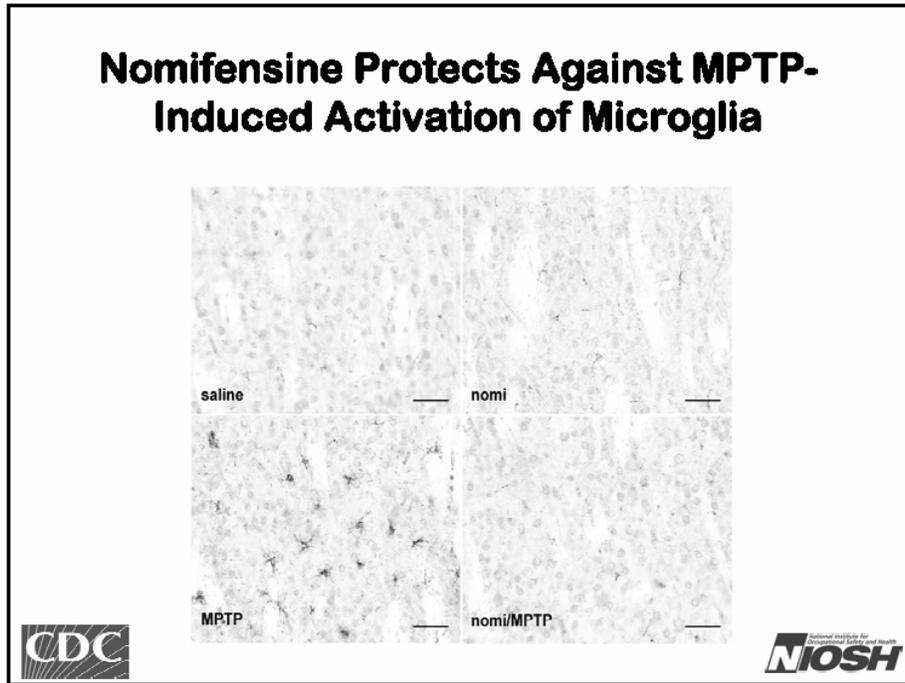








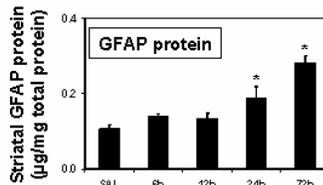
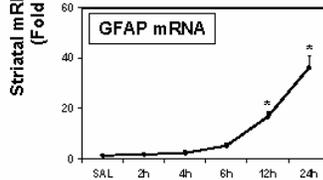
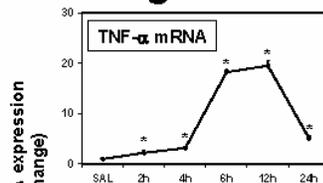


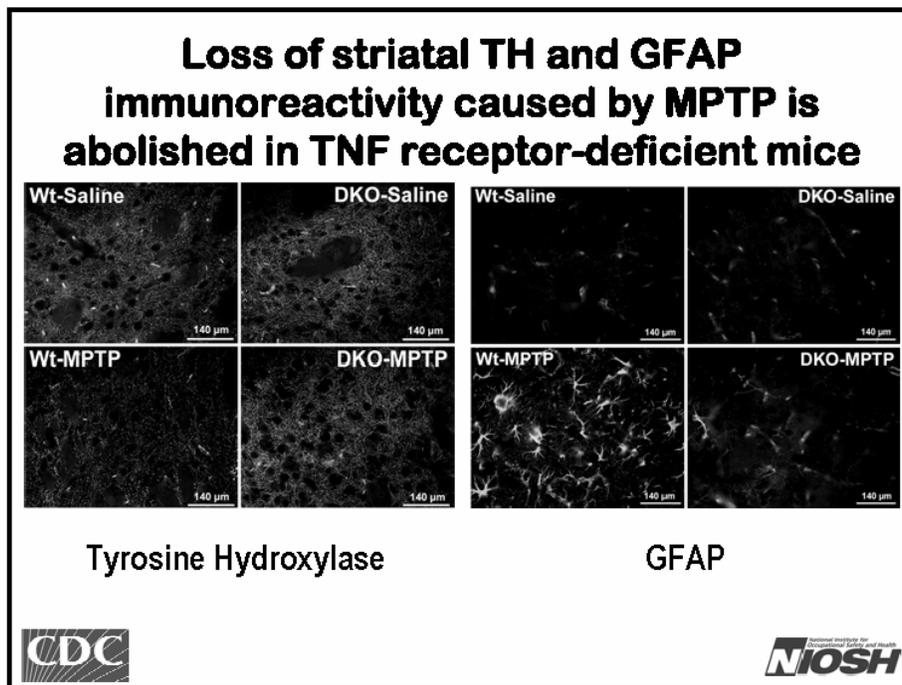
Tumor Necrosis Factor- α

- Proinflammatory Cytokine in the Periphery
- Effects Mediated through 2 receptors
- Role in CNS unknown
- Enhanced Expression in Brain Linked To:
 1. Parkinson’s Disease
 2. HIV-dementia
 3. Activation of Microglia



MPTP-mediated expression of TNF- α precedes gliosis

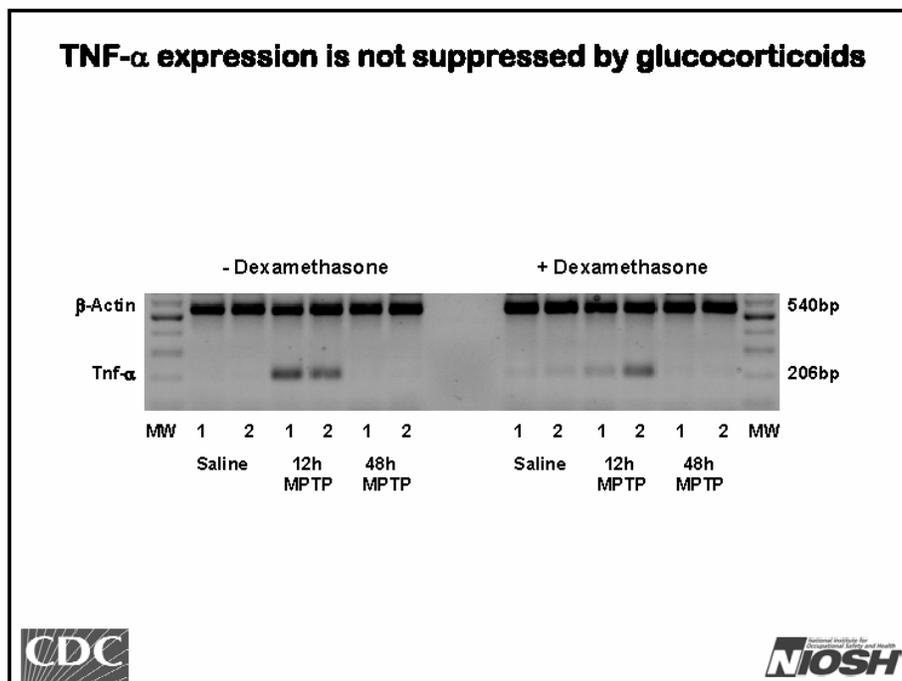
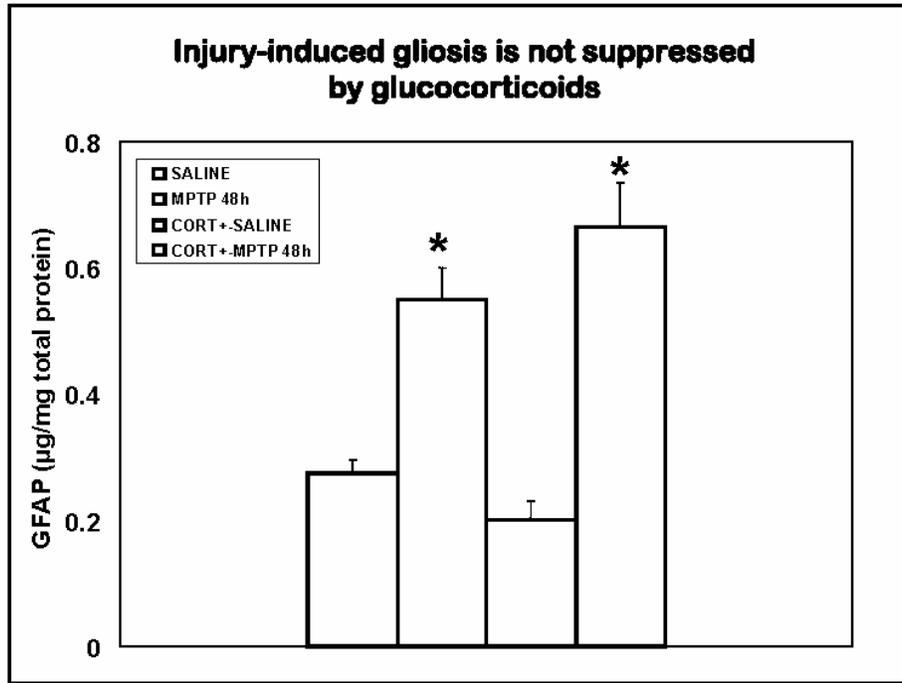




OK, TNF- α is “Bad” but can be suppressed in the periphery by Glucocorticoids

Will Glucocorticoids suppress brain damage and TNF- α in the CNS?

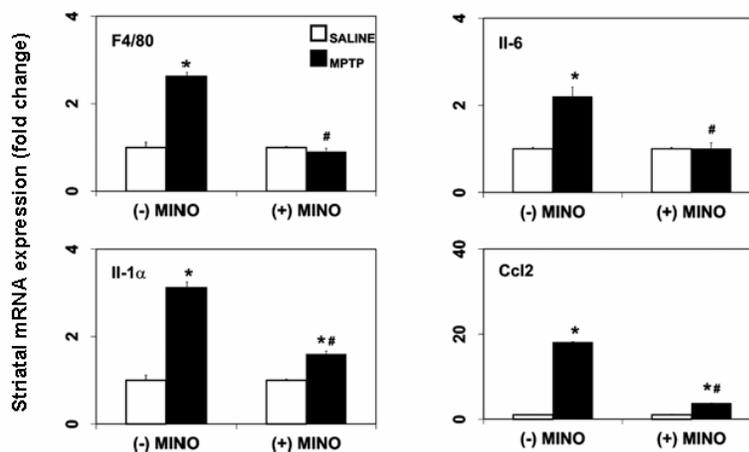


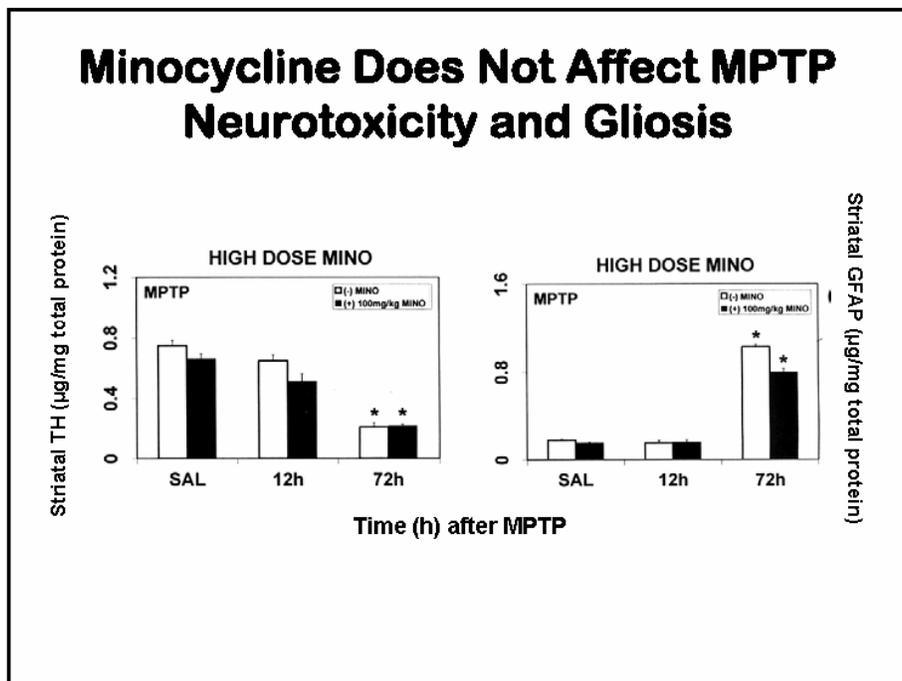
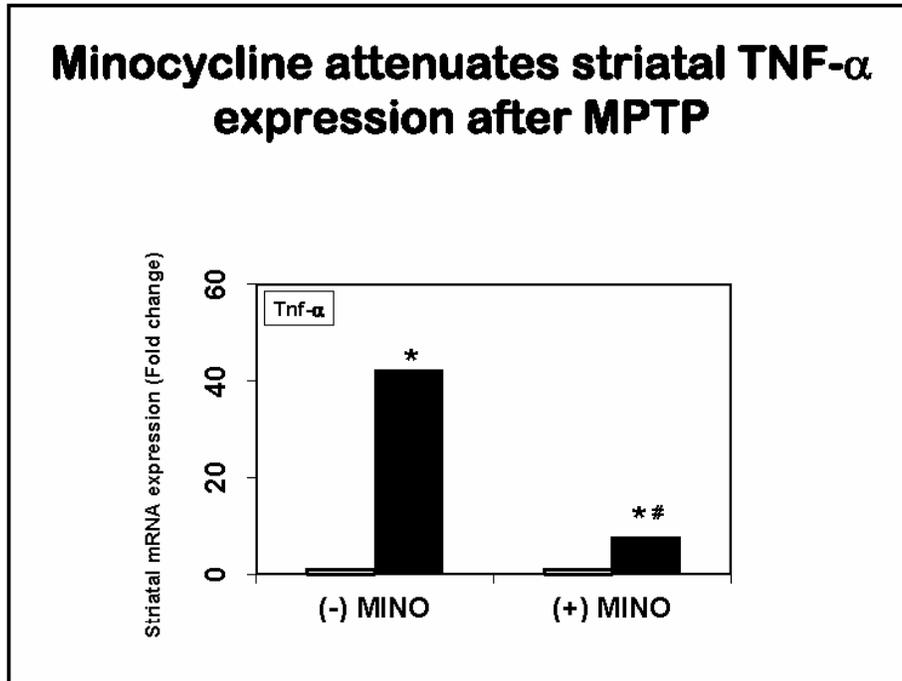
Use minocycline to block microglial activation?

- **Broad spectrum tetracycline derivative antibiotic**
- **Anti-inflammatory properties**
- **Reported to block microglial activation**
- **Reported to block nigral cell loss after (high dose) MPTP**



Minocycline suppresses the striatal expression of microglial factors following MPTP

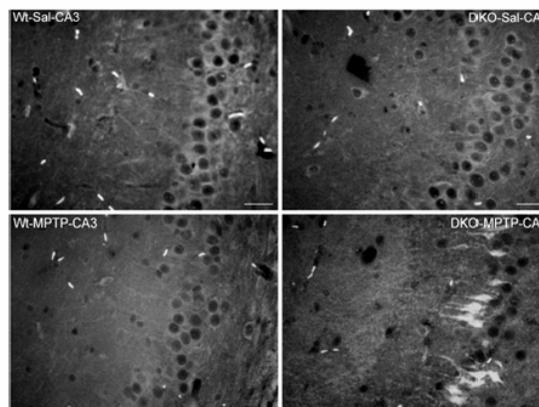




OK...TNF is bad but...not so fast..



**Role of TNF- α in Neurodegeneration is *NOT*
Simple: F-J Staining is seen in hippocampus of
TNF receptor-deficient mice treated with MPTP**



A few words from Richard Ransohoff on *in vivo* vs. *in vitro* cytokine data

“....comparisons of *in vitro* studies of explanted CNS cells with *in vivo* data (e.g. in-situ hybridization) show that tissue disruption and cell culture dysregulates the chemokine system. Therefore, it is Perilous to extrapolate the situation *in vivo* from results *in vitro*.”

Ubogu, et al., Trends in Pharmacol. Sci. 27: 49, 2006



Some take home messages

1. Data exist suggestive of involvement of dysregulated immune signaling in GWVI
2. Neuro-immune and immune-neuro interactions are complex and reciprocal; they pose multiple targets for diagnosis and therapy
3. Expanded serum cytokine profiling and immune function tests of GW veterans may aid in revealing the GWI phenotype
4. Therapeutics that affect immune signaling in the periphery may not modulate CNS immune signals or they may inappropriately disrupt normal beneficial effects of cytokine signals in the CNS



Presentation 6 – Floyd Bloom

Neuroplasticity and GWVI

Floyd E. Bloom, MD
Neurome, Inc. &
The Scripps Research Institute

RAC- GWVI

May 15, 2006

Floyd Bloom

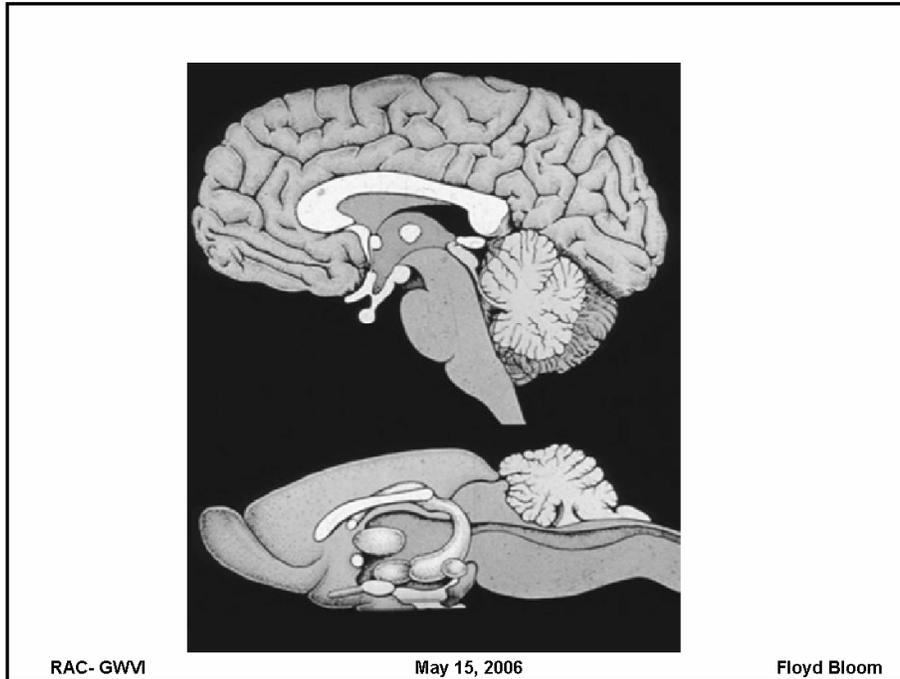
Neuroplasticity & GWVI

- Mammalian brains have considerable plastic ability.
- In response to functional signals attempts to repair can occur.
- Aberrant sprouting may result in long-lasting changes in circuitry.

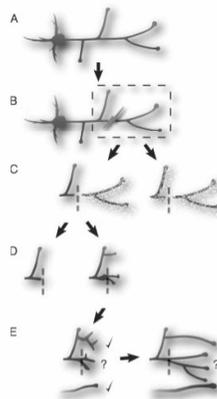
RAC- GWVI

May 15, 2006

Floyd Bloom



Mammalian brains have considerable (local) plastic ability.



Chuckowree, Dickson & Vickers,
Neuroscientist 10:240,2005

Mammalian brains have considerable plastic ability.

Brain Research, 59 (1973) 169-179 169
© Elsevier Scientific Publishing Company, Amsterdam - Printed in The Netherlands

PROLIFERATION OF NOREPINEPHRINE-CONTAINING AXONS IN
RAT CEREBELLAR CORTEX AFTER PEDUNCLE LESIONS

VIRGINIA M. PICKEL, HELMUT KREBS and FLOYD E. BLOOM

*Laboratory of Neuropharmacology, Special Mental Health Research Division, NIMH, Saint Elizabeths
Hospital, Washington, D.C. 20032 (U.S.A.)*

RAC- GWVI

May 15, 2006

Floyd Bloom

Mammalian brains have considerable plastic ability.

Reprinted from THE JOURNAL OF COMPARATIVE NEUROLOGY
Vol. 155, No. 1, May 1, 1974 © The Wistar Institute Press 1974

Axonal Proliferation Following Lesions of Cerebellar
Peduncles. A Combined Fluorescence Microscopic
and Radioautographic Study

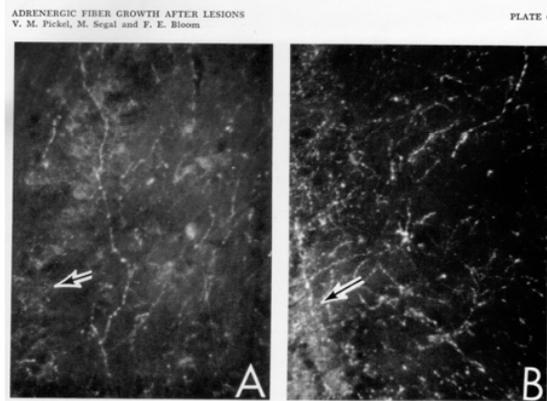
VIRGINIA M. PICKEL, MENAHEM SEGAL and FLOYD E. BLOOM
*Laboratory of Neuropharmacology, Special Mental Health Research
Division IRP, National Institute of Mental Health, Saint Elizabeths
Hospital, Washington, D.C. 20032*

RAC- GWVI

May 15, 2006

Floyd Bloom

Mammalian brains have considerable plastic ability.

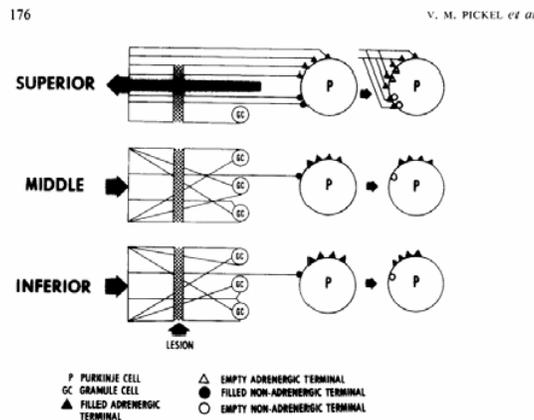


RAC- GWVI

May 15, 2006

Floyd Bloom

Mammalian brains have considerable plastic ability.



RAC- GWVI

May 15, 2006

Floyd Bloom

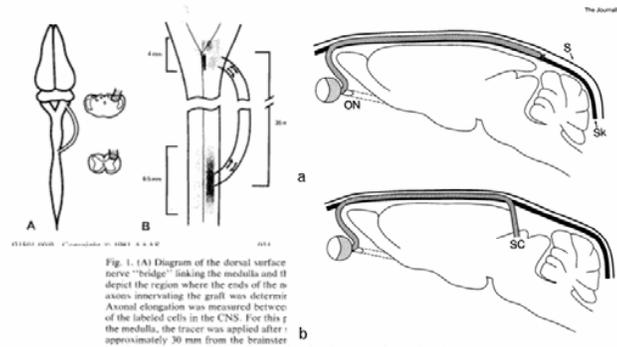
Mammalian brains have considerable plastic ability.

- Injured peripheral nerves will regrow in periphery, not in CNS.
- Injured central axons can regrow through peripheral nerve bridges.

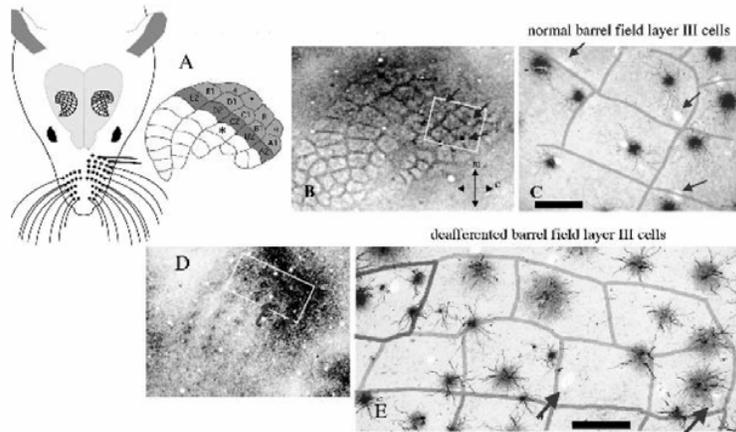
Mammalian brains have considerable plastic ability.

A.J. Aguayo

Axonal Elongation into Peripheral Nervous System "Bridges"
After Central Nervous System Injury in Adult Rats



In response to functional signals
attempts to repair can occur.



RAC- GWVI

May 15, 2006

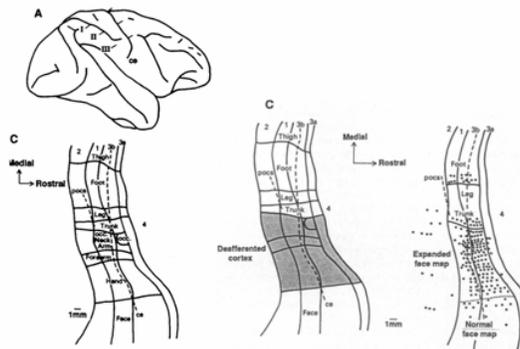
Floyd Bloom

In response to functional signals
attempts to repair can occur.

**Massive Cortical Reorganization After Sensory
Deafferentation in Adult Macaques**

TIM P. PONS,* PRESTON E. GARRAGHTY, ALEXANDER K. OMMAYA,
JON H. KAAS, EDWARD TAUB, MORTIMER MISHKIN

Science 252: 1857, 1991



RAC- GWVI

May 15, 2006

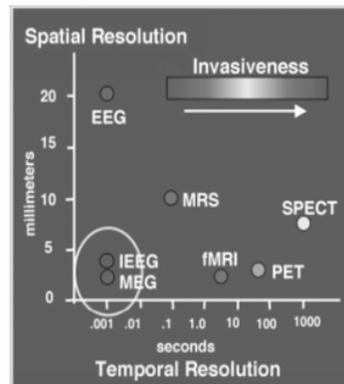
Floyd Bloom

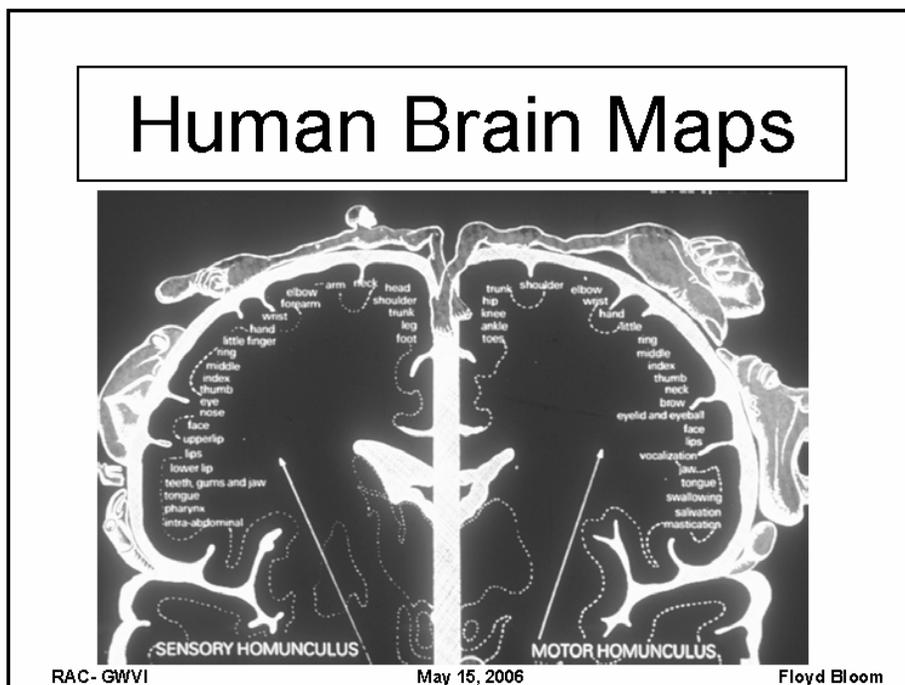
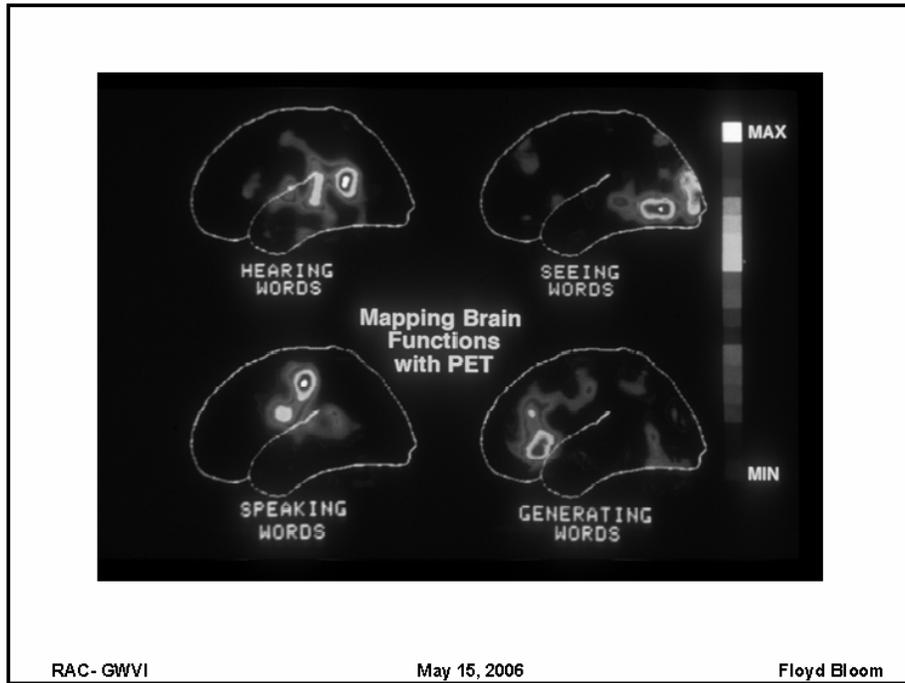
In response to functional signals
attempts to repair can occur.

- To study effects in living subjects
new ways to map have been
developed: fMRI and NeuroMEG
- These show human brain's plastic
ability.



Spatial and Temporal Resolutions for Various Functional Imaging Modalities





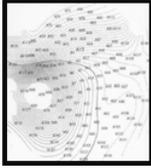
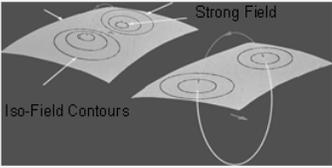
 **MEG: Basic Concepts**

The current dipole generating the measured magnetic field lies midway between the two extrema

- The greater the distance between the extrema, the deeper the dipole

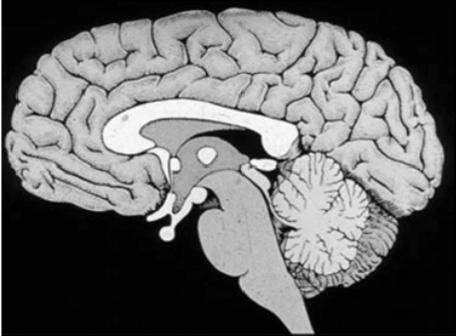
Magnetic Field Pattern

Recording Surface Weak Field Strong Field



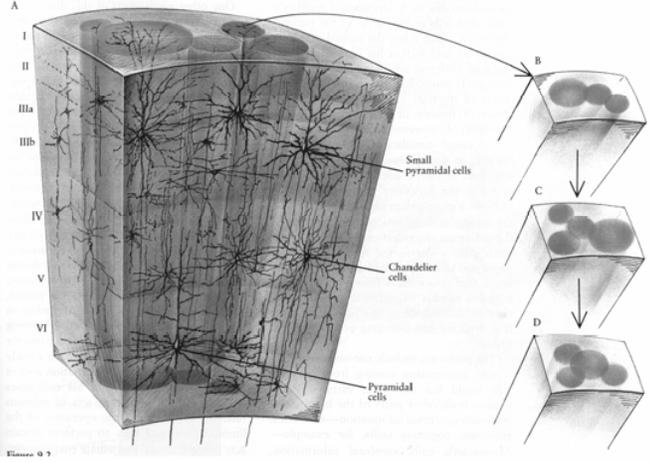
RAC- GWVI May 15, 2006 Floyd Bloom

 **MEG: Basic Concepts**



RAC- GWVI May 15, 2006 Floyd Bloom

 **MEG: Basic Concepts**



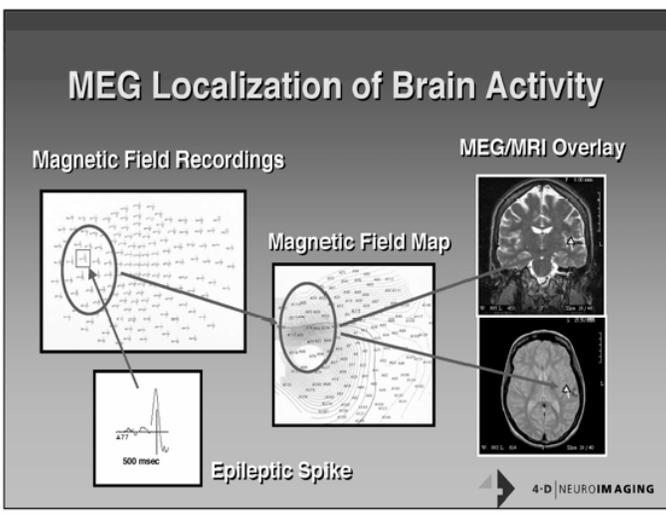
A
I
II
IIIa
IIIb
IV
V
VI

Small pyramidal cells
Chandelier cells
Pyramidal cells

B
C
D

RAC- GWVI May 15, 2006 Floyd Bloom

 **MEG Localization of Brain Activity**



Magnetic Field Recordings MEG/MRI Overlay

Magnetic Field Map

Epileptic Spike

4-D | NEUROIMAGING

RAC- GWVI May 15, 2006 Floyd Bloom

Aberrant Responses Can Be Intra-Neuronal



www.elsevier.com/locate/neuroscience

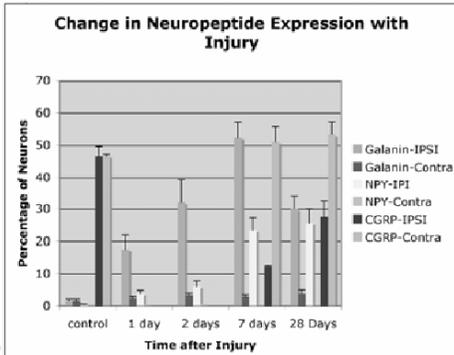
PII: S0306-4522(01)00148-8

Neuroscience Vol. 105, No. 1, pp. 249-265, 2001
© 2001 IBRO. Published by Elsevier Science Ltd
Printed in Great Britain. All rights reserved.
0306-4522/01/105249-17\$05.00

EFFECT OF PERIPHERAL NERVE INJURY ON DORSAL ROOT GANGLION NEURONS IN THE C57 BL/6J MOUSE: MARKED CHANGES BOTH IN CELL NUMBERS AND NEUROPEPTIDE EXPRESSION

T.-J. S. SHU, T. TANDRUP, E. BERGMAN, Z.-Q. D. XU, B. ULFHAKE* and T. HÖKfelt**

*Department of Neuroscience, Karolinska Institute, S-141 77 Stockholm, Sweden



RAC- GWVI

May 15, 2006

Floyd Bloom

Aberrant Responses Can Be Intra-Neuronal

Annu. Rev. Neurosci.
2005. 28:377-401

The Plastic Human Brain Cortex

Alvaro Pascual-Leone, Amir Amedi,
Felipe Fregni, and Lotfi B. Merabet

Center for Non-Invasive Brain Stimulation, Department of Neurology, Beth Israel
Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215;
email: ap Leone@bidmc.harvard.edu

RAC- GWVI

May 15, 2006

Floyd Bloom

Neuroplasticity and GWVI

Floyd E. Bloom, MD
Neurome, Inc. &
The Scripps Research Institute

Presentation 7 – Dan Clauw

The Cause(s) and Potential Treatments of Chronic Multisymptom Illnesses Following the First Gulf War

Daniel J. Clauw, MD

Professor of Medicine, Division of Rheumatology
Director, Chronic Pain and Fatigue Research Center
Assistant Dean for Clinical and Translational Research,
Medical School
University of Michigan

Outline of Talk

- **Background of CMI and “old” findings**
- **Relevant preliminary data**
- **Treatment of CMI**

Chronic Multi-symptom Illnesses (CMI)

- Term coined by the CDC in 1999 to describe multiple somatic symptoms in Gulf War veterans (Fukuda et. al. JAMA 1999)
- This study and subsequent studies in the general population using factor analytic techniques (e.g., Doebbling et. al. Am J Med 2000) identified 3 – 4 symptom factors that cluster in the populations
 - Multifocal pain
 - Fatigue
 - Cognitive difficulties
 - Psychological symptoms
- This and subsequent studies demonstrated that approximately 10 – 15% of the population suffers from a syndrome characterized by two or more of these symptoms

“Systemic” Chronic Multisymptom Illnesses

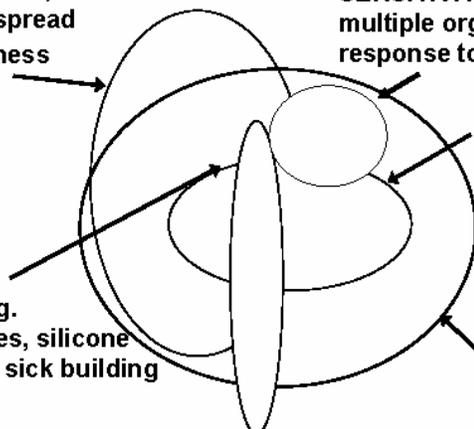
FIBROMYALGIA
2 - 4% of population;
defined by widespread
pain and tenderness

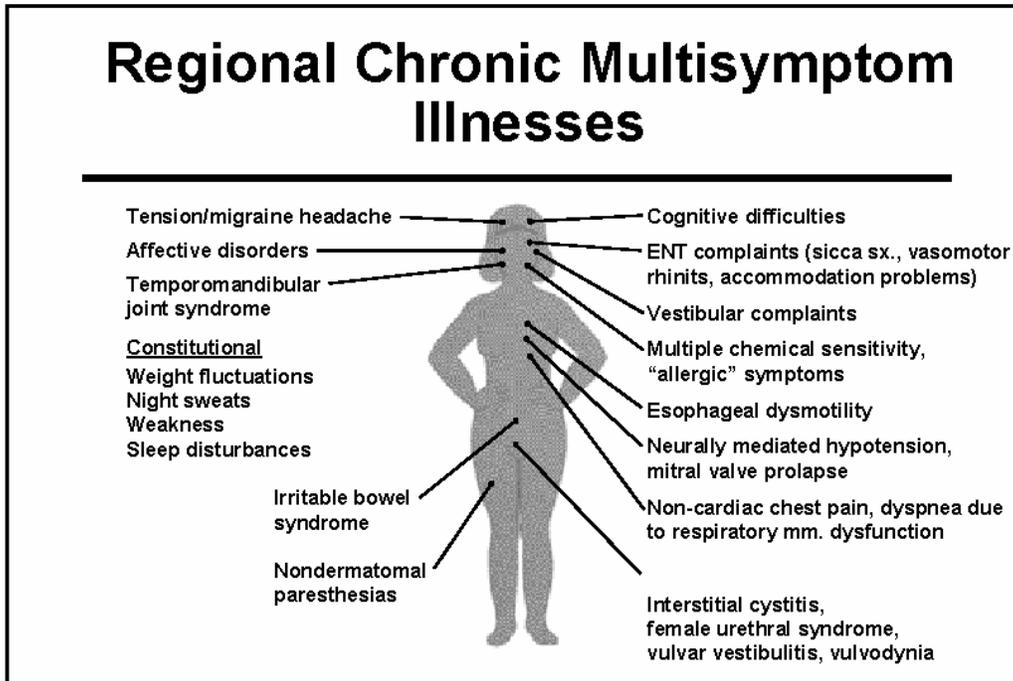
**MULTIPLE CHEMICAL
SENSITIVITY** - symptoms in
multiple organ systems in
response to multiple substances

**CHRONIC FATIGUE
SYNDROME** 1% of
population; fatigue and
4/8 “minor criteria”

**EXPOSURE
SYNDROMES** e.g.
Gulf War Illnesses, silicone
breast implants, sick building
syndrome

**SOMATOFORM
DISORDERS** 4% of
population; multiple
unexplained
symptoms - no
organic findings





In Addition to the CMI Seen Commonly in the General Population, is There a Superimposed "Neurological Damage" Disorder?		
	Yes	No
Population-based	1/200 Nonspecific (Haley 1999; Kang 2002) 2-3x ALS (Horner 2003)	Several others Increased in all veterans (Weisskopf 2005)
Case-control Neurological study	Nonspecific (Haley 1997)	(Sharief 2002; Lee 2005)
Abnormal functional imaging	Abnormal MRS (Haley 2000)	Abnormal fMRI Abnormal imaging in CMI
Abnl autonomic fxn	(Haley 2004)	(Stein 2004)

What Causes CMI?

- **Genetics**
- **“Triggers”**
- **Mechanisms**
 - Relationship between physiologic and psychologic factors
 - Disordered sensory processing
 - Autonomic/neuroendocrine dysfunction

Genetics of Fibromyalgia

- **Clearly is a strong familial predisposition**
 - Most recent work by Arnold et al suggest >8 odds ratio (OR) for first-degree relatives, and much less familial aggregation (OR 2) with affective disorders¹
- **Genes that may be involved**
 - 5-HT_{2A} receptor polymorphism T/T phenotype²
 - Serotonin transporter³
 - Dopamine D4 receptor exon III repeat polymorphism⁴
 - COMT (catecholamine o-methyl transferase)
 - Shown to be involved in pain transmission⁵
 - Slightly different in FM⁶
 - Predictive of development of TMD⁷

1. Arnold et al. *Arthritis Rheum.* 2004;50:944-952; 2. Bondy et al. *Neurobiol Dis.* 1999;6:433-439; 3. Offenbaecher et al. *Arthritis Rheum.* 1999;42:2482-2488; 4. Buskila et al. *Mol Psychiatry.* 2004;9:73; 5. Zubieta et al. *Science.* 2003;299:1240-1243; 6. GURSOY et al. *Rheumatol Int.* 2003;23:104-107; 7. Diatchenko et al. *Hum Mol Genet.* 2005;14:135-143.

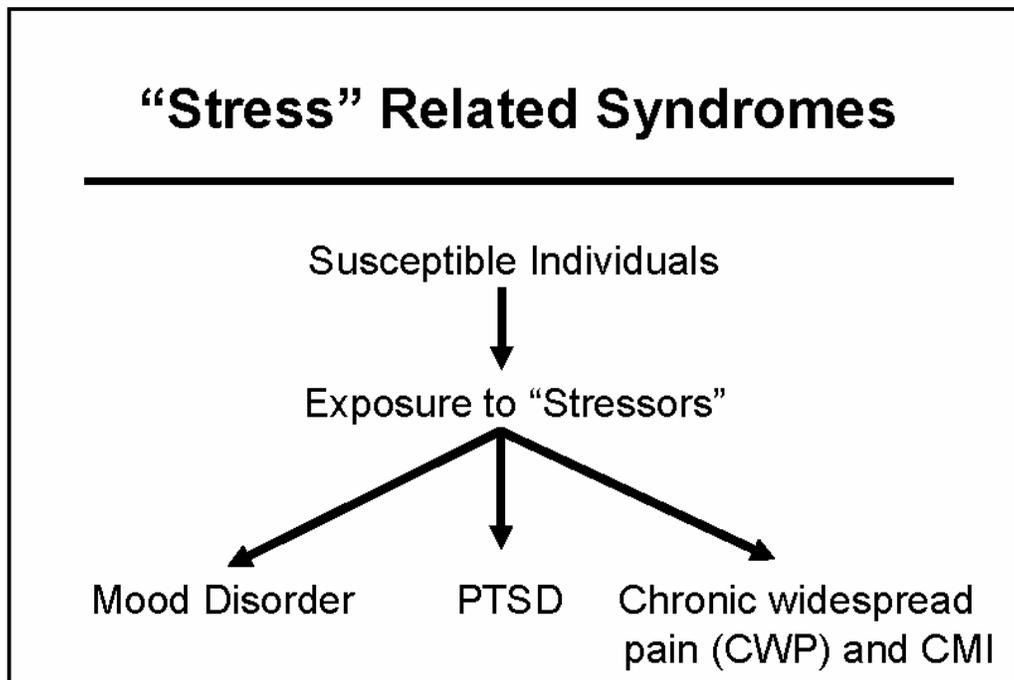
What Causes CMI?

- **Genetics**
- **“Triggers”**
- **Mechanisms**
 - Relationship between physiologic and psychologic factors
 - Disordered sensory processing
 - Autonomic/neuroendocrine dysfunction

“Stressors” capable of triggering these illnesses – supported by case-control studies

- Infections (e.g., parvovirus, EBV, Lyme, Q fever; not common URI)
- Physical trauma (automobile accidents)
- ? Psychological stress / distress
- Hormonal alterations (e.g., hypothyroidism)
- Drugs
- **Certain catastrophic events (*war, but not natural disasters*)** (Clauw, Engel, Aronowitz, Jones, Kipen, Kroenke, Ratzan, Sharpe, Wessely. *J Occup Environ Med*, 2003)

Clauw, Chrousos; *Neuroimmunomodulation*, 1997



- ## What causes CMI?
-
- **Genetics**
 - **“Triggers”**
 - **Mechanisms**
 - Relationship between physiologic and psychologic factors
 - Disordered sensory processing
 - Autonomic/neuroendocrine dysfunction

What causes CMI?

- **Genetics**
- **“Triggers”**
- **Mechanisms**
 - Relationship between physiologic and psychologic factors
 - Disordered sensory processing
 - Autonomic/neuroendocrine dysfunction

Summary (Scientific)

- Recent research is giving significant insights into the underlying mechanisms of Chronic Multisymptom Illnesses such as Fibromyalgia, Irritable Bowel Syndrome, TMD syndrome
 - **CNS disorder**
 - **Triggered by a variety of “stressors”**
 - **Abnormalities in brain function, especially in**
 - Sensory processing
 - Autonomic nervous system
 - Hypothalamic pituitary adrenal axes
- Very few mechanistic studies have compared GWI to those with CMI that are in general population, but this is an essential “control” group to interpret findings of physiological studies in GWV

Summary (Personal)

- The notion that the Gulf War and other post-deployment syndromes are *either*
 - “Psychological” *or* “physiological”
 - Due to “stress” *or* “toxins”is both inaccurate and counter-productive
- Psychological = Physiological
- The evidence that purely psychological stressors are responsible for triggering or worsening CMI is weak
- Stress is a toxin, and toxins are stressors

Outline of Talk

- Background of CMI and “old” findings
- Relevant preliminary data
- Treatment of CMI

New Information

- **The fact that established CMI syndromes do not nearly entirely explain the increased symptoms in Gulf War veterans is largely an artifact of how we define these illnesses**
- Nearly any functional neuroimaging study identifies robust differences between CMI patients and controls
- A revision of the “stress” theory is necessary
 - Original theory was that abnormalities in function of autonomic and HPA systems *caused* illness
 - Present and emerging evidence suggests that baseline differences in the function of these systems act as a *diathesis* to put individuals at higher risk for developing these illnesses

Distribution of chronic pain among non-deployed and deployed veterans (from CSP #458)

Chronic Pain Characteristic	% Deployed (n)	% Non-Deployed (n)
No Pain	41.27% (393)	54.05% (569)
1-2 Pain Areas	26.33% (275)	25.48% (293)
3+ Pain Areas, Not Widespread	11.29% (126)	9.04% (107)
Widespread Pain	21.11% (255)	11.43% (151)
Any Pain	59.07% (661)	46.20% (555)

21 missing observations. Frequencies are actual, and percents are weighted.

New Information

- The fact that established CMI syndromes do not nearly entirely explain the increased symptoms in Gulf War veterans is largely an artifact of how we define these illnesses
- Nearly any functional neuroimaging study identifies robust differences between CMI patients and controls
- A revision of the “stress” theory is necessary
 - Original theory was that abnormalities in function of autonomic and HPA systems *caused* illness
 - Present and emerging evidence suggests that baseline differences in the function of these systems act as a *diathesis* to put individuals at higher risk for developing these illnesses

A Revision of the “Stress” Theory

- In a DoD funded study published in 2004, we hypothesized that a subset of healthy individuals deprived of routine exercise would develop pain, fatigue, and other somatic symptoms (true – about half did), and that autonomic and neuroendocrine responses would change as individuals developed symptoms (they didn't) (Glass, Psychosomatic Research, 2004)
- However, baseline differences in HPA and autonomic function predicted who developed symptoms when they were deprived of exercise
- Subsequently, a large, population-based study showed that baseline HPA function predicted the subsequent development of chronic widespread pain (McBeth Arthritis Res Ther 2005)
- Two ongoing models of post-stress symptoms of relevance to CMI

The Predictors of Pain and Other Somatic Symptoms, and Psychological Sequelae, and Decrements in Performance Following Sleep or Exercise Deprivation

- This ongoing DoD-funded study has recruited 36/128 total subjects, and is a 2 x 2 x 2 design to examine the independent and synergistic effects of exercise and sleep deprivation, as well as the neurobiological measures that predict symptom development.
- The ongoing study has four treatment conditions: control (regular exercise and no sleep restriction), exercise deprivation alone, sleep restriction alone and both exercise deprivation and sleep restriction. We have tested 36 subjects to date (11 control, 6 exercise deprivation, 9 sleep restriction, 10 both exercise deprivation and sleep restriction).

Association Between Baseline Autonomic and HPA Function and Subsequent Development of Symptoms

	McG Sens	McG VAS	MFI- gen	PVT lapses	CESD	Anxiety
AM cortisol	-0.238	-0.263	-0.457	-0.466	-0.306	-0.599
ULF	-0.539	-0.706	0.317	-0.512	-0.091	-0.173
VLF	-0.547	-0.671	0.186	-0.526	-0.198	-0.317
TP	-0.548	-0.697	0.312	-0.534	-0.120	-0.224

The Predictors of Pain and Other Somatic Symptoms, and Psychological Sequelae, Following a Motor Vehicle Collision

- Patients being evaluated in the Emergency Department (ED) after MVC were recruited into an ongoing multicenter study which includes ED baseline assessment and 1-month outcome evaluation.
- ED assessment includes salivary cortisol collection and 20 minute Holter monitor recording. Outcome evaluation includes assessment of persistent MVC-related neck or back pain, significant PTSD symptoms (IES-R score ≥ 33), and significant depressive symptoms (CES-D ≥ 27).
- Cortisol samples were assayed using the Diagnostic Products Corporation Coat-a-Count cortisol kits. HF HRV was assessed using HF power spectral analysis (0.15 to 0.4-Hz).
- To date, follow-up data has been obtained in 48 of 49 enrolled patients who have reached the 1 month follow-up time point (98%, 28 female, 20 male, age 18-84, mean 36.4 years).

Association between mean ED HRV and presence of early and persistent pain and psychological sequelae

Mean ED HF HRV ¹ by Group	Pain 3-7 days after MVC	Moderate or severe neck and/or back pain at 1 Month	PTSD at 1 Month
Present	146±118	185±119	614±661
Not present	566±534	539±583	278±225
t	3.494	2.618	-2.038
p value	.002	.015	.028

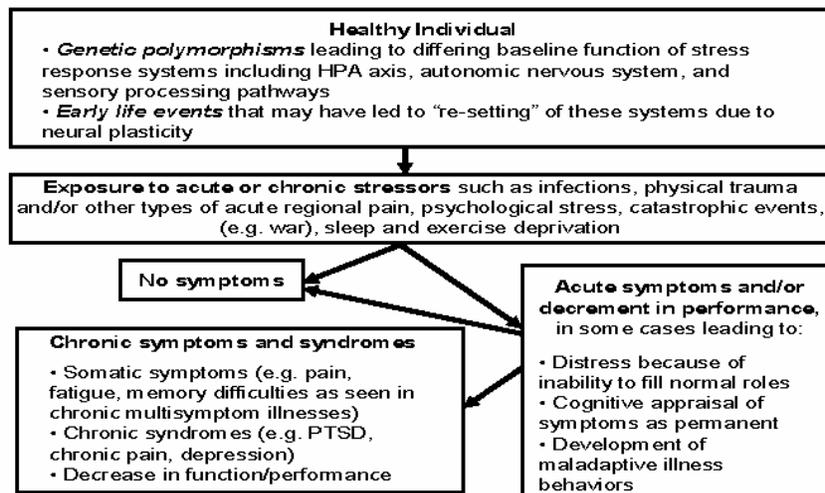
¹High frequency heart rate variability, ²Defined by Neck pain and back pain summed NRS scores ≥ 10 , ³Defined by IES-R score ≥ 33

Association between mean ED cortisol level and persistent pain and psychological sequelae

1 month after MVC

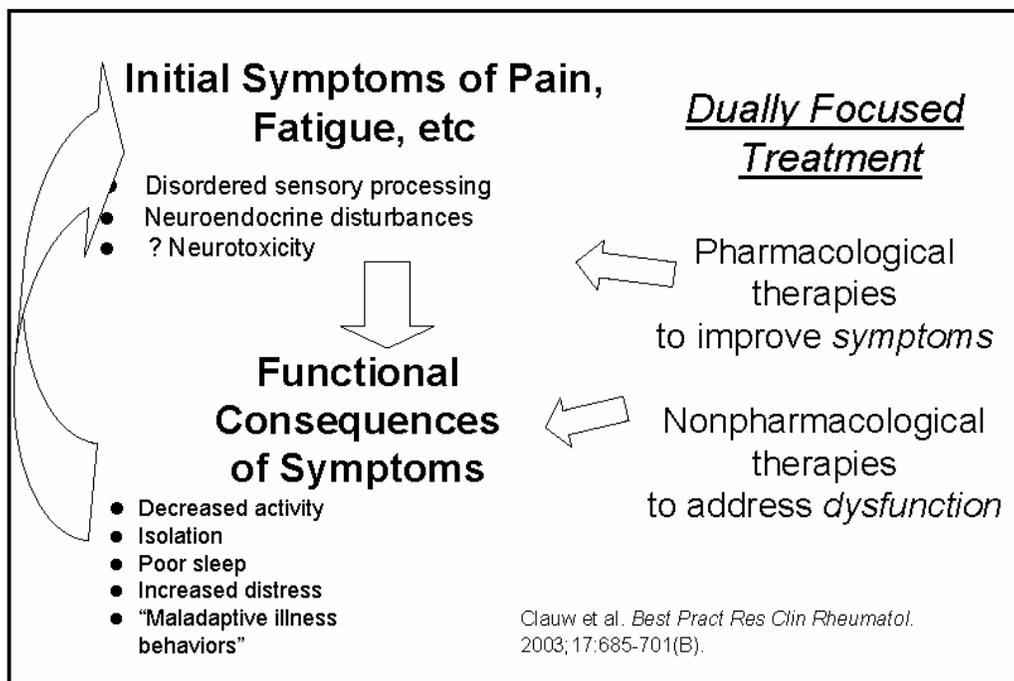
1 Month Outcome (n)	ED Cortisol (ug/mL)
No Symptoms (26)	.33 ± .46
Pain Only (10)	.27 ± .29
PTSD ± Pain (5)	.18 ± .10
Depression ± Pain (2)	1.48 ± 2.0
Depression & PTSD ± Pain (5)	.52 ± .50
ANOVA F statistic (p value)	2.777 (.039)

The Etiology of Post-“Stress” Syndromes



Outline of Talk

- Background of CMI and “old” findings
- Relevant preliminary data
- Treatment of CMI

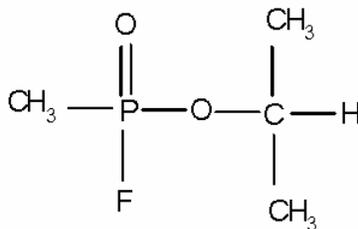


Presentation 8 – Mohamed Abou-Donia

Gene Expression Profiles Following Sarin Exposure

Mohamed B. Abou-Donia, Ph.D.
Duke University Medical Center
Durham, North Carolina

Test Compound



Sarin

Sarin-Induced Neurotoxicity

Sarin causes the following neurotoxic effects:

1. Cholinergic Neurotoxicity
2. Organophosphate-Induced Delayed Neurotoxicity (OPIDN)
3. Organophosphate-Induced Chronic Neurotoxicity (OPICN)
 - a. Acute, high-level exposure
 - b. Low-level Exposure

Cholinergic System

Neurotransmitter: *Acetylcholine (ACh)*

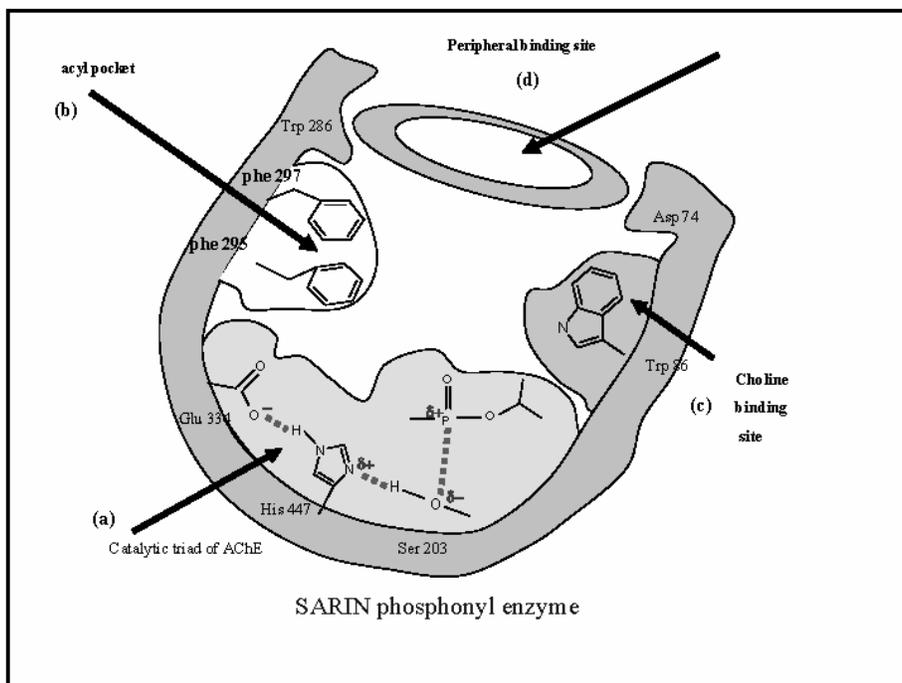
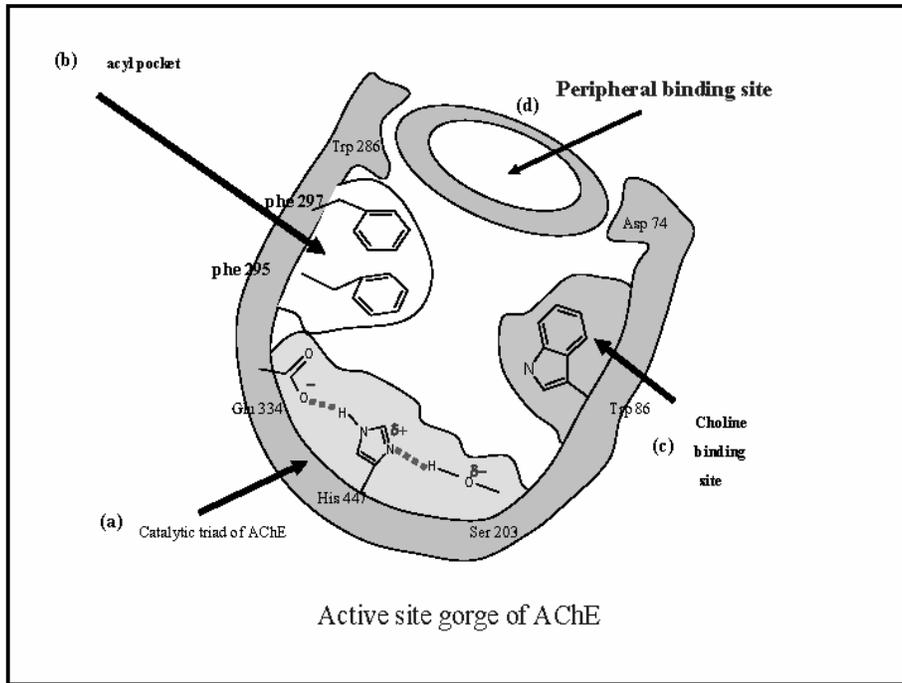
Synthesis: *Choline acetyltransferase (ChAT)*

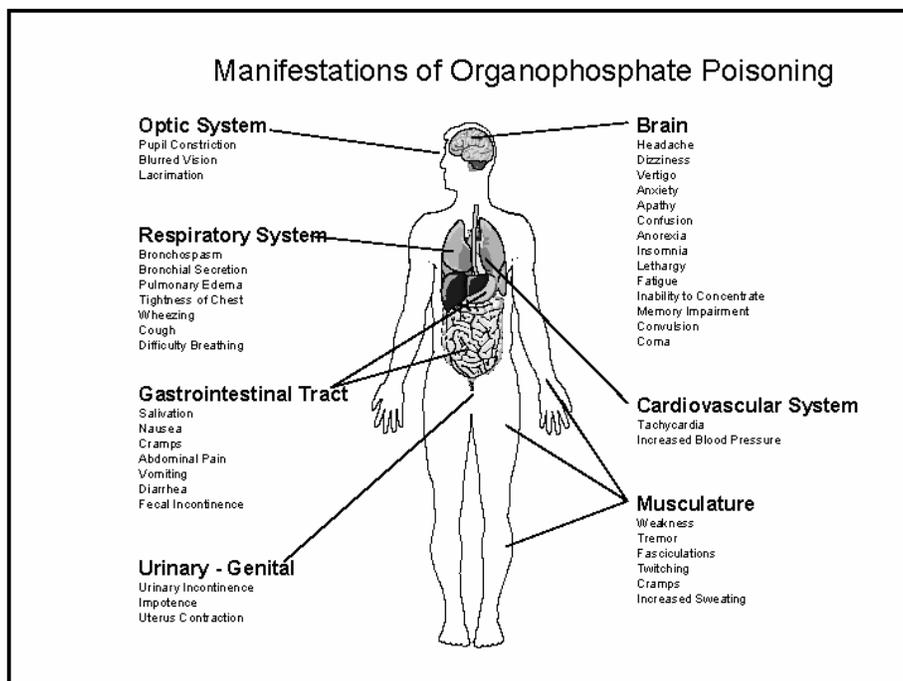
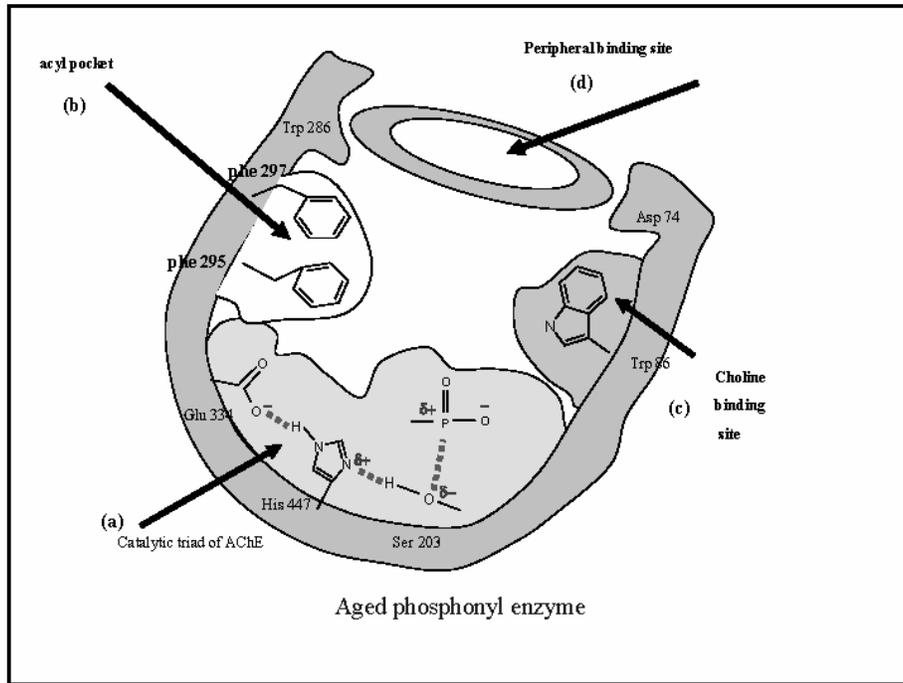
Choline + AcetylCoA + ChAT → ACh

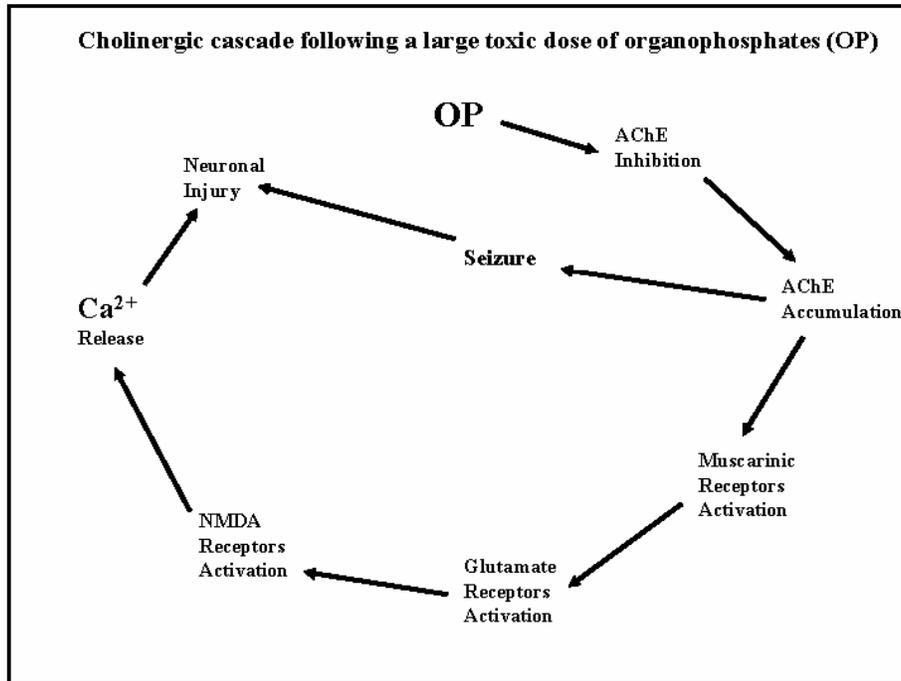
Action: *Stimulation of Muscarinic and nicotinic acetylcholine receptors*

Hydrolysis: *Acetylcholinesterase (AChE)*

ACh + AChE → Choline + Acetic acid







Human Exposure to Sarin

1. High-level (Acute) Exposure:

a) *Matsumoto City, Japan*

At midnight of June 27, 1994

b) *Tokyo subway trains*

At 8:05 AM, on March 20, 1995

2. Low-level Exposure:

In 1991 during the Persian Gulf War

U.S. military personnel were exposed to low-level sarin during the destruction of Iraqi munition-containing sarin at Khamisiyah

Matsumoto (Cholinergic)

1. Sarin release was at midnight on June 27, 1994
2. About 600 persons were exposed
3. Fifty eight were admitted to hospitals
4. Seven died
5. Miosis was the most common symptom
6. Severe cases developed CNS symptoms and cardiomyopathy
7. A few victims complained of arrhythmia and showed cardiac contraction.

Tokyo (Cholinergic)

Patients: 58 , (Zuzuki, et al. 1999)

Severe Poisoning:

Reduced consciousness, miosis, flushing, respiratory distress, *fasciculation, tachycardia, high blood pressure* (nicotinic responses), and flaccid paralysis. Plasma AChE activity: 35% of normal. (Hospital)

Mild poisoning:

Headaches, dizziness, nausea, chest discomfort, abdominal cramps, marked miosis.

Tokyo (Cholinergic)

Route of exposure:

Absence of bradycardia, excessive secretions, which are common in dermal or ingestion exposure, may be related to exposure to gas.

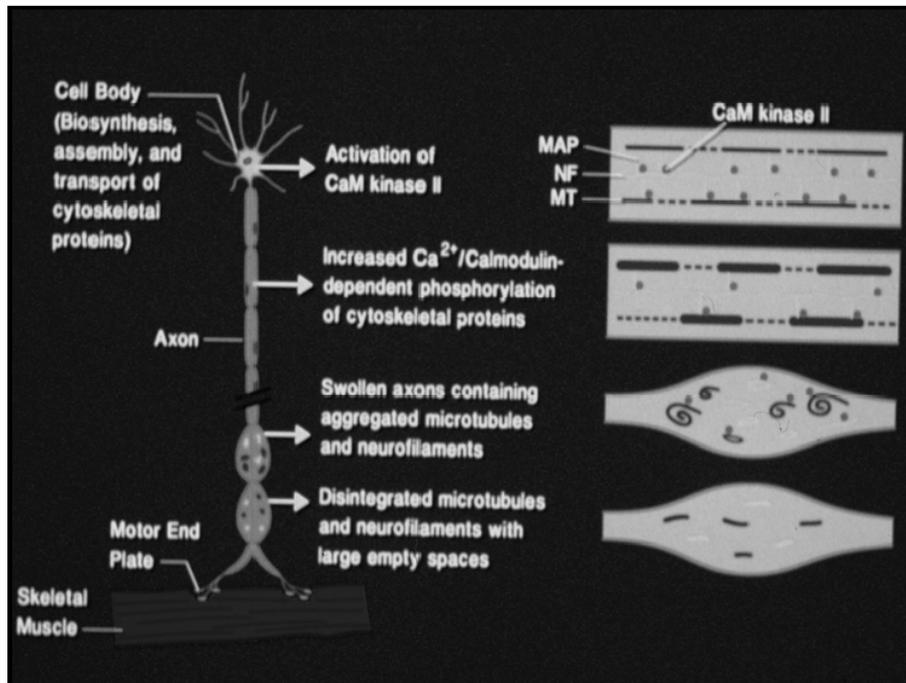
Treatments:

1. Atropine eye drops for marked miosis.
2. Pralidoxime Iodide (2-PAM)

OPIDN

Characteristics of OPIDN:

1. A latent period, ranging between 6 and 14 days.
2. Neuropathological lesions are in the medulla of the brain, spinal cord, and sciatic nerve.
3. Primary degeneration of the axon, followed secondary degeneration of myelin (Wallerian).
4. Species and age sensitivity.
5. Inhibition of neurotoxicity target esterase (NTE).



Sarin-Induced OPIDN

The patient: A 51-year man inhaled sarin in the Tokyo subway, died 15 Months later.

neuropathologic examination:

marked *nerve decreased* in the sural nerve, moderate *nerve fiber loss* in the sciatic nerve, unremarkable dorsal root ganglia, dorsal roots and posterior column of the spinal cord.

Conclusion:

Pathology is consistent with OPIDN.

Sarin-Induced Chronic Neurotoxicity (OPICN)

1. Results from direct action of sarin on brain neuronal and glial cells.
2. Leads to neurological dysfunctions characterized by:
 - a) Cognition impairment.
 - b) Locomotor and sensory deficits.
 - c) Body weakness and incoordination.
 - d) Behavioral abnormalities.

OPICN

1. Six to eight months after Tokyo poisoning, some victims showed delayed effects on psychomotor performance, visual nervous system, and the vestibulo-cerebellar system, (Yokoyama et al., 1998).
2. Females were more sensitive than males in exhibiting delayed effect on the vestibulo-cerebellar system.

OPICN

1. **Three years and nine months** after the **Tokyo** attack, some victims and rescue workers complained of chronic decline of memory (Nishiwaki et al, 2001).
2. **Three years** after the **Matsumoto** attack, some victims complained of fatigue, shoulder stiffness, weakness, blurred vision (Nakajima et al., 1999)
3. Others complained of insomnia, had bad dreams, husky voice, slight fever, and palpitation.

OPICN

“Chronic decline of psychomotor function and memory still exist in Tokyo subway workers, 7 years after the sarin exposure”

K. Miyaki et al., J. Occup. Health
47:,299-304 (2005)

The Gulf War Syndrome

1. Between the invasion of Kuwait by Iraq on August 2, 1990, and March 1991, the U.S. had 697,000 military personnel in the Persian Gulf region.
2. Since their return, several thousands, complained of the following chronic symptoms:
headache, loss of memory, fatigue, muscle and joint pain, ataxia, skin rash, respiratory difficulties, and gastrointestinal difficulties.
3. A recent report showed that PGW veterans are twice more likely to develop amyotrophic lateral Sclerosis (ALS) than other military personnel.

HYPOTHESIS

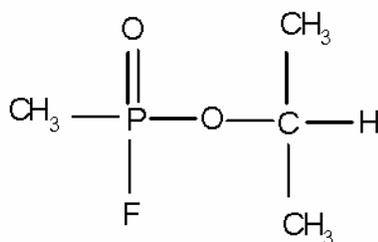
Exposure to low level sarin, alone or in combination with other chemicals and/or stress was involved in the development of the Persian Gulf War Veterans' illnesses.

Specific Aims

To investigate the neurological deficits in the adult male rat, following exposure to sarin:

1. Clinical signs
2. Brain AChE and Plasma BChE
3. M2 muscarinic receptors
4. Integrity of the blood blood barrier (BBB)
5. Brain neuropathological alterations

Test Compound



Sarin

The stock solution of Sarin (1.9 mg/ml) in saline was stored frozen at -80°C prior to use.

Experimental

Treatment: A dose-response, time course study

Male Sprague-Dawley rats (225 g) were given a single i.m. injection of:

1. Saline, 0.1 ml/kg (Control)
2. Sarin, 1.0 x LD₅₀ (100 µg/kg)
3. Sarin, 0.5 x LD₅₀ (50 µg/kg)
4. Sarin, 0.1 x LD₅₀ (10 µg/kg)
5. Sarin, 0.01 x LD₅₀ (1 µg/kg)

6. Groups of 15 animals from each treatment and control were sacrificed at: 24 hour, 7 days, 30 days, and 10 months.

Clinical Condition

Sarin at 1 x LD50:

Severe tumors, seizures, and salivation within 3 – 5 min. of treatment. Half of the animals died within 15 minutes.

Sarin at 0.5 x LD50:

Tremors by 15- 30 min. no animals died.

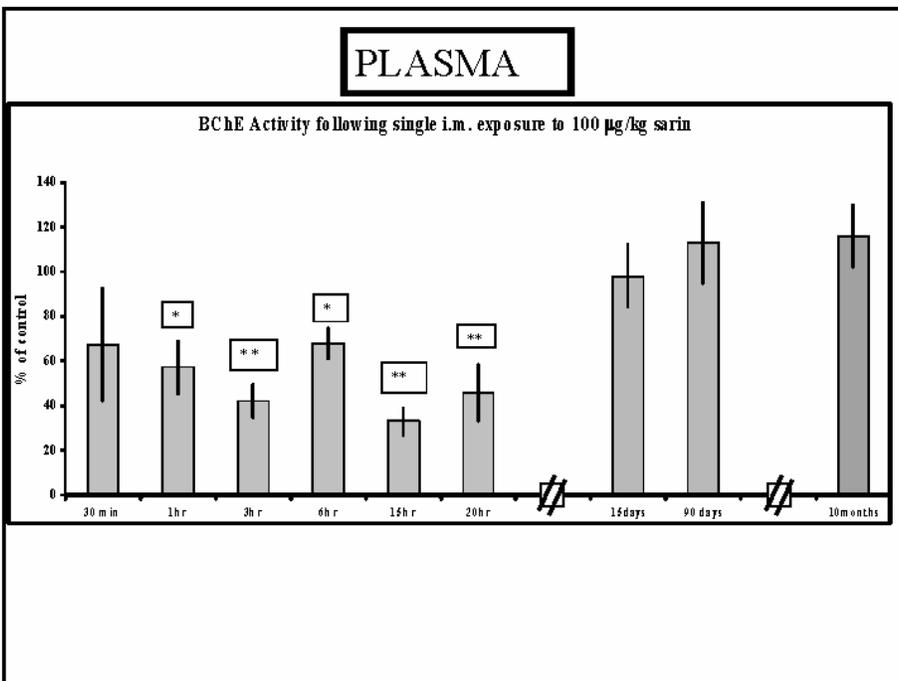
Sarin at 0.1 and 0.01 x LD50:

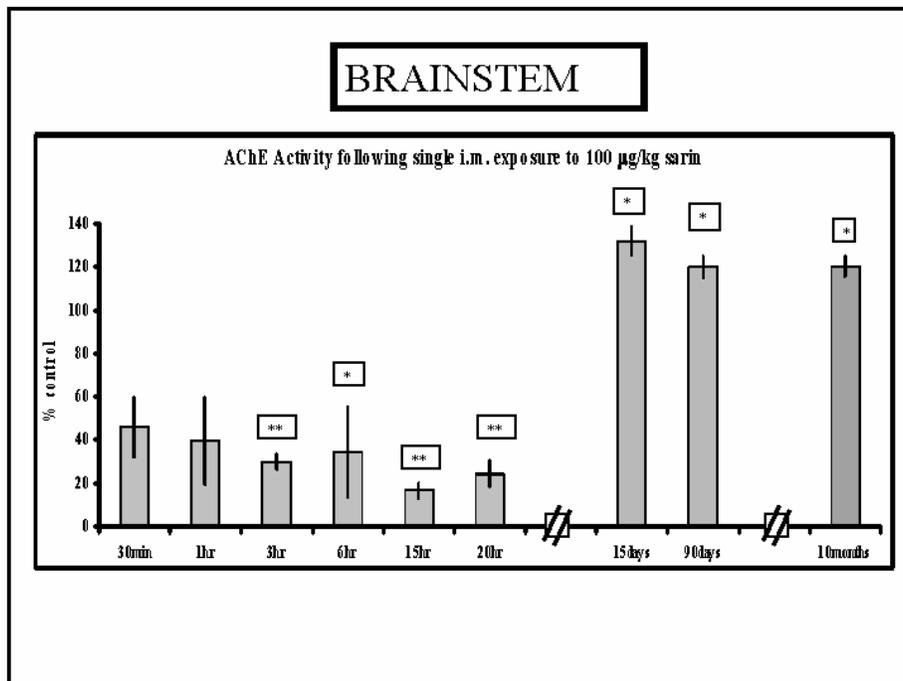
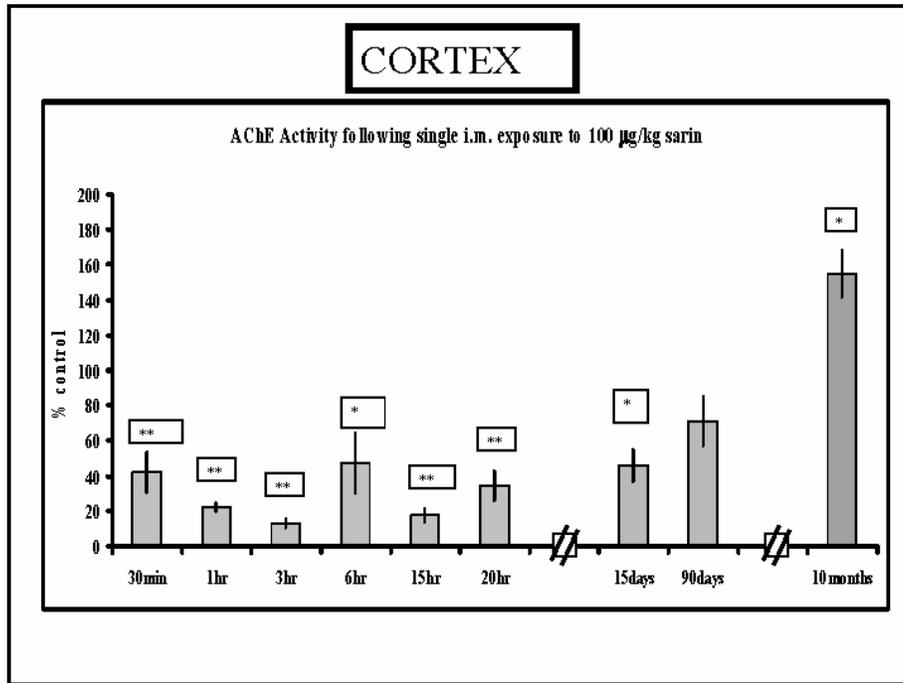
No toxicity signs.

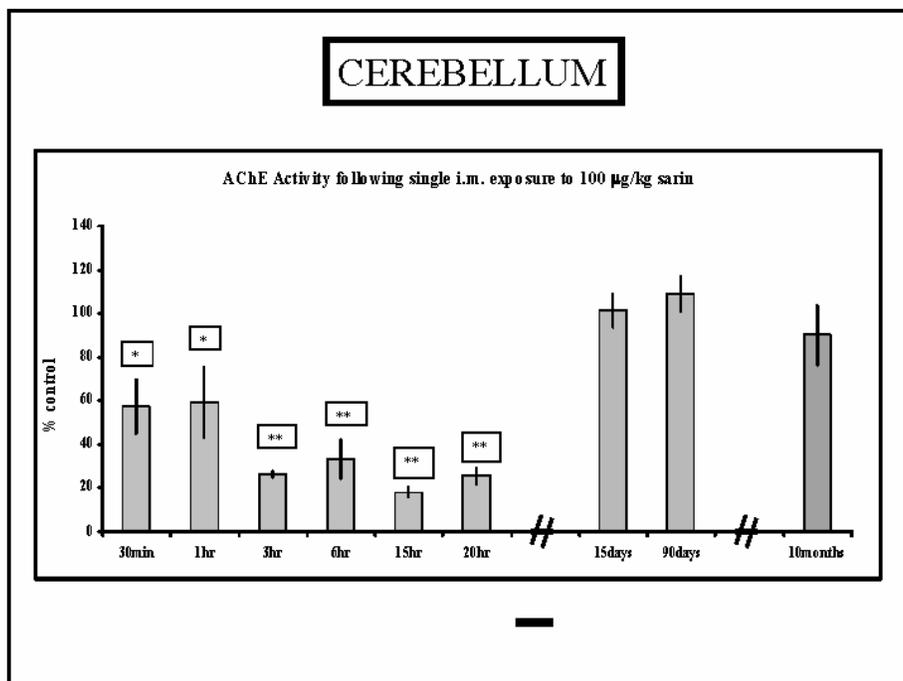
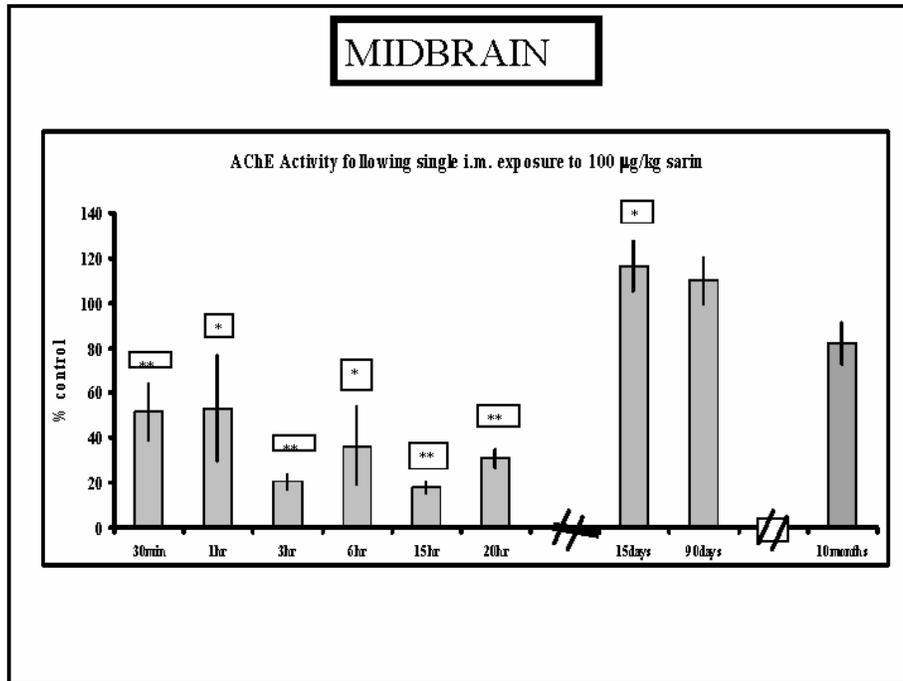
Effect on enzymatic activity

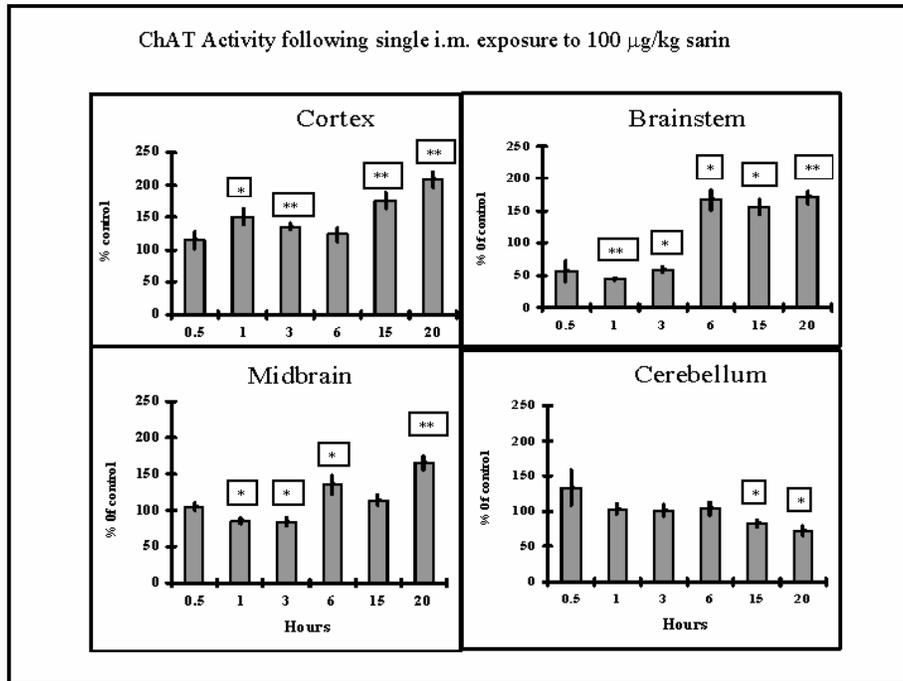
The effect of sarin exposure was determined on the following enzymes:

1. Plasma butyrylcholinesterase (BChE)
2. Brain acetylcholinesterase (AChE)
3. Brain Choline acetyltransferase (ChAT)

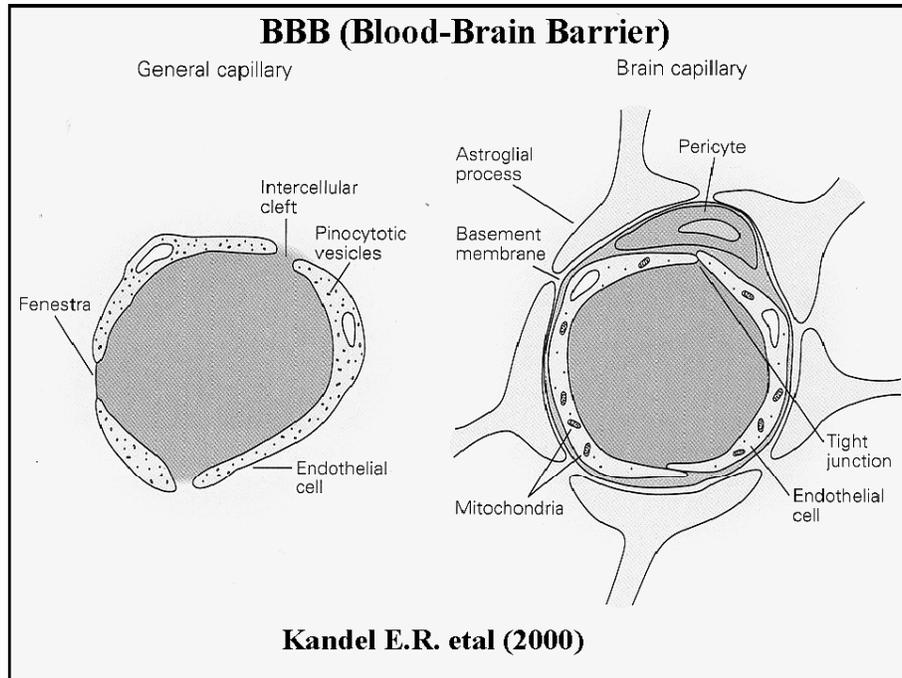








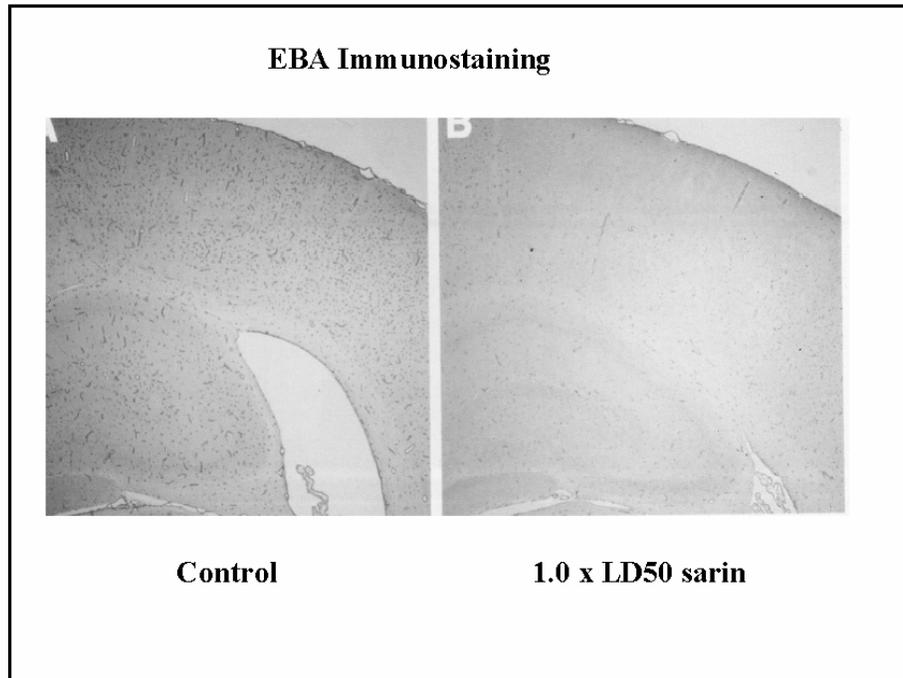
Effect of Sarin on the Blood Brain
Barrier



Blood Brain Barrier studies

Alterations in the permeability of the BBB were assessed by immunohistochemical staining of *endothelial barrier antigen (EBA)* (using SMI-71 antibody).

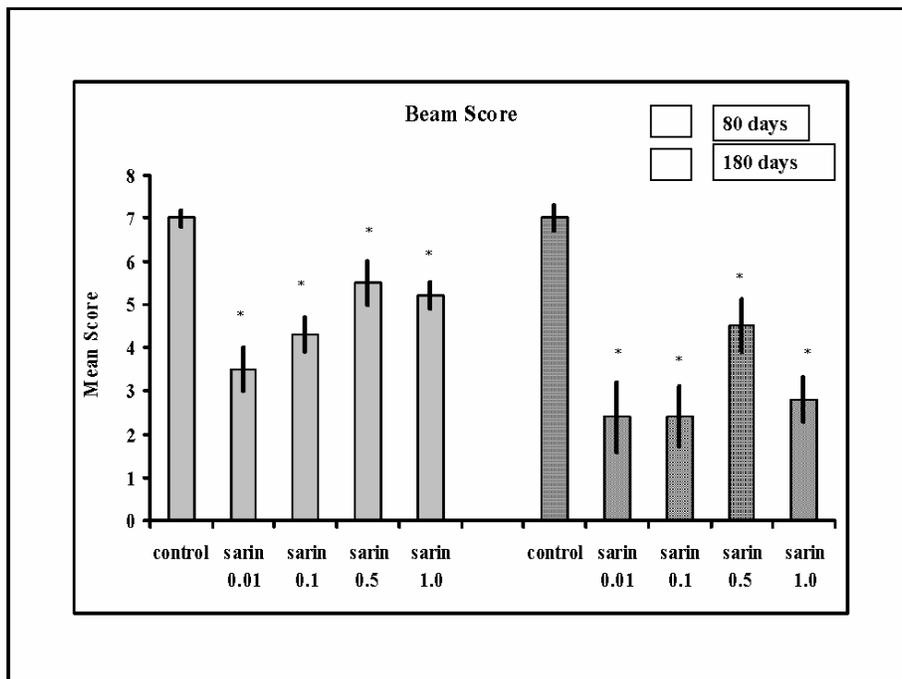
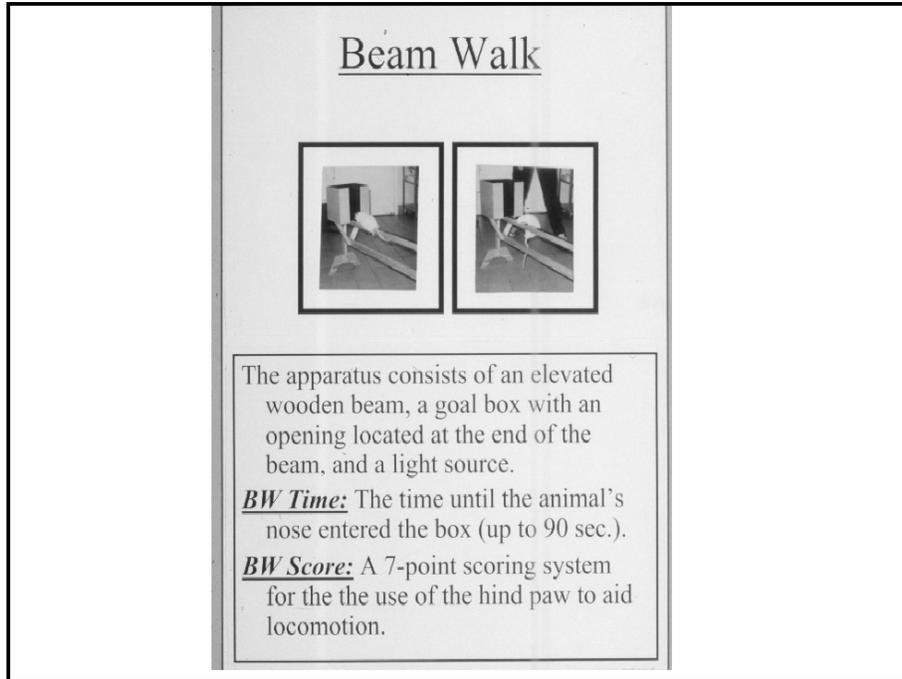
This staining visualizes BBB protein in brain capillaries and in smaller vessels invading the brain parenchyma.

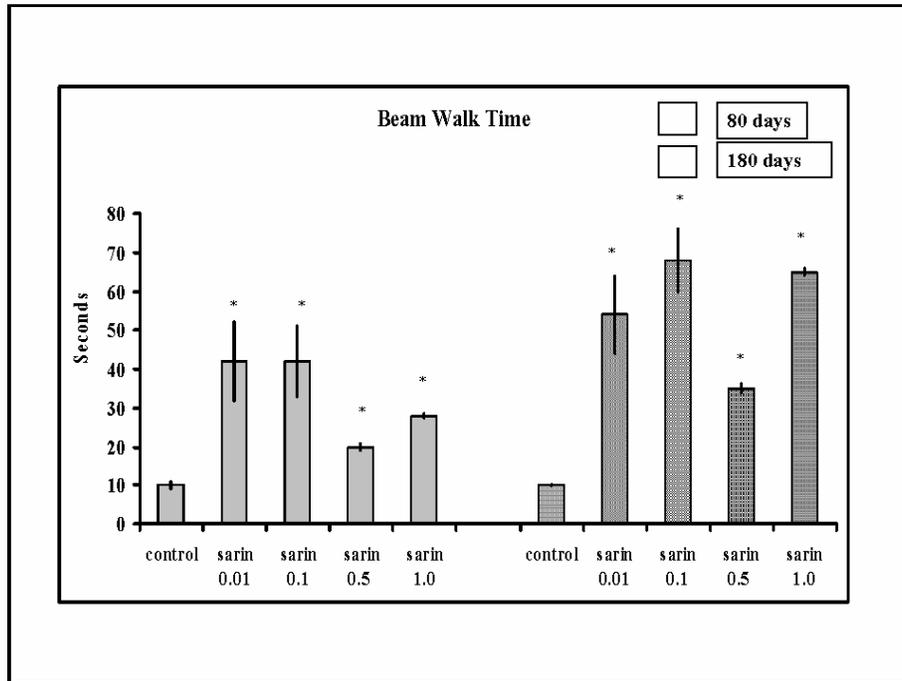


Effect of Sarin on Sensorimotor Performance

Control and treated animals were evaluated for sensorimotor performance using the following tests:

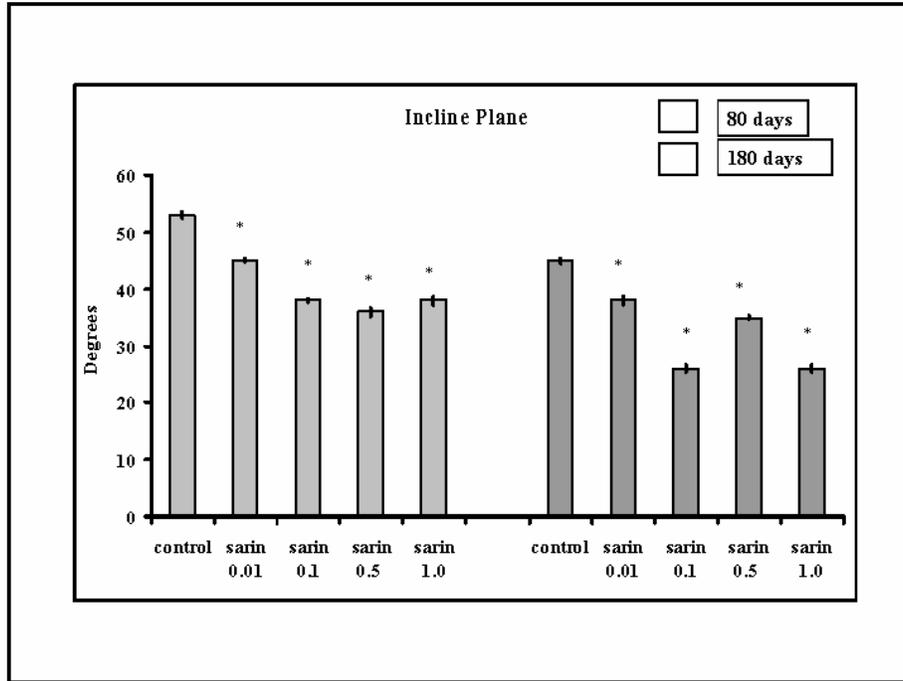
1. Beam walk performance and time
2. Incline plane performance
3. Forepaw grip time





Incline Plane

Description
Rats are placed on a flat plane in the horizontal position, with the head facing the side of the board to be raised.
The angle at which the rat begins to slip is recorded.

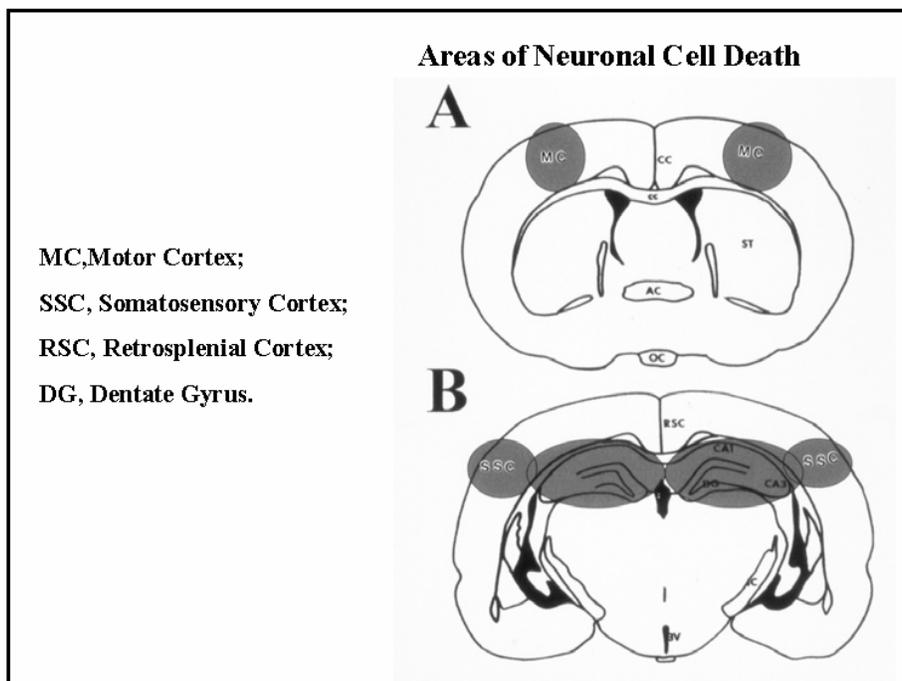
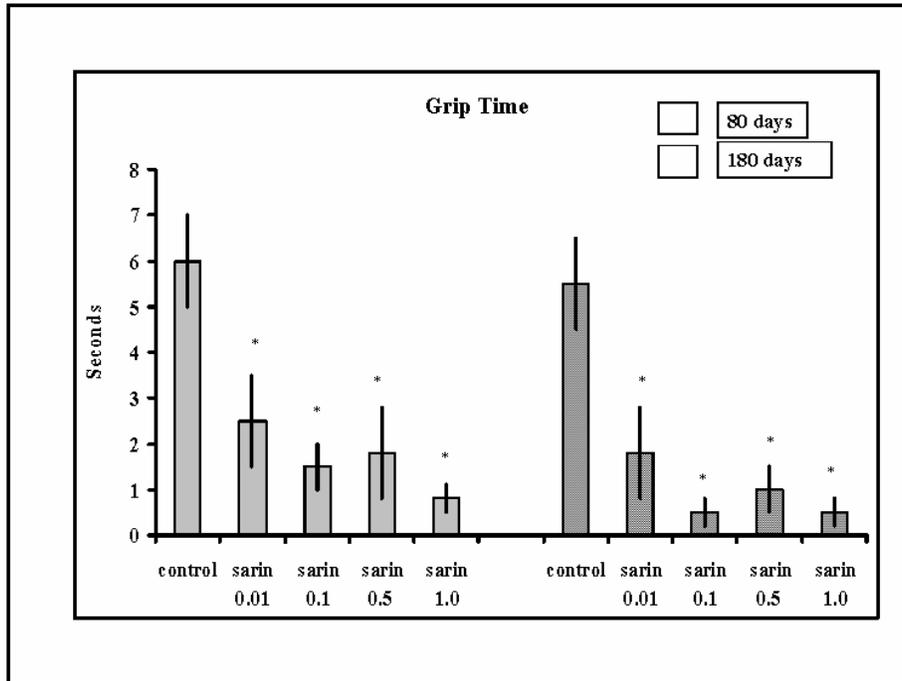


GRIP TIME

PURPOSE: To assess forepaw grip strength

PROCEDURE:

1. Have the rats grip a 5-mm diameter wood dowel
2. Time to release grip is recorded in seconds.



Neuropathological Studies

NEUROPATHOLOGICAL STUDIES

INCLUDED:

1. Cerebral cortex
2. Hippocampus
3. Cerebellum

AChE-Induced Neuronal Cell Death

1. An increased AChE protein, in Alzheimer disease causes aggregation of β amyloid peptide, causing neuronal cell death (Inestrosa et al., 1996; Calderon et al., 1988).
2. Over expression of AChE activates caspases, leading to apoptosis.

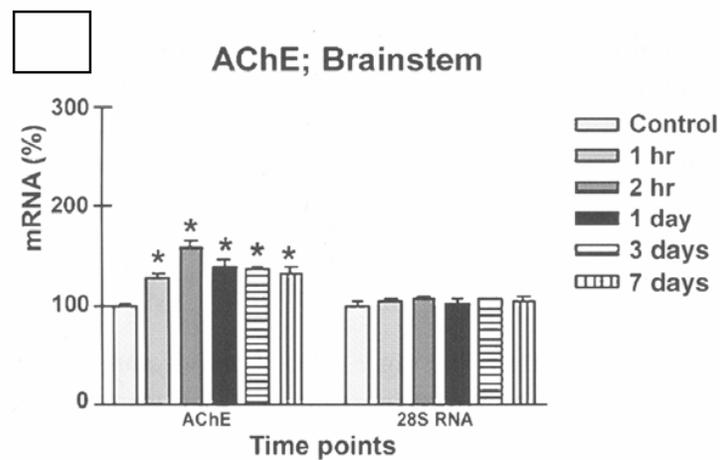
Sarin-Induced Expression of mRNA Coding of Acetylcholinesterase

Dose: 0.5 x LD₅₀ im sarin

Time points: 1 and 2 hours, 1, 3, and 7 days.

Results:

Sarin produced immediate and persistent induction of AChE mRNA levels in various regions of the brain.



Introduction

Gene profiling studies have the promise to delineate global alterations in molecular expression as toxic effects and mechanisms of action of chemicals with multitude effects, such as sarin GB:

(*O*-isopropyl methylphosphonofluoridate)

Specific Aim

To study sarin-induced global expression profiles at:

- 1. 15 min; after 0.5 x LD₅₀ sarin**
- 2. 30 min; after 1.0 x LD₅₀ sarin**
(Intramuscular LD₅₀ = 100 µg/kg)

**Using Affymetrix : Rat Neurobiology U34
Chips in male Sprague-Dawley rats,**

Results: Clinical condition

Rats given 1 X LD₅₀ exhibited:

1. Excessive salivation, severe tremors, seizures, and convulsions within 5-10 min.
2. Prolonged convulsion ensued for 3 hr.
3. One half of the animals died within 3 hr.
4. The remaining animals survived the 3-month experiment.

RNA Isolation

1. At each time-point, animals were euthanized, brain dissected out, and separated into the cortex, cerebellum, midbrain and brainstem.
2. RNA was extracted using Trizol solution
3. About 200-250 µg/µl of total RNA was applied on RNA chip and analyzed on the Agilent Bioanalyzer 2100.

Chips

1. Rat Neurobiology U34 array of the Affymetrix gene chip was used.
2. It allowed monitoring the relative abundance of more than 1200 mRNA transcripts.
3. It contains genes representing different cell types, signaling pathways, and other functional and structural groups relevant to the nervous system.

Affymetrix chip hybridization

1. The double-stranded cDNA from total RNA was synthesized and isolated from the rat tissue.
2. Biotin-labeled cRNA was generated by *in vitro* transcription from the DNA.
3. The cRNAs were hybridized to the oligonucleotide probes on the probe arrays for a 16 h incubation at 45 C.
4. The DNA chips were scanned with the Affymetrix gene chip scanner.

Data Analysis

Duke University Bioinformatics Shared Resources Consortium

1. Affymetrix Microarray Software Solutions were used to identify the list of genes showing statistically significant levels of alterations.
2. Partek clustering and treeview analysis program was used to identify clustering of genes that showed alteration.

RESULTS

Number of genes showed predominated alterations:

1. At 15 minutes ($0.5 \times LD_{50}$) a total of 65 genes
2. At 3 months ($1.0 \times LD_{50}$) a total of 36

RESULTS: 15 Minutes

At 15 minutes (0.5 X LD₅₀) the following classes of altered genes predominated:

1. Ion channel and Cell adhesion molecule (8 genes).
2. Cytoskeletal proteins (8 genes).
3. Neuropeptides and their receptors (5 genes each).

RESULTS : 15 Minutes

The following categories had 2 genes each:

1. Cholinergic signaling
2. Energy metabolism
3. GABAnergic signaling
4. Glutamergic and aspartate signaling
5. Mitochondria associated proteins
6. Myelin proteins
7. Neurotransmission and related transporters
8. Serotonergic signaling, and
9. Tyrosine phosphorylation molecule

RESULTS : 15 Minutes

The following categories had 1 gene each:

1. ATPases and ATP-based transporters
2. Catecholaminergic signaling
3. Cyclic nucleotide signaling
4. Mitochondria associated proteins
5. Nitric Oxide signaling
6. TNF beta family, and
7. Transcription factors

RESULTS : 15 Minutes

Other altered genes at 15 minutes (0.5 XLD₅₀) were:

1. Cholinergic signaling
2. Calcium channels
3. Calcium binding proteins
4. Transporters
5. Chemokines
6. GABAergic
7. Glutamatergic
8. Aspartate
9. Catecholaminergic
10. Nitric oxide synthase
11. Purinergic
12. Serotonergic signaling molecules

Results: 15 Minutes (% of Control)

Receptors

- Nicotinic ACh receptor (150 ± 9)
- Muscarinic ACh receptor (214 ± 4)
- Glutamate receptor (177 ± 2)
- NMDA receptor-like long variant ($142 \pm 2\%$)
- GABA-A receptor α -subunit (130 ± 5)
- Dopamine receptor (225 ± 3)
- A1 adenosine receptor (201 ± 1)
- Purinergic receptor (193 ± 2)
- Tyrosine Kinase receptor (238 ± 3)

Results: Clinical condition

Rats given 0.5 X LD₅₀ exhibited:

1. Did not develop any of the signs seen in animals given.
2. They were inactive 1 X LD₅₀.
3. All animals survived the experimental period

Results: 15 Minutes

Down-regulated genes (4 out of 27) ranging from $46 \pm 11\%$ - 38%

1. Cyclic nucleotide signaling
2. Detoxification molecules
3. Mitochondria associated proteins
4. Neurotransmission and neurotransmitter transporters

Results: 15 Minutes

1. Metabolism Enzyme:
Cytochrome P-450 ($65\% \pm 4\%$)
2. Detoxification Enzyme:
Glutathione *S*-transferase ($71\% \pm 2$)

Results: 15 Minutes

Mitochondrial Associated Proteins:

Bax apoptosis exposure (75 ± 5)

Bcl-2-related ovarian killer protein (BOK, 70 ± 7).

Nitric oxide signaling:

Nitric oxide synthase (Nos-2, $167\% \pm 1$)

Results: 3 Months

- A total of 38 genes were altered
- An equal number of gene showed up-regulation and down regulation (50%)

Results: 3 Months

Calcium/calmodulin Protein Kinase II

<u>Brain Region</u>	<u>% of control</u>
Brainstem	140 ± 5
Cerebellum	182 ± 2
Cortex	35 ± 3
Midbrain	62 ± 6

Results: 3 Months

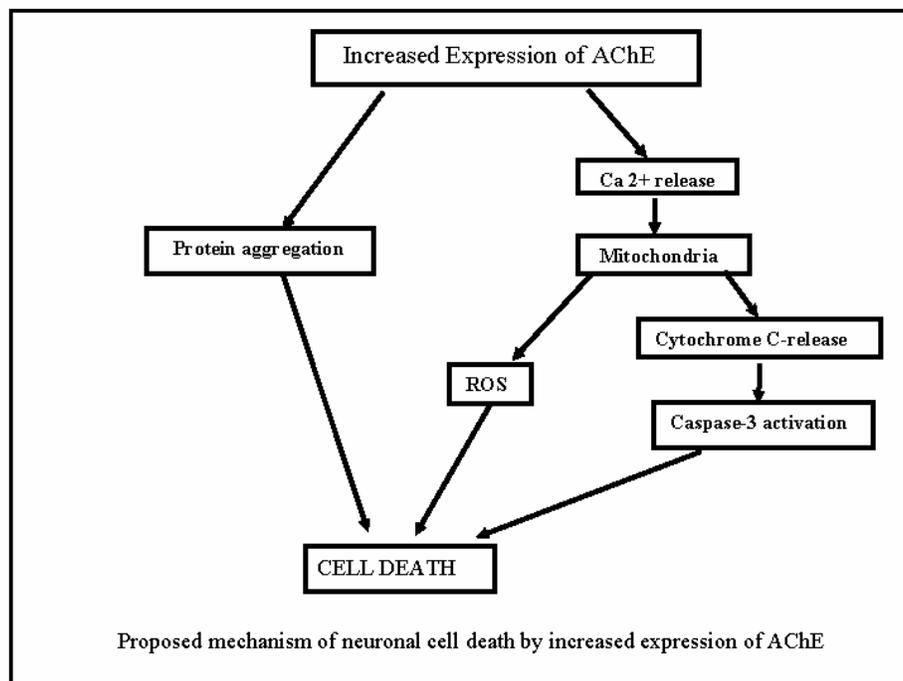
Receptors

1. GABA A receptor (166 ± 2)
2. Glutamate receptor, AMPA subtype (236 ± 1).

RESULTS : 3 Months

At 3 months ($1.0 \times LD_{50}$) the following classes of altered genes predominated:

1. Calcium channels
2. Calcium binding proteins
3. Cytoskeletal proteins
4. Cell adhesion molecule
5. GABAnergic signaling molecules



Presentation 9 – James Baraniuk

**“A Chronic Fatigue Syndrome Related
 Proteome in Cerebrospinal Fluid”**

Baraniuk JN,* Casado B,*§ Maibach H,*
 Clauw DJ,*† Pannell LK,‡ Hess S.‡

BMC Neurology 5:22, 2005

*Georgetown University
 §Swiss Federal Institute of Technology
 †University of Michigan
 ‡NIDDK

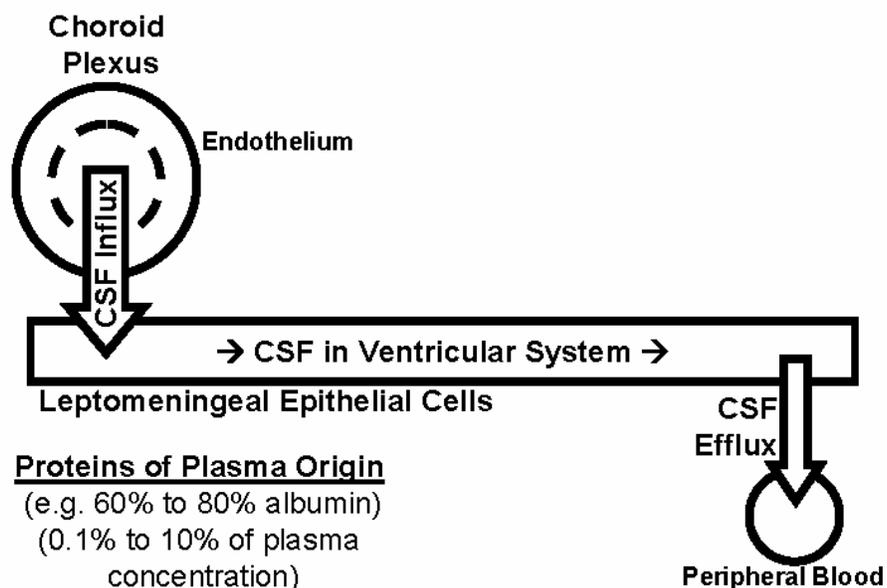
DNA → mRNA → Protein

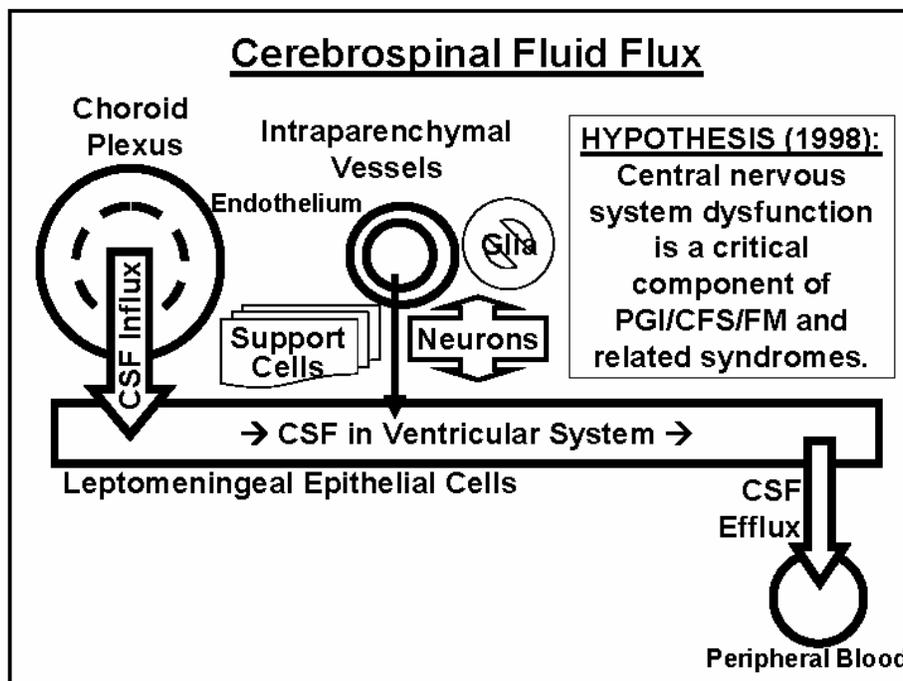
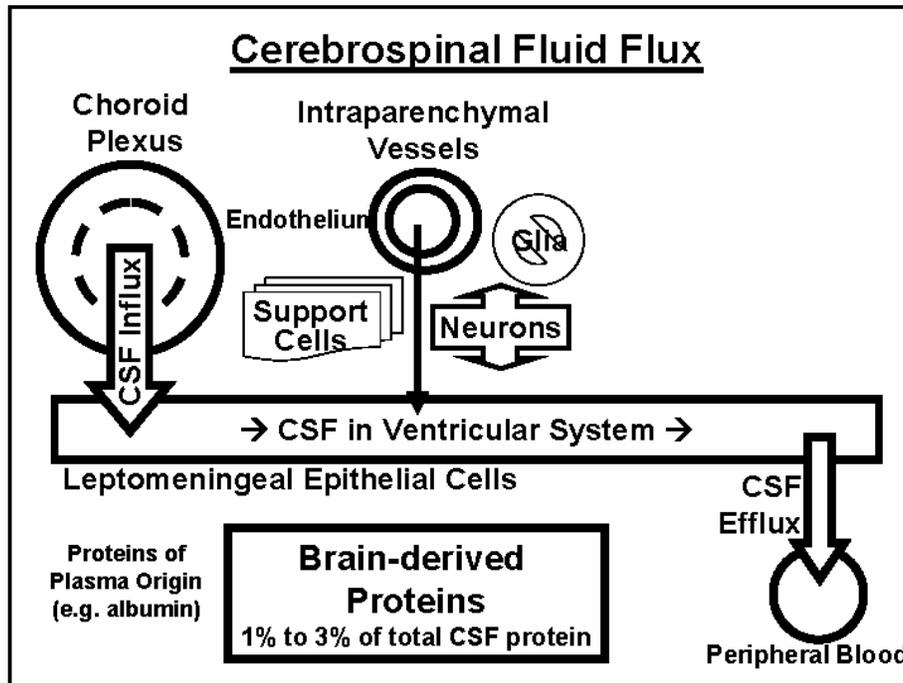
<u>Genomics</u>	<u>mRNA Microarray</u>	<u>Proteomics</u>
Examine genes in DNA Single point mutations (SNPs)	Examine mRNA expressed at one point in time	Examine the proteins in a cell, tissue, fluid sample
What you are born with	mRNA is made into proteins	Proteins determine what is happening now
Potential Risk Factors	Different expression between “Disease” and “Control”	Comparison of “Disease” and “Control”
Diathesis		Disease-related set of proteins or “Proteome”
Population Studies	Gene microarrays	

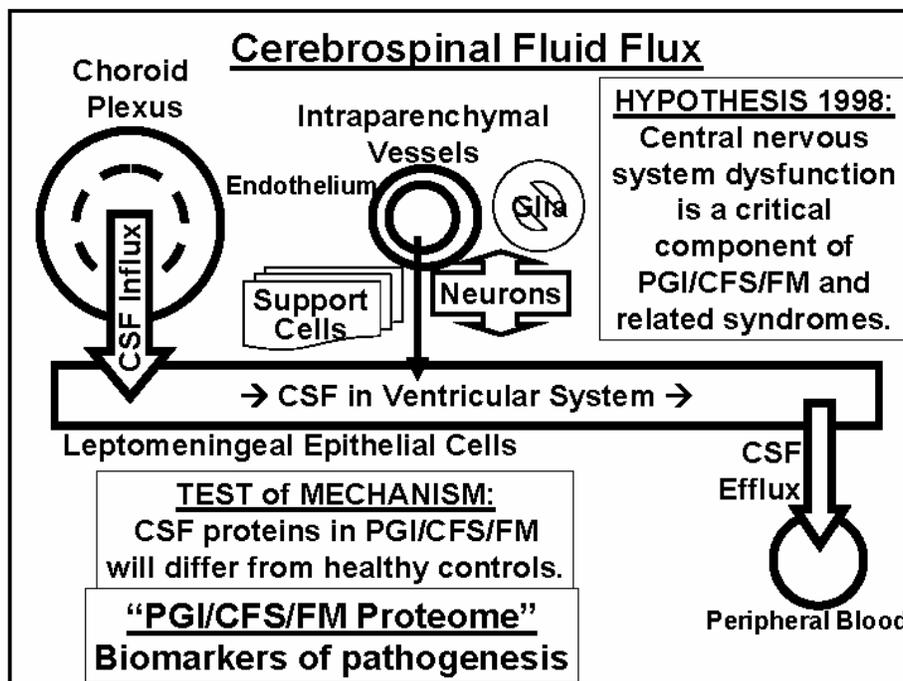
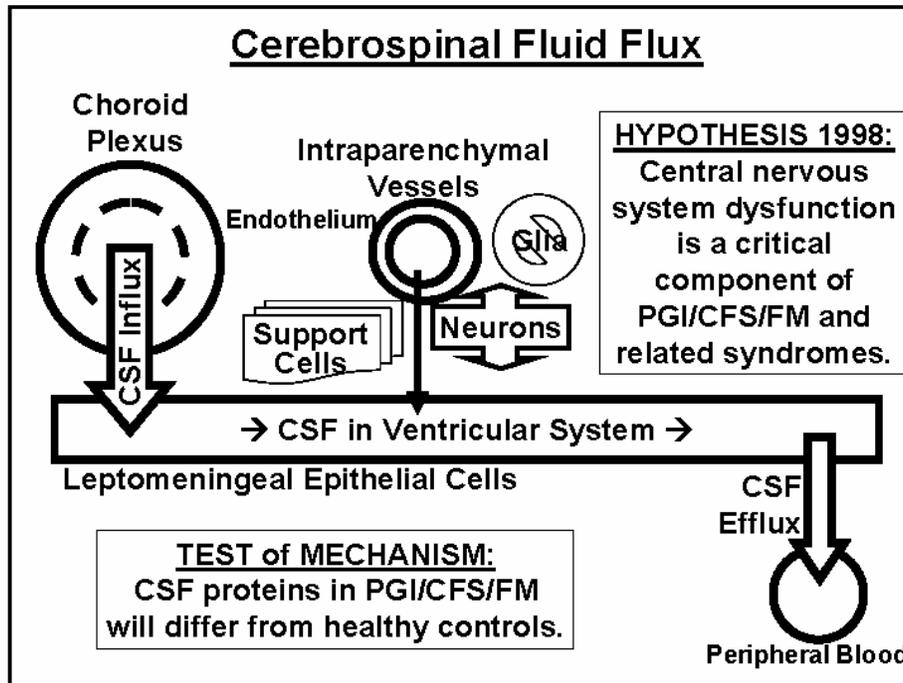
Outline

- Cerebrospinal fluid (CSF)
- Study design
- Patient groups for proteomic analysis
- Tandem Mass Spectrometry (MS-MS), Bioinformatics
- Statistical Analysis
- Implications
- Funding Sources
 - United States Department of Defense Award DAMD 170020018
 - Public Health Service Award RO1 AI42403
 - General Clinical Research Center Program 1 M01-RR13297-01A1
- Site:
 - Georgetown University G-CRC and Proteomics Laboratory

Where Does Cerebrospinal Fluid Come From?







Georgetown “CMI” Study; Dan Clauw, PI

- **Recruited Subject Groups:**
 - **Veterans** with Persian Gulf Illness (PGI, GWI, CMI)
 - **Fibromyalgia** (FM; positive controls, ACR Criteria)
 - **Healthy controls** (HC)

- **Multidimensional Evaluation:**
 - Psychiatric, psychometric
 - HPA axis, hyperalgesia, fMRI
 - Autonomic and exercise responses
 - Blood biomarker and lumbar puncture
 - Assess for PGI, CFS, FM, MCS, IBS, and other syndromes

Cerebrospinal Fluid

- One anesthetist for reproducible technique
- Lumbar punctures at same time of the morning
- Narrow gauge (22G) catheters
- Few, mild adverse events (headaches)

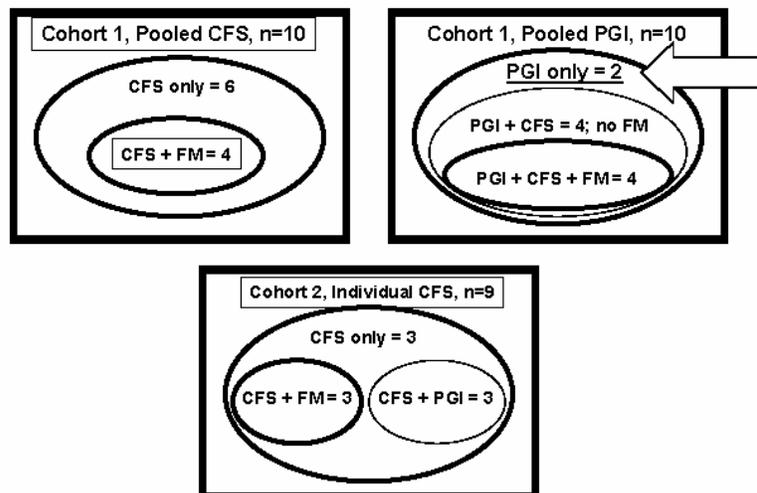
- Tubes 2, 3, 4
- Centrifuged to remove cells
- Aprotinin (antiprotease) added
- Frozen at -80°C

2 Distinct Proteomic Analysis Groups

- | | |
|---|--|
| <ul style="list-style-type: none">• <u>Cohort 1:</u>• <u>Pooled Samples</u>
• Healthy controls (HC)• PGI• CFS
• N = 10 CSF specimens per group• 3 samples | <ul style="list-style-type: none">• <u>Cohort 2:</u>• <u>Individual Samples</u>
• N= 12 HC• N = 9 "CFS"
• 21 separate proteomic analyses• Statistical comparisons |
|---|--|

Final overall analysis of all HC vs. all CFS/PGI/FM ("CFS")

Overlapping Syndromes: "Psycho – Semantics" of Case Definitions



Cohort Characteristics					
Group	N	Age (yr)	Male	CESD Affective Dysfunction	Pain Threshold (kg)
COHORT 1 (Pooled Samples)					
HC Pool	10	34.4 (29.1 to 39.7)	80%	4.3 (0.6 to 7.9)	7.69 (5.72 to 9.65)
CFS Pool	10	39.9 (34.3 to 45.5)	20% ^{***}	17.6 ^{***} (12.1 to 23.0)	4.01 ^{**} (2.86 to 5.16)
PGI Pool	10	43.5 (38.7 to 48.3)	60%	18.1 ^{**} (8.7 to 27.5)	4.89 [*] (3.64 to 6.14)
COHORT 2 (Individual Samples)					
HC	12	41.3 (33.6 to 48.9)	75%	-	7.17 (5.71 to 8.64)
CFS	9	39.1 (32.2 to 46.0)	33%	-	4.97 [§] (3.75 to 6.19)

^{*}p<0.05, ^{**} p<0.01, ^{***} p<0.001 compared to HC Pool results; [§] p<0.05 compared to HC individuals; ANOVA followed by Student's t-tests.

- ### Clinical Summary
- CFS / PGI / FM groups had extensive overlap, with only 2 “pure” PGI subjects.
 - CFS was the single most common “syndrome” in these subjects.
 - CFS / PGI / FM subjects had:
 - Worse QOL (SF-36), fatigue (MFI), and affective dysfunction (CESD)
 - Lower pain thresholds (systemic hyperalgesia)

Proteomics: Proteins → Peptides

- CSF proteins digested into peptides with trypsin
- Trypsin peptides separated by capillary liquid chromatography (CapLC)
- → Tandem mass spectrometry (MS-MS)
 - 1st MS: quadrupole MS to separate peptide ions
 - 2nd MS-MS: time-of-flight MS to sequence peptides

Peptide Sequences to Protein Functions

- 2nd MS-MS spectra → sequence each peptide
- Peptide sequences → MASCOT software
- MASCOT → protein identification for each sample

- Protein functions and interactions →
- Protein Information Resource (PIR)
- <http://pir.georgetown.edu>

Proteins from Pooled Samples
(Cohort 1)

Proteins that were detected in **BOTH** the
pooled PGI and pooled CFS specimens

AND

were **ABSENT** from the pooled healthy
control specimen

defined the

“Cohort 1 CFS-related Proteome”

Cohort 1 Pooled CFS” Proteome

Cohort 1
“Pooled CFS” Proteome

α 2-Macroglobulin
Ceruloplasmin / ferroxidase II
Orosomucoid 2
Autotaxin / phosphodiesterase 1 α
Amyloid precursor-like protein 1
BEHAB

Complement C4A, C4B
PEDF
Gelsolin
Carnosine dipeptidase 1 (CNDP1)

Proteins from Individual Samples
(Cohort 2)

- **Statistical analysis**
- Lists of proteins from each individual sample
 - **Multilogistic analysis and modeling (GLM)**
 - **Support Vector Machine Learning (SVM-PSO-LOO)**
- Identify the unique set of proteins found in CFS/PGI/FM but not healthy controls

Proteins from Individual Samples
(Cohort 2)

- **“Detectability”:**
 - All proteins detected and identified by 2nd MS-MS.
 - Peptides identified above the lower limits of detection
- **Frequency of detection:**
 - The frequencies or prevalences of each protein in the healthy control group (HC) and CFS/PGI/FM group.
 - Qualitative analysis (ANOVA).
- Proteins detected significantly more frequently in CFS/PGI/FM than HC group formed the:
“CFS/PGI/FM related proteome”.

Cohort 2 “CFS/PGI/FM” Proteome

**Cohort 2
 “CFS” Proteome**

Keratin 16
 α2-Macroglobulin
 Ceruloplasmin / ferroxidase II
 Orosomuroid 2
 Autotaxin / phosphodiesterase 1α
 Amyloid precursor-like protein 1
 BEHAB
 Keratin 6C
 Keratin 17
 Orosomuroid 1
 Keratin 10
 Complement C4B
 PEDF
 Gelsolin
 Carnosine dipeptidase 1 (CNDP1)
 Keratin 14

Comparison of Cohort 1 and 2 Proteomes

Cohort 1 “Pooled CFS” Proteome	Cohort 2 “CFS” Proteome
	Keratin 16
α2-Macroglobulin Ceruloplasmin / ferroxidase II Orosomuroid 2 Autotaxin / phosphodiesterase 1α Amyloid precursor-like protein 1 BEHAB	α2-Macroglobulin Ceruloplasmin / ferroxidase II Orosomuroid 2 Autotaxin / phosphodiesterase 1α Amyloid precursor-like protein 1 BEHAB
	Keratin 6C Keratin 17 Orosomuroid 1 Keratin 10
Complement C4A, C4B PEDF Gelsolin Carnosine dipeptidase 1 (CNDP1)	Complement C4B PEDF Gelsolin Carnosine dipeptidase 1 (CNDP1)
	Keratin 14

Odds of matching 10 proteins: 10^{-15}

Multilogistic Proteomic Biosignature (B1/5) Model

IF any 1 of these 5 proteins was detected:

Keratin 16
 α 2-Macroglobulin
Orosomuroid 2
Autotaxin / phosphodiesterase 1 α
Pigment Epithelium Derived Factor (PEDF)

THEN

CFS was present with
OR=34.5
(1.49 to 809.61; p=0.0072, Fisher's Exact test)

AND

CFS status = gender + (B1/5)
80% concordance

First objectively defined, predictive model
for these illnesses.

Pathophysiological Implications

Protease – Antiprotease
Imbalance

- α 2-Macroglobulin
- Orosomuroid 1 and 2

Structural Injury

- Gelsolin (apoptosis)
- Amyloid APLP1
- C4B (C3)

Oxidant Injury

- Ceruloplasmin
- Carnosine dipeptidase 1

Vascular Dysregulation

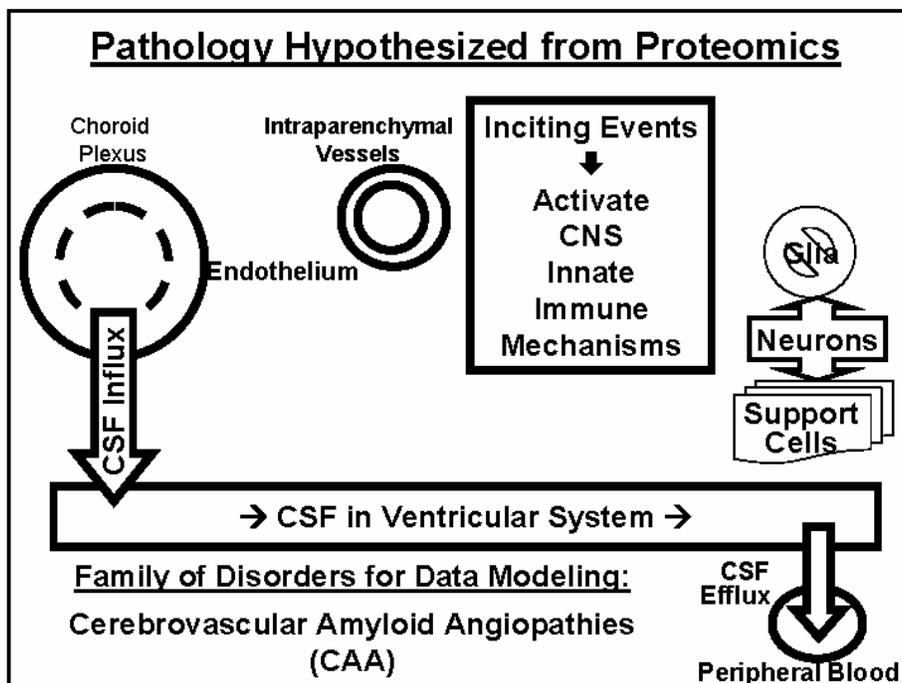
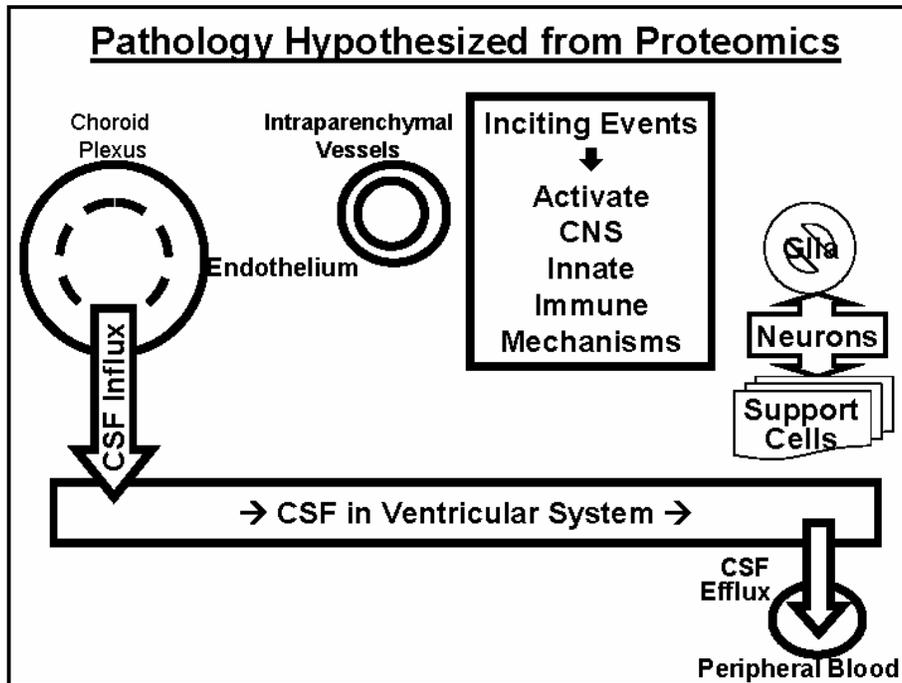
- Autotaxin
- Pigment Epithelium Derived Factor (EPDF)
- Vasoconstriction (ischemia)
- Endothelial proliferation (repair)

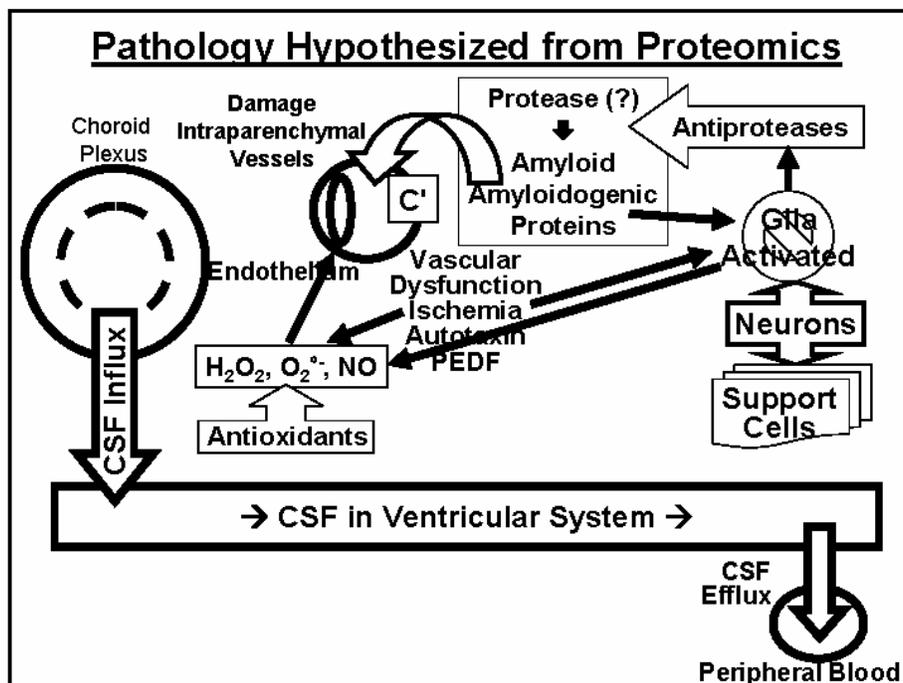
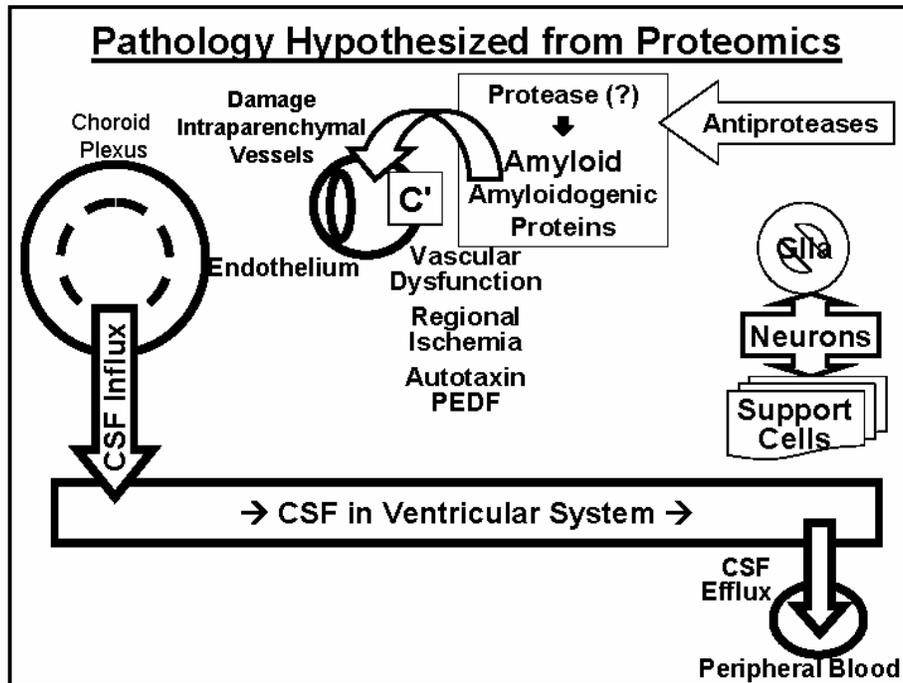
Leptomeningeal Activation

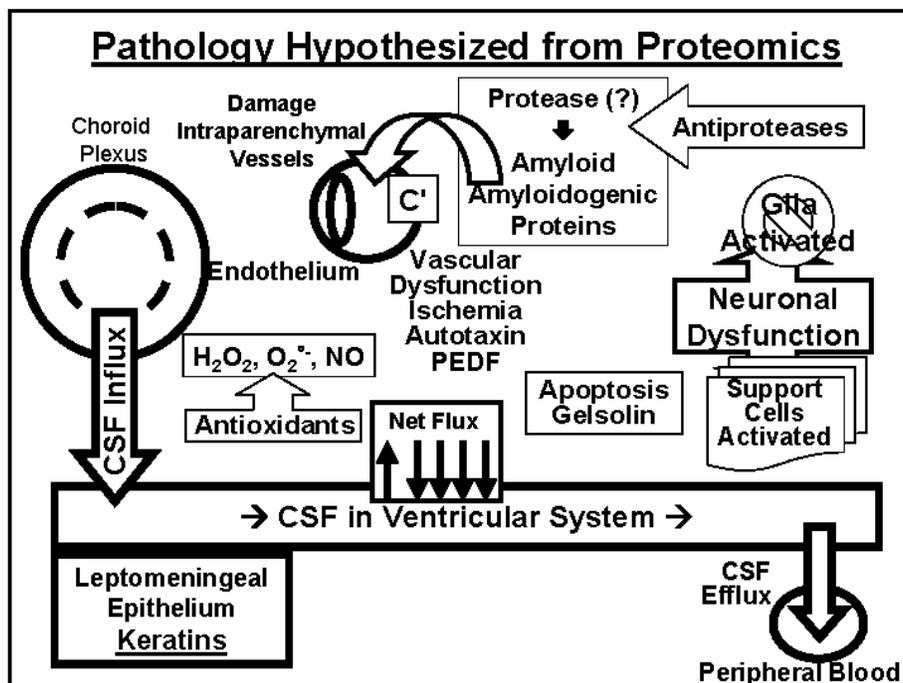
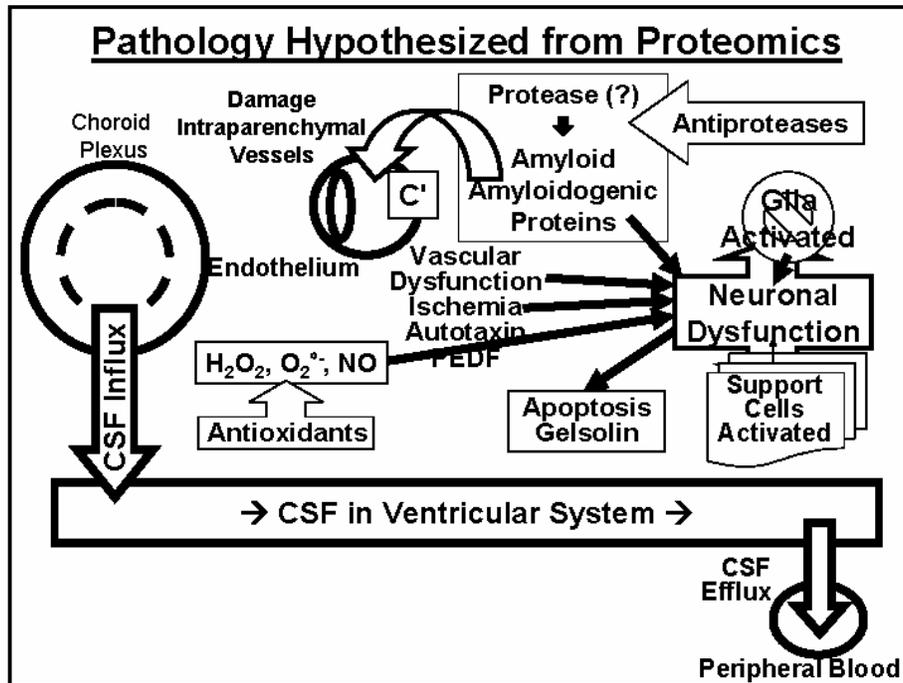
- Keratins 4, 10, 16, 17

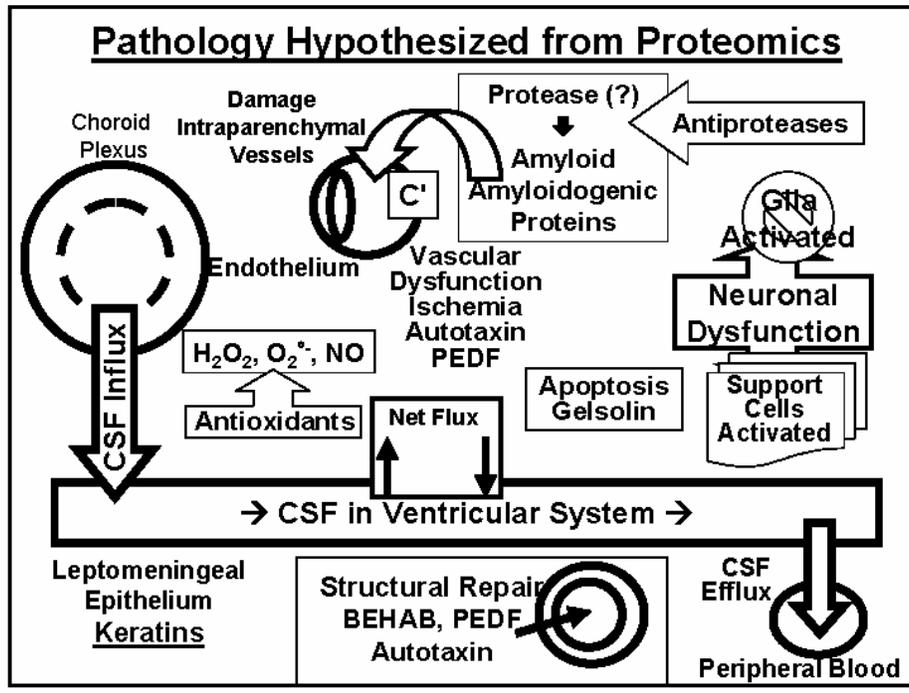
Structural Repair

- Brain-enhanced hyaluronan binding (BEHAB)









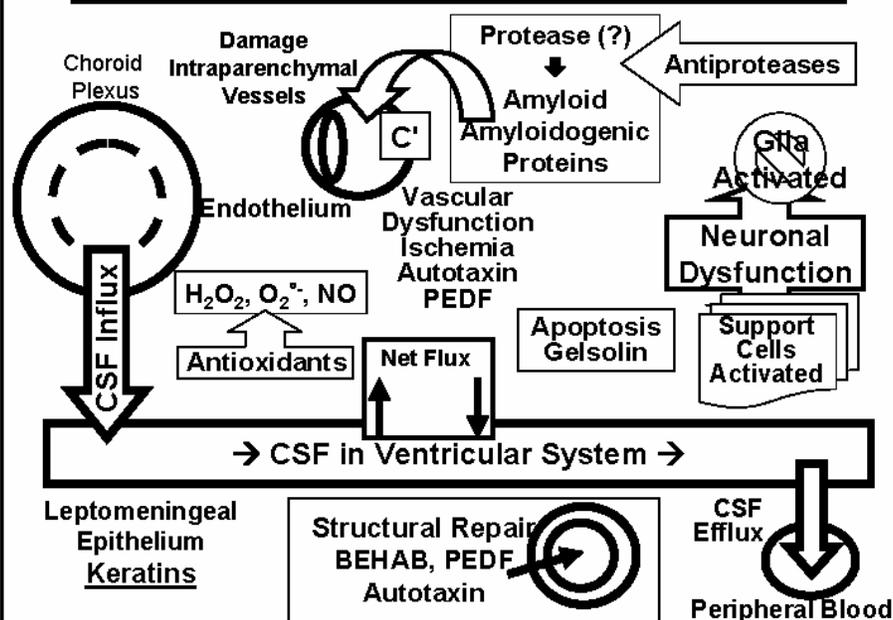
Conclusions: “Psycho-Semantics”

- Subjects met case designation criteria for several syndromes.
 - CFS / PGI / FM / IBS / MCS / hyperalgesia / dysautonomia . . .
- The high degree of overlap suggests that some pathophysiological mechanisms may be shared between syndromes.
- The patients are not “psycho” (the doctors are).

Conclusions: Proteomic Modeling

- Proteomic analysis of 2 different cohorts of CFS/PGI/FM subjects qualitatively identified a subset of cerebrospinal fluid proteins.
 - “CFS/PGI/FM Proteome”
- Multilogistic modeling identified a biosignature (**B1/5**) where the presence of 1 out of 5 proteins was sufficient to predict CFS status.
- This is the first objectively defined model predicting CFS/PGI/FM status.
 - OR=34.5; 80% concordance

Conclusions: Reversible, Non-Lethal CAA?



<u>DNA → mRNA → Protein</u>		
<u>Genomics</u>	<u>mRNA Microarray</u>	<u>Proteomics</u>
<ul style="list-style-type: none"> •Examine genes in DNA •Single point mutations (SNPs) •What you are born with •Potential •Risk Factors •Diathesis •<u>Population Studies</u> 	<ul style="list-style-type: none"> •Examine mRNA expressed at one point in time •mRNA is made into proteins •Different expression between "Disease" and "Control" •<u>Gene microarrays</u> 	<ul style="list-style-type: none"> •Examine the proteins in a cell, tissue, fluid sample •Proteins determine what is happening now •Comparison of "Disease" and "Control" •Disease-related •<u>"Proteome"</u>
<div style="border: 1px solid black; padding: 5px; width: fit-content; margin: 0 auto;"> <p><u>Snap shots of one point in time.</u> Poor agreement (17%) RNAi Post-translational modifications</p> </div>		

Presentation 10 – Beatrice Golomb

**Update on Research in
Persian Gulf War Veterans
Illnesses
May 2006**

Beatrice Alexandra Golomb, MD, PhD

Epidemiology

UK: “Operation Granby”: no special
syndrome

Kang Group: Chronic multisymptom illness
10 years later

UK: Mortality by deployment experiences

Khamisiyah Model and Outcomes

Op Granby Study

- Conclusion: "There is nothing ... in this analysis to support the media publicised suggestion of a specific Gulf War Syndrome or any unique Gulf service related illness" "We found no evidence of a unique "Gulf War Syndrome." "All illnesses are accounted for by well-established ICD-10 classifications. We have not found any medically unexplained conditions."
- Subjects: 3233 Gulf Veterans who attended Op Granby (UK) medical assessment program. Mean age 34 (58% under this age)
- Methods: "Over a period of 10 years, 3233 veterans have been assessed" with "in-depth interviews, full medical examination and appropriate investigation." "All diagnoses have been made according to ICD-10 classifications. All psychiatric diagnoses have been confirmed by consultant psychiatrists"

Bale 2005. J Royal Naval Medical Service 91: 99-111.

Op Granby Study

Outcome: The categories used were

1. Well completely (asymptomatic)
2. Well with symptoms but "no disease"
3. Well with incidental diagnoses:
 - Only psychiatric
 - Only organic
 - Both
4. Unwell:
 - Only psychiatric
 - Only organic
 - Both

Bale 2005. J Royal Naval Medical Service 91: 99-111.

Op Granby Study

Findings:

- “75% of veterans were well”
- Of the 25% unwell, 83% of ill health was “accounted for” by a psychiatric disorder.
- “The most commonly seen disorders amongst veterans here were of a psychiatric nature, confirming our previous findings. The most frequently seen condition was PTSD 12% with or without comorbidity and 93% were Gulf Service related”.
- “3% of veterans had an organic conditions that could be linked to Gulf deployment. The most common were respiratory, digestive, and skin disorders. Only 11 of these cases could be linked to the use of medical countermeasures.”

Bale 2005. J Royal Naval Medical Service 91: 99-111.

Op Granby Study

Alternative Analysis: The categories used were:

1. Well completely (asymptomatic): 10%
2. Well with symptoms but “no disease”: 27%
3. Well with incidental diagnoses: 39%
 - Only psychiatric: 7%
 - Only organic: 29%
 - Both: 3%
4. Unwell: 25%
 - Only psychiatric: 14%
 - Only organic: 4%
 - Both: 6%

Thus: 27% + 29% + 3% + 4% + 6% (69%) have symptoms or organic diagnoses; excluding concurrent psych: 60%

Bale 2005. J Royal Naval Medical Service 91: 99-111.

Op Granby Study

Limitations:

- No epidemiological assessment of relation of exposures to symptoms OR to conditions: but concludes that most conditions not be linked to exposures or countermeasures. Appears to assume its conclusion.
- Presumes attachment of an ICD-10 code implies an “explanation” – thus no “medically unexplained conditions”
- No control group against which to assess rates
- [codes include things like: shoulder pain; toxic myopathy]
- Cites in discussion negative findings (no overall increase mortality or cancer); fails to cite or dismisses findings suggestive of health problems (e.g. immune findings shown to differ in GWV)

Bale 2005. J Royal Naval Medical Service 91: 99-111.

Op Granby Study: Discussion

“Many veterans came here with known diagnoses but wanted reassurance that their problems were not related to service in the Gulf. Having been given such reassurances, these veterans went away satisfied as per data gathered from anonymised patient satisfaction questionnaires” [data not shown]

“Some studies have suggested neurological disorders and parasympathetic autonomic nervous system dysfunction as a result of Gulf service but such studies have been poorly controlled and numbers recruited extremely small” [They did not do parasympathetic measures; or have a control group, so no basis for comment]

“There have been media claims of disorders of the immune system.... Elsewhere no evidence of abnormal immunological responses in Gulf War veterans has been found (Everson 2002). Another study suggested there might be altered immune cellular activation in Gulf veterans. Their results were debatable given they were 9 years after service in the Gulf.” [Comment: there is a control group; and they have no basis to refute: they did not present immune measures or have a control group]

Bale 2005. J Royal Naval Medical Service 91: 99-111.

Chronic Multisymptom Illness Complex (CMI) in GWV 10 Years Later

Finding: Chronic medical illness (CMI) continues to be substantially more prevalent among deployed veterans than among nondeployed veterans 10 years after the War, but it manifests similarly in both groups

Blanchard MS 2006 Chronic Multisymptom Illness Complex in Gulf War I Veterans 10 years Later. Am J Epi 163: 66-75

CMI in GWV 10 Years Later

Subjects: National Health Survey of Gulf War Era Veterans and their Families in 1995-6. From Kang et al stratified random sample surveys sent to 15K deployed and 15K nondeployed veterans among cohort of 693K Gulf deployed and 800K nondeployed era personnel.

- 11441 deployed, 9476 era participated.

Outcome: CMI: Modified Fukuda CDC definition, >6mo of sx from 2/3 categories of fatigue, mood/cognition, "musculoskeletal pain". But used responses to spec sx questions to define this. E.g. unusual fatigue following exertion that lasts for at least 24 hours' or "feeling depressed"

-- To be "severe" at least 1 sx in each case defining cluster had to be severe.

Factors that analysis assessed in relation to CMI:

- QOL: SF-36
- Medical History: "Have you ever been told by a physician that you have"
- Medical Conditions: HTN (conservative, BP >160/100 or hxHTN+(Meds or BP>140/90), leukopenia <3.5Kcells/mm³, renal insuffic >1.5 creat, leukocytosis >11K, waist:hip ratio cutoff not given, Metabolic syndrome by NCEP: glc>110, waist >40men 35 women, TG>150, HDL<40, and HTN
- Psychiatric d/o by CIDI structured interview

Problem: picked med conditions to be those with prewar assoc to CMI (p >.25); expect these also related in any popn then.

Blanchard MS 2006 Chronic Multisymptom Illness Complex in Gulf War I Veterans 10 years Later. Am J Epi 163: 66-75

CMI in GWV 10 Years Later: More CMI; especially more severe CMI

CMI:	Deployed n=1035	Nondeployed n=1116	OR	95% CI
All cases	327 (28.9%)	165 (15.8%)	2.2	1.6-2.9
Mild-mod	257 (24.8%)	142 (14.7%)	1.9	1.4-2.6
Severe	70 (7.0)	23 (1.6)	4.7	2.3-9.5

Blanchard MS 2006 Chronic Multisymptom Illness Complex in Gulf War I Veterans 10 years Later. Am J Epi 163: 66-75

CMI 10 yrs later: CMI are sicker

	<u>Deployed</u>				<u>Nondeployed</u>			
	CMI <i>n=327</i>	Not <i>n=708</i>	OR	P-or-CI	CMI <i>n=165</i>	Not <i>n=951</i>	OR	P-or-CI
PCS	43	53		<0.001	44	52		<0.001
MCS	43	51		<0.001	46	54		<0.001
≥1 nonroutine clinic visit in last yr	71	52	2.3	1.4-3.5	74	54	2.4	1.5-3.9
≥1 hospitalization in last yr	5.1	3.3	1.6	0.63-4.0	9.6	4.5	2.3	1.02-5.1
Mean # prescripx meds	1.2	0.57		0.002	1.3	0.81		0.004
≥1 psychotropic meds	10	3.0	3.8	1.7-8.2	17	3.7	5.5	2.8-10.7

Blanchard MS 2006 Chronic Multisymptom Illness Complex in Gulf War I Veterans 10 years Later. Am J Epi 163: 66-75

CMI: Relation to Medical Conditions

CMI linked to:	Deployed			Nondeployed		
	%CMI vs %non	OR	CI	%CMI vs %non	OR	CI
- Fibromyalgia	5.2 > 0.7	7.4	2-27	3 > 0.8	3.9	1.2-13
- CFS	5.7 > 0	∞	n/a	0.6 > 0.0	∞	n/a
- Dyspepsia	16 > 6	3.2	1.6-6.1	12 > 4.8	2.6	1.3-5.4
- Arthralgias	9.8 > 3.5	3.0	1.4-6.7	13.4 > 5.4	2.7	1.4-5.2
- Metabolic synd	25 > 13	2.2	1.3-3.7	30 > 19	1.9	1.2-3.1
- HTN	14 > 9	1.6	0.9-2.9	20 > 14	1.4	0.8-2.5
- ObstruxLungDs	4.4 = 4.5	.99	.4-2.5	7.8 > 5.6	1.4	0.7-3.1
- DM	3.7 < 4.5	0.8	0.3-2.3	5.2 > 3.0	1.8	0.6-5.0
- Waist:hip, men	.93 > .91	p = 0.004		0.95 > 0.92	p = 0.001	

BUT conditions examined = those w/prewar reln to CMI w/ p < 0.25 (& prevalence > .2)
 Blanchard MS 2006 Chronic Multisymptom Illness Complex in Gulf War I Veterans 10 years Later. Am J Epi 163: 66-75

Chronic Multisymptom illness Complex in Gulf War I Veterans 10 Years Later

CMI link to:	Deployed			Nondeployed		
	%CMI > non	OR	CI	%CMI > non	OR	CI
PTSD	6.1 > 2.0	3.2	1.2-8.7	7.5 > 1.1	7.1	2.3-22
Major depression	16 > 4.2	4.4	2.3-8.8	14 > 3.1	5.3	2.6-11
Anxiety disorder	22 > 6.1	4.3	2.4-7.8	22 > 5.5	4.8	2.7-8.7
1 or more mental d/o	39 > 12.7	4.4	2.7-6.9	38 > 11	5.1	3.1-8.3
Alcohol dependence	3.3 > 0.7	4.8	1.1-21	0.5 > 0.4	1.3	.22-7.4
Nicotine dependence	19 > 8.1	2.6	1.4-4.8	16 > 5.6	3.3	1.7-6.3

Blanchard MS 2006 Chronic Multisymptom Illness Complex in Gulf War I Veterans 10 years Later. Am J Epi 163: 66-75

Chronic Multisymptom illness Complex in Gulf War I Veterans 10 Years Later

Conclusion:

- CMI occurs in deployed and nondeployed
- It is more common in deployed, especially severe form
- “Similar relations to physical and mental illness in both groups”

However: differences as well as similarities may be worthy of note:

- GW -> More symptom based diagnoses focused on fatigue and muscle
- Nondeployed -> More Diagnoses “expected” to produced sx

Limitation: Included only conditions assoc with CMI prewar. Since GW should not protect against CMI induced by these, expect also related postwar. Didn't look for conditions only related postwar; further limits conclusion of similar relations to phys illness in these groups.

Blanchard MS 2006 Chronic Multisymptom Illness Complex in Gulf War I Veterans 10 years Later. Am J Epi 163: 66-75

Mortality as a Function of Deployment experiences in UK GWV

Finding:

Overall no difference in death rate in UK GWV vs Era controls*.

Within GWV: Association of exposure to mortality strongest for handling DU, anthrax, and handling pesticide (& scud missile passed nearby). Pattern of deaths differs:

Pesticides, anthrax, scud -> nondisease deaths;

DU, anthrax (and pertussis) -> disease deaths

PB, CW not included

*Recall sick people not selected for deployment; “healthy warrior”

Macfarlane, Hotopf et al 2005. Long term mortality among Gulf War veterans: is there a relationship with experiences during deployment and subsequent mortality? Int J Epidem advance copy pub Oct 26, 2005

Mortality vs Exposures: UK GWV

Subjects: 51,753 UK GWV; 50,808 Era. GWV in-Gulf btn 9-90 and 6-91 vs random sample of nondeployed era personnel "group-matched" for age-band, sex, service branch, rank, fitness for active service(for army and RAF). Recall prior Unwin study. #s given are after losses/exclusions: initially 53,462 each group.

Exposures examined:

- Vaccinations (pertussis; plague; anthrax; "all" (all 3 of these))
- Handled pesticides. Living quarters sprayed with pesticides. Personal pesticide or insecticide use.
- DU
- Smoke from oil fires
- Scud missile passed nearby
- Came under small arms fire
- (Not PB; CW)

Outcomes: death; disease death; nondisease death

Analysis: Mortality Risk Ratio by Cox proportional hazards

Adjustment: age-group, gender, service (regular/reservist), branch (army, Navy, RAF), smoking, alcohol consumption

Macfarlane, Hotopf et al 2005. Int J Epidemiol advance copy Oct 26, 2005

Mortality vs Exposure: UK GWV

Exposure	Disease Deaths		NonDisease Deaths	
	Adj MRR	95% CI	Adj MRR	95% CI
DU	1.99	0.98-4.0	0.78	0.2-2.5
Anthrax vaccine	1.34	0.8-2.2	1.16	0.6-2.2
Pertussis	1.15	0.7-1.8	0.85	0.4-1.6
Scud passed nearby	0.98	0.6-1.6	1.38	0.7-2.6
Smoke from oil fires	0.96	0.6-1.5	0.65	0.4-1.2
Personal pesticides	0.87	0.5-1.4	0.95	0.5-1.7
Handled pesticides	0.85	0.3-2.1	2.05	0.9-4.6
Pest sprayed quarters	0.78	0.4-1.4	1.31	0.7-2.5
Plague	0.82	0.5-1.3	1.01	0.6-1.8
Came under small arms fire		0.89	0.4-1.9	0.46 0.2-1.3
PB, CW	Not assessed			

Adjustment: age-group, gender, service (regular/reservist), branch (army, Navy, RAF), smoking, alcohol consumption. Self reported exposure to DU: ? How asked
 Macfarlane, Hotopf et al 2005. Long term mortality among Gulf War veterans: is there a relationship with experiences during deployment and subsequent mortality? Int J Epidemiol
 advance copy pub Oct 26, 2005

Mortality vs Exposures: UK GWV			
	% Exposed	Adj MRR	95% CI
DU	7	1.48	0.8-2.6
Anthrax vaccine	72	1.21	0.8-1.8
Handled pesticides	7	1.19	0.7-2.2
Scud passed nearby	24	1.15	0.8-1.7
Pertussis	33	1.05	0.7-1.5
Pest sprayd Living quarters		22	1.030.7-1.6
Personal pesticides	53	0.91	0.6-1.3
Plague	45	0.90	0.6-1.3
All vaccines	25	0.88	0.6-1.3
Smoke from oil fires	65	0.83	0.6-1.2
Came under small arms fire		18	0.620.3-1.2
PB, CW	Not assessed		
Attributable risk 3% DU (.07*.48), 15% AxVax (.72*.21), if MRR upheld			
<small>Macfarlane, Hotopf et al 2005. J Epidem advance copy Oct 26, 2005</small>			

Do Symptoms Relate to Death?		
Symptom Tertiles	Adj MRR	95% CI
All Deaths		
Lowest	1	
Middle	0.51	0.2-1.4
Highest	1.17	0.6-2.4
Disease Deaths (will require ongoing eval for 40% incr to be signif – if sustained)		
Lowest	1	
Middle	0.3	0.06-1.4
Highest	1.4	0.6-3.4
NonDisease Deaths		
Lowest	1	
Middle	0.93	0.2-3.7
Highest	0.88	0.3-3.1
Comment: Symptom Nonreporters Do Worse than Those Citing Middle Symptoms. Very ill may not put self at risk (My GWV is getting pilot lessons; healthy people die of accidents)		
<small>Macfarlane, Hotopf et al 2005. Long term mortality among Gulf War veterans: is there a relationship with experiences during deployment and subsequent mortality? Int J Epidem advance copy pub Oct 26, 2005</small>		

Mortality Relations

Conclusions:

Some exposures may be linked to increased mortality

- **Need to Look at PB (and CW?)**
- **Adjust for other exposures?**

High sx reporting may be linked to mortality; need larger N

Low sx reporting may be linked to mortality (reporting vs hyporesponsive)

Macfarlane, Hotopf et al 2005. Long term mortality among Gulf War veterans: is there a relationship with experiences during deployment and subsequent mortality? Int J Epidem advance copy pub Oct 26, 2005

“Khamisiyah” Outcomes

2 studies:

- I. **Little association between potential exposure (2000 plume model) to sarin or cyclosarin and self-perceived health status after adjusting for covariates.**
- II. **Comparing notified to nonnotified subjects, there were no significant differences in bed days, activity limitations, clinic visits, or hospital visits. 5 among 71 self-reported medical conditions and symptoms were significantly different – 4 of which were lower in notified subjects.**

Mahan, Page, Bullman, Kang 2005. Part I. Morbidity Associated with Potential Exposure. Military Medicine 170: 935-944.

Page, Mahan, Kang, Bullman 2005. Part II. Morbidity Associated with Notification of Potential Exposure. Military Medicine 170:945-951

“Khamisiyah” Outcomes

Subjects: From Kang stratified sample of “15,000” each Gulf and NonGulf, use subsample of 1200 respondents to the National Health Survey intended to have equal numbers exposed and nonexposed Army veterans; and notified and nonnotified Army subjects. “but inadvertent inclusion of nonarmy subjects meant final sample only 1056, of whom 72% (756) responded. 73% of notified subjects responded; 70% of nonnotified.

Exposure: 1997 or 50Km Khamisiyah notification; & “exposure” by 2000 plume model

Outcomes: # bed days; activity limitations “attributable to health”; #doctor visits in last 12 mo; #hospitalizations in past 12mo; overall health status (e.g. good or fair); selected medical conditions; selected symptoms; birth defects yes/no; life events scale; PTSD checklist.

Analysis: Chi2 to compare prevalence for notified/non. Cochran-Mantel-Haenszel test to compute adjusted RRs notified vs non.

Adjustment: exposure or notification status, initial health survey response, age in 1991 (binarized <30 vs >30), gender, race (white or hispanic vs all other; or for exposure, white, afr am, or all other incl hispanic), rank (enlisted vs officer or warrant officer), Army active duty vs Army Reserve or National Guard -- All but 1st two used to compute a propensity score which, divided into quintiles, was used to adjust for these factors in the Cochran-Mantel-Haenszel analysis.

-- No interaction on outcomes noted btn exposure and notification status; so looked at notification separately, adjusted for exposure.

Mahan, Page, Bullman, Kang 2005. Part I. Morbidity Associated with Potential Exposure. Military Medicine 170: 935-944.
Page, Mahan, Kang, Bullman 2005. Part II. Morbidity Associated with Notification of Potential Exposure. Military Medicine 170:945-951

“Khamisiyah” Outcomes

Conclusion:

“There were few adverse health effects associated with notification regarding potential exposure to nerve agents, a finding that contradicts the prevailing view” (not mine!!)

“Those who may be planning future notification efforts may nonetheless take some comfort in the fact that there were few adverse effects seen in this study”

Comment:

Suggests against major impact of suggestibility.

“Khamisiyah” Outcomes

Limitations:

Misclassification potentially severe: bias to the null.

- a. **Modeling excessively broad and founded on numerous posthoc assumptions; finding of no association, contrast with findings for sarin exposure in Aum Shinrikyo**
- b. **Many in model likely had little or no exposure. Others outside of plume may have had exposure in other CW settings. Contrast prior findings with CW in those at Khamisiyah, vs larger “Khamisiyah” group.**

Also: No adjustment for other variables like PB that may account for much of illness, and eliminate excess variability

Mahan, Page, Bullman, Kang 2005. Part I. Morbidity Associated with Potential Exposure. Military Medicine 170: 935-944.

Page, Mahan, Kang, Bullman 2005. Part II. Morbidity Associated with Notification of Potential Exposure. Military Medicine 170:945-951

French Gulf War Veterans

Finding: French PGWV do report symptoms at high rates. No controls

Background:

- **1995: 1st compensation demand for GW problem filed in France.**
- **June 2000; French Assn of Gulf War Victims (Avigolfe) founded.**
- **Oct 2000: Working Group in charge of analysis of health data from French GWV created by French govt; rec'd epi study of “all French PGWV in order to describe their complaints & objectively measure their disorders through a standardized clinical examination”**
[Comment: there is no standardized clinical examination that can objectively measure all disorders]
- **Jan 2002: French Ministry of Defense in collab with Ministry of Health solicited the “INSERM” to conduct an “exhaustive investigation of all French PGWV”, to “examine self-reported symptom data among GWV; describe main forms of exposure reported in theater; symptoms and diseases that appeared during and after; and determine if unexpected statistical associations of such symptoms could suggest the presence of a new specific syndrome”**

Salamon et al 2006. Health consequences of the first Persian Gulf War on French troops. Int J Epi, advanced access published 1-24-06

French Gulf War Veterans

Subjects: all civilian and military personnel who served in the Gulf from 8-90 to 7-91. Based on: census w/in dif milit units btn 00 and 01 by army staff headquarters; list of all decorated GW subjects by Hx Dept of the Army (list created 1991); census of participating organizations for civilians. "Finally, several subjects willing to participate in the study spontaneously contacted us and were added to the list, once we verified their participation in the Gulf War" (suggests lists were not exhaustive)

Design: Survey beginning Feb 02; data collex completed 6-04

Survey: 12-page, self-administered.

Exposures: sandstorms; oil fire smoke; CW or BW alerts; vaccinations; meds.

Covariates: Sociodemog; milit hx; living conditions; dates departure/return; places of operation

Health: diseases and sx appearing B4, during, after the war:

Sx: List of 49 symptoms appearing after the mission rated by Hopkins Symptom Checklist. Frequency of sx coded never, <1ce/mo, 1-3x/mo, <1ce a day, daily; except 6 coded yes/no.

Diseases: ICD-10-CM used to code self-reported disease

Perceived stress: 4 items of Cohen and Williamson Scale

Children's health: miscarriages, @children, diagnoses before & after mission

Salamon et al 2006. Health consequences of the first Persian Gulf War on French troops. Int J Epi, advanced access published 1-24-06

French Gulf War Veterans

Subjects: 5666 French troops deployed by MOD data.

20261 Fr troops deployed by their MOD. Address available for 52% (10,478). Of these 5666 (54%) participated: 2695 Army; 770 Navy; 1895 Air Force; 306 Other branches. Participation rate 28% (5666/20261) and varied by branch: 21% Army to 41% Navy.

Difficult to contact those who retired from army btn 1991 and 2002.

Refusal rate "low" (5% of people contacted by 6-04), mainly due to only briefly present or perceived self in good health.
(HOWEVER: loss to f/u may follow opposite pattern)

Mean age 41 at time of participation. Mean duration deployment 118 days, mainly Saudi Arabia 76%; and Iraq 33%; Kuwait 12%.

Primary exposures: Sandstorms 74%; and CW or BW alerts 63%.

Salamon et al 2006. Health consequences of the first Persian Gulf War on French troops. Int J Epi, advanced access published 1-24-06

French GWV: Factor Analysis

Analysis:

Pearson’s correlation matrix among the 43 sx coded in 5 levels (excluded Ss with missing values for any sx; and those with no sx)

Principal components analysis: applied to correlation matrix

Retained factors: by Kaiser criterion, Eigenvalues >1; or if factor had at least 2 symptom frequencies that loaded >0.3 on this factor.

Factor loading can be interpreted as correlation coeff measuring the association btn symptom frequencies and the factor

Salamon et al 2006. Health consequences of htefirst Persian Gulf War on French troops. Int J Epi, advanced access published 1-24-06

French GWV: “Bottom Line”

Reported symptoms (total group): though 86% considered selves in good health

Symptom	%	Symptom	%
Headaches	83	Probs with mouth, gums, teeth	29
Sleep difficulty	71	Shortness of breath	27
Irritability	69	Rapid heart rate	25
Backache	63	Depression	24
Memory prob	56	Difficult with speech	23
Fatigue	55	Unintended wt gain >10lb	22
Word finding	53	Loss strength	21
Numbness/tingling	45	Nausea/vomiting	21
Gastralgia	43	Panic/anxiety	19
Joint pain	41	Swollen glands	18
Diarrhea	37	Confusion	18
Muscle pain	34	Shaking	18
Sweats	32	Chem sensitivity	14
Chest pain	30	Slowness healing	8
Rashes	29	Teeth loss	7

Symptom rates: Max in Army; Least in Navy

Salamon et al 2006. Health consequences of the first Persian Gulf War on French troops. Int J Epi, advanced access published 1-24-06

French GWV: Factor Analysis

Factor analysis results (for what it's worth)

7 factors. 1. General; 2. Neurocog/opp resp; 3. Musculoskel vs mood

Factor 1: General, almost all sx load

Factor 2:

- a. Neurocognitive (memory, word finding, confusion, difficulty w speech) orthogonal to:***
- b. Respiratory (wheezing, persistent cough, sore throat)***

Factor 3:

- a. Mood (Depression, panic, anxiety): orthogonal to***
- b. Musculoskeletal (muscle pain, joint pain, joint stiffness, joint swelling)***

Other:

- a. Auditory and tinnitus (load in 5 of the factors)***
- b. Rapid HR and shortness of breath***

Salamon et al 2006. Health consequences of the first Persian Gulf War on French troops. Int J Epi, advanced access published 1-24-06

French GWV

Real Results: High rates sx reporting, no controls

- Higher rates (than other GW studies) of headaches, back pain, sleeping disorders
- Similar rates memory, irritability, fatigue

Limitations:

- Selection/ self-selection: 71% of respondents still in the service (healthy); vs ill may choose to participate; vs ill may not have the energy to fill out a 12 page survey and get it in
- Self-report: recall / reporting bias
- Lack of control group: no risk ratios
- No exposure/outcome associations provided

Salamon et al 2006. Health consequences of the first Persian Gulf War on French troops. Int J Epi, advanced access published 1-24-06

French Gulf War Veterans

Discussion:

“Even if French troops did not face the same exposures as US or UK troops, a “new” symptom cluster, involving disparate organ symptoms, was not highlighted by our analysis” in which “the associations of symptoms were quite similar to those derived among other PGWV and appropriate non-deployed control veterans”

“Until 2000, France did not receive any specific complaints and no study on French PGWV was undertaken” [reminder: absence of evidence is not evidence of absence]

“Fourteen years after the end of the Gulf War it is very difficult to describe deployment circumstances, living conditions, and exposures and to link them to illness”

“Further studies, based on data collected, are in progress and results will be reported in the near future”

Salamon et al 2006. Health consequences of the first Persian Gulf War on French troops. Int J Epi, advanced access published 1-24-06

Incarceration in GWV

Subjects: 3695 Iowa PGWV and nondeployed. 4886 randomly drawn from 1 of 4 groups: GW regular military; GW National Guard / Reserve; nonGW regular Military; nonGW National Guard/ Reserve

Design: Structured Phone Interview of Iowa personnel: PGW and nondeployed

“Outcomes: Sx of medical conditions; psychiatric disorders; health care utilization.”

Outcome Here: Incarceration

Result: “GW deployment carried no increased risk of subsequent incarceration overall”: rate “lower than nondeployed” (after GWV; higher before GWV) (If were true: like HRT, can’t say what true effect of deployment is)

Ever incarcerated: 25.9% GWV; 22.9% nonGWV. **BUT:** GWV higher pre GW; non higher post-GW; incarceration linked to age at 1990; and no comparison of age in GWV vs nonGWV groups.

Ever incarcerated had higher freq psychiatric and medical comorbidity; and health care utilization.

Associated with male gender; lower education; enlisted rank; lower level milit preparedness; discharge from service; smoking; antisocial traits; having used illegal drugs.

Participation in combat: OR 1.6, (1.0-2.5).

Black 2005. Incarceration and veterans of the first Gulf War. Military Medicine 170: 612-8

“Systematic Review”

Goal: Summarize findings from studies that have assessed multisymptom conditions in GWV and an unexposed comparison group

Method: Studies from Jan 1990-May 2004 by search electronic databases.

Included if compared prevalence of chronic fatigue; MCS; CDC GWI; FM; or sx of either fatigue or numbness/tingling in GWV and non GWV. 23 pubs.

Thomas et al 2006. Psychological Medicine X: 1-13.

“Systematic Review”

Condition	#studies	OR	CI
CFS	10	3.8	2.2-6.7
Fatigue symptoms	16	3.7	2.9-4.8
MCS	7	3.6	2.0-6.2
GWI/CMI (CDC)	5*	3.6	2.8-4.8
Numbness/tingling	6	2.4	1.8-3.1
FM	2	1.8	1.6-2.1

*Duplicated unwin sample

Comment: “The methodological quality of the studies varied but the later and larger studies were of a high methodological standard with robust sampling strategies, adequate response rates and good adjustment for confounders:

Conclusion: “The results support the hypothesis that deployment to the GW is associated with greater reporting of multi-symptom conditions”

Thomas et al 2006. Psychological Medicine X: 1-13.

“Systematic Review”

Goal: Summarize findings from studies that have assessed multisymptom conditions in GWV and an unexposed comparison group

Method: Studies from jan 1990-May 2004 by search electronic databases.

Included if compared prevalence of chronic fatigue; MCS; CDC GWI; FM; or sx of either fatigue or numbness/tingling in GWV and non GWV. 23 pubs.

Thomas et al 2006. Psychological Medicine X: 1-13.

“Systematic Review”

Condition	#studies	OR	CI
CFS	10	3.8	2.2-6.7
Fatigue symptoms	16	3.7	2.9-4.8
MCS	7	3.6	2.0-6.2
GWI/CMI (CDC)	5*	3.6	2.8-4.8
Numbness/tingling	6	2.4	1.8-3.1
FM	2	1.8	1.6-2.1

*Duplicated unwin sample

Comment: “The methodological quality of the studies varied but the later and larger studies were of a high methodological standard with robust sampling strategies, adequate response rates and good adjustment for confounders:

Conclusion: “The results support the hypothesis that deployment to the GW is associated with greater reporting of multi-symptom conditions”

Thomas et al 2006. Psychological Medicine X: 1-13.

“Systematic Review”

Goal: Summarize findings from studies that have assessed multisymptom conditions in GWV and an unexposed comparison group

Method: Studies from jan 1990-May 2004 by search electronic databases.

Included if compared prevalence of chronic fatigue; MCS; CDC GWI; FM; or sx of either fatigue or numbness/tingling in GWV and non GWV. 23 pubs.

Thomas et al 2006. Psychological Medicine X: 1-13.

“Systematic Review”

Condition	#studies	OR	CI
CFS	10	3.8	2.2-6.7
Fatigue symptoms	16	3.7	2.9-4.8
MCS	7	3.6	2.0-6.2
GWI/CMI (CDC)	5*	3.6	2.8-4.8
Numbness/tingling	6	2.4	1.8-3.1
FM	2	1.8	1.6-2.1

*Duplicated unwin sample

Comment: “The methodological quality of the studies varied but the later and larger studies were of a high methodological standard with robust sampling strategies, adequate response rates and good adjustment for confounders:

Conclusion: “The results support the hypothesis that deployment to the GW is associated with greater reporting of multi-symptom conditions”

Thomas et al 2006. Psychological Medicine X: 1-13.

Military Deployment & Psychiatric Illness

Finding: Gulf war veterans have a significantly higher prevalence of psychiatric diagnoses with twice the prevalence of anxiety disorders and depression; no signif increase in somatization.

Independent predictors include lower rank, female, unmarried (single or divorced)

Fiedler 2006. Military deployment to the Gulf War as a risk factor for psychiatric illness among US troops. Brit J Psychiatry 198:453-459

Military Deployment & Psychiatric Illness

Subjects: 59% of random sample of 1765 GWV; 51% of random sample of 1832 era veterans.

Design: Phone interview. Administer CIDI interview.

Comment: Positive predictive value of CIDI vs Structured Clinical Interview: ranges .21 for GAD; to .95 for social phobia.

10% of calls monitored, “100% concordance” but not stated to be independent.

Not stated if interviewers blinded to GW status.

Outcomes: Psychiatric conditions by CIDI

Primary Exposure: Gulf War vs Era sample

Fiedler 2006. Military deployment to the Gulf War as a risk factor for psychiatric illness among US troops. Brit J Psychiatry 198:453-459

Military Deployment & Psychiatric Illness

Analysis:

1st pass compare studied groups to total sample; & GWV to era (NDV) (chi-2)

1st pass compare 12-mo prevalence of psych conditions by gender and GW status

Regress on conditions that look different: stepwise forward regression model with inclusion/exclusion of $p < .15$ and $p > 0.2$

Outcomes not regressed (not diff): somatization (fainting, abd pain, menstr problems with other medical explanations “ruled out”!)

Outcomes regressed:

1. Any anxiety d/o including PTSD, GAD, agoraphobia, OCD, panic attack, social phobia
2. Major depression
3. Drug or alcohol dependence

Adjustment variables: race, education, age, marital, active duty status, enlisted rank, other deployments

Fiedler 2006. Military deployment to the Gulf War as a risk factor for psychiatric illness among US troops. Brit J Psychiatry 198:453-459

Military Deployment & Psychiatric Illness

Results:

Comparability:

“comparable age” but GWV “somewhat younger” (age 39 vs 42). Less educated.

Less white. Less married: More enlisted. More active duty.

More never married, 39% vs 29%

Diagnoses:

“Little difference” in rates of:

- Alcohol dependence
- Specific phobia
- Mania
- Somatization

2-3 x increase in rates of:

- Anxiety disorders (the rest)
- Depression
- Drug dependence

Fiedler 2006. Military deployment to the Gulf War as a risk factor for psychiatric illness among US troops. Brit J Psychiatry 198:453-459

Military Deployment & Psychiatric Illness			
Disorder	GWV	Era	NCS (National Comorbidity Survey)*
Alcohol	4.6	3.1	10.7
Drug	1.2	0.1	3.8
Major Depr.	15.1	7.8	7.7
Any Anxiety d/o	16.0	9.7	(not given)
GAD	6.0	2.7	2.0
Panic attack	1.6	0.5	1.3
OCD	2.8	1.1	(not given)
PTSD	3.4	0.9	5.0
Any psychiatric d/o	26.1	16.1	(not given)
	GW		
FINAL MODEL	OR	95%CI	Other retained variables
Anxiety	1.8	1.3-2.5	enlisted rank, army, female
Depression	2.1	1.5-2.9	enlisted rank, female, low education
Dependence	Dropped vbl		Other deployment, enlisted rank, male, nonmarried

* NCS: Assessed 12 mo prevalence in adult males
 Fiedler 2006. Military deployment to the Gulf War as a risk factor for psychiatric illness among US troops. Brit J Psychiatry 198:453-459

Military Deployment & Psychiatric Illness
Conclusions:
<ul style="list-style-type: none"> • Depression & anxiety increased • Low rates of PTSD and of drug/ alcohol abuse • No material increase in somatization, alcohol abuse
Limitations:
<p>Small samples; low “response rate” (higher “cooperation rate” if reached, 87% and 77%; but more if white, noncommissioned and warrant, commissioned officers; signif dif age, gender, branch by response rate)</p> <p>CIDI a limitation -- if you believe DSM better</p>
Observation: Rates elevated in conditions (depression and anxiety – conditions with very high cross-correlation generally > 0.5) that have somatic symptoms (e.g. fatigue, sleep, concentration) in diagnosis: interesting to look separately at psychic vs somatic symptoms
Fiedler 2006. Military deployment to the Gulf War as a risk factor for psychiatric illness among US troops. Brit J Psychiatry 198:453-459

PB & sarin: effects on Ach levels in brain

Conclusion: no long term effect on choline and acetylcholine when average across brain regions

Subjects: male Sprague Dawley rats 250-300g

Design: PB, sarin, both or neither x 3 weeks; then sacrifice animals at 2, 4, or 16 weeks thereafter

Exposure:

PB: 80mg/L in drinking water vs tap water x 3 weeks: or tap water control

Sarin: 62.5 microgm/kg injex 3x/week x 3 weeks; or saline injection control

	Sample Size			
	2 weeks	4 weeks	16 weeks	
Group 1: neither	11	10		8
Group 2: PB	10	12	9	
Group 3: Sarin	12	12	8	
Group 4: both	12	10	7	

Send by air freight 1 week prior to sacrifice

Then pulse injection of radiolabeled choline just before sacrifice; with microwave fixation of the brain 1 minute later

Shih 2006 Cerebral acetylcholine and choline contents and turnover following low dose acetylcholinesterase inhibitor treatment in rats. Arch Toxicol.

PB & sarin: effects on Ach levels in brain

Accessory outcome: signs of cholinergic toxicity: fasciculation, tremor, convulsions, salivation, lacrimation, eyebulb protrusion, general activity and coordination

Outcome: Gas chromatography mass spectrometry for brain regional measure of:

1. Brain (tissue) ACh;
2. Brain (tissue) choline;
3. *Choline as measure choline uptake from the blood (labeled ch)
4. *ACh as measure of Ach turnover (labeled ACh)

Measured of these in HC, infundibulum, mesencephalon, neocortex, piriform cortex, and striatum at baseline; then change value

AChE inhibition: Did NOT measure AChEi; but reportedly prior study showed 49% inh with PB; 66% inh with sarin; 73% inh with both

Analysis: ANOVA for brain factors "region" and "Treatment"; Tukey Kramer multiple comparison test if signif

ANCOVA: using the region's values for each variable as covariates

Shih 2006 Cerebral acetylcholine and choline contents and turnover following low dose acetylcholinesterase inhibitor treatment in rats. Arch Toxicol.

PB & sarin: effects on Ach levels in brain

Result:

- 0. No cholinergic toxicity signs (also: no msr AChE inhib)**
- 1. Regional differences in the outcome variables**
- 2. No treatment/region interaction**
- 3. No duration/region interaction**
- 4. Treatment differences:**
 - At 2 then esp 4 weeks: increase ACh in sarin groups (signif less if both); increase Ch signif only if both**
 - At 16 weeks: trend decrease ACh in sarin group (looks similarly large, but not signif); trend increase choline uptake in PB group (NS)**

Shih 2006 Cerebral acetylcholine and choline contents and turnover following low dose acetylcholinesterase inhibitor treatment in rats. Arch Toxicol.

PB & sarin: effects on Ach levels in brain

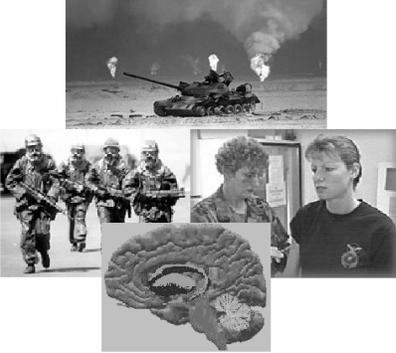
Limitation:

- 1. No msr AChE inhib; not clear how close to 1st signs**
- 2. Looks only at choline and Ach (even then very small samples)**
- 3. To look at cholinergic function: need to include many other elements including amount ACh per vesicle, number vesicles released in a signaling event; nicotinic and muscarinic Ach receptor density by type and brain region and time (and coexposure), binding affinity, receptor sensitivity;**
- 4. Small sample sizes even for the measures used: trends esp at 16 weeks, maybe signif with larger sample?**
- 5. Changes appear to be evolving (including reversing) with time; need to look further out in time**
- 6. Single size, gender; single species (not human)**

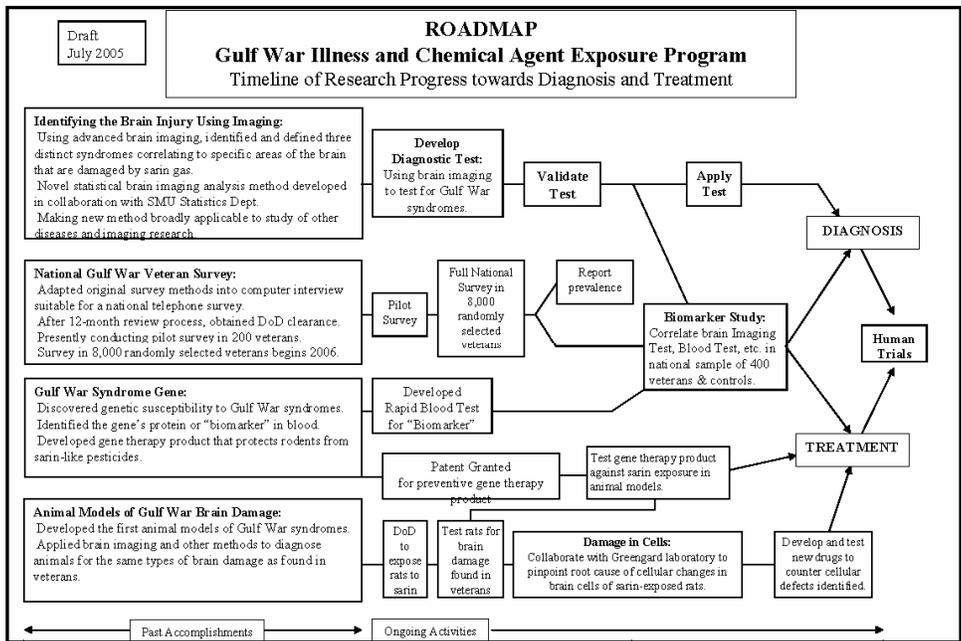
Shih 2006 Cerebral acetylcholine and choline contents and turnover following low dose acetylcholinesterase inhibitor treatment in rats. Arch Toxicol.

Presentation 11- Robert Haley

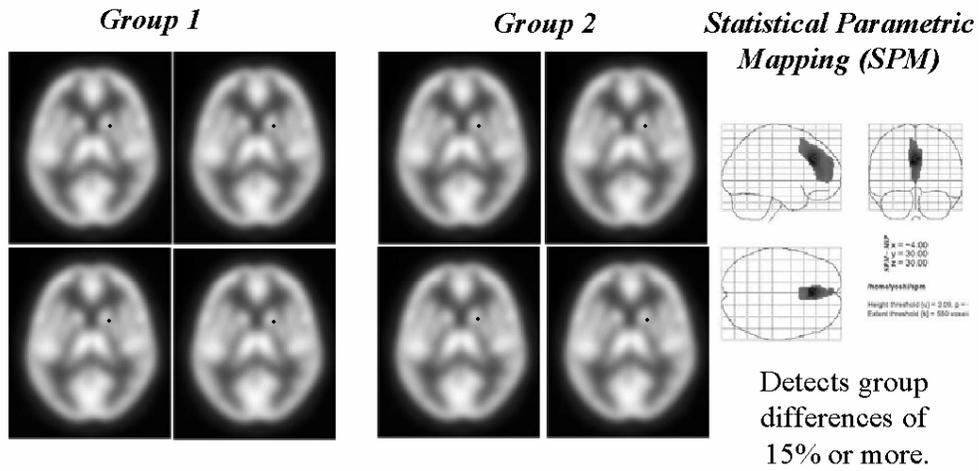
Plan for Gulf War Illness and Chemical Exposure Research Program



Robert W. Haley, M.D.
 Department of Internal Medicine
 University of Texas
 Southwestern Medical Center
 Dallas, Texas



Analysis of Group Differences in Brain Imaging Experiments



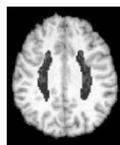
The Statistical Development Team



Wayne Woodward Bill Schucany Dick Gunst Robert Haley
Pat Carmack Jeff Spence

SPM Approach	New Approach
<ul style="list-style-type: none"> • Spatial normalization • Count normalization to the whole brain average $CBF_v / CBF_{wb} = nrCBF_v$ • Brett transformation of Talairach coordinates to MNI space (brain surface) • Smoothing with an 8-14 mm gaussian kernel over all 200,000 voxels • Group comparisons with canned GLM programs 	<ul style="list-style-type: none"> • Spatial normalization • Count normalization to a white matter volume $CBF_v / CBF_{wm} = nrCBF_v$ • Carmack transformation of Talairach coordinates to MNI space (ventricular surface) • Geostatistical spatial modeling to extract larger uncorrelated blocks for analysis • Group comparisons with SAS modeling

Count Normalization to a White Matter Standard Region vs the Global IC Average



Centrum semiovale

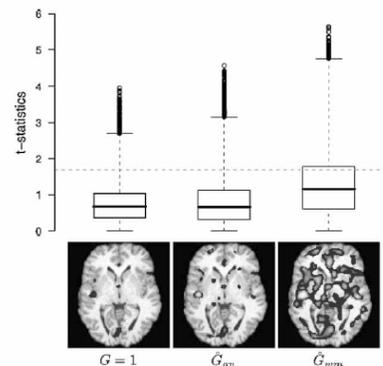


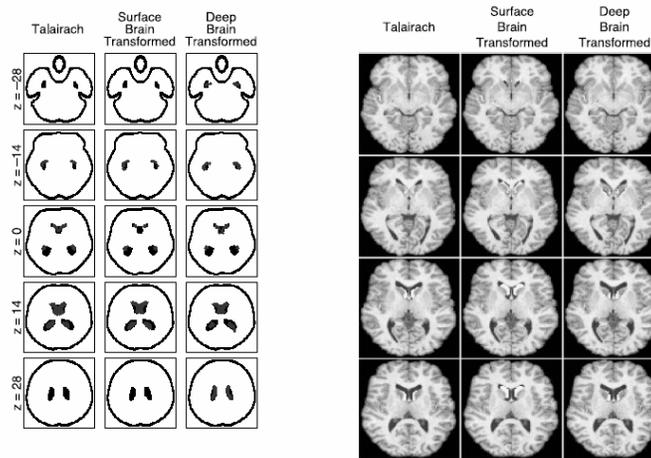
Table 2
 Results of SPM analysis (cluster-level *P* values) using three measures of global signal

Location	$G = 1$		\hat{G}_{av}		\hat{G}_{wm}	
	Cluster size	$P_{corrected}$	Cluster size	$P_{corrected}$	Cluster size	$P_{corrected}$
Lt. Mid. Frontal	–	–	–	–	82	0.029
Lt. Insula	–	–	5	0.835	86	0.025
Lt. Sup. Frontal	–	–	–	–	94	0.018
Rt. Sup. Frontal	–	–	2	0.931	85	0.026
Lt. Med. Fronta	–	–	–	–	88	0.023

In each case, the *t* statistic threshold is 4.22 on 34 degrees of freedom.

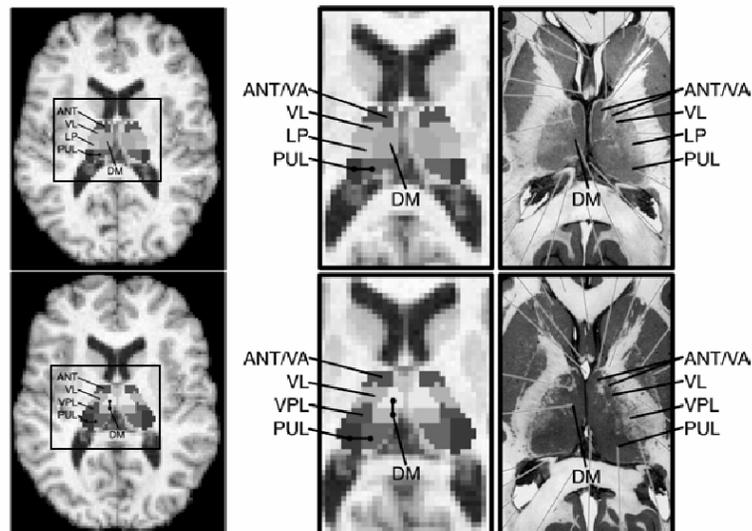
Spence et al. *NeuroImage* 2006 (in press)

Improved Algorithm for Locating Deep Brain Structures: The Carmack Transformation



Carmack et al. *NeuroImage* 2004; 20:367-371

Anatomic Accuracy Achieved by Spatial Modeling of High Resolution SPECT using the Carmack Transformation: Thalamic Nuclei



Appendix B

Public Submission 1 – Kirt Love

On May 15th and 16th I was pressed to confine my remarks to 5 minute presentations in the evening. To make a 28 slide presentation.

The bulk centered around the need for a restructuring of assets in the Gulf War community that would be more conducive to both veterans and medical circles. That current dogma was taking the words "Gulf War" out of the literature and replacing it with deployment health. That Gulf war medical research and treatment was in danger of being white washed from the vernacular.

In my presentation I covered the Gulf War registry decline, Public Laws that had lapsed, the barely functioning WRIISC, Adverse drug reactions, Glutamate receptors in conjunction with substance abuse, ALS Glutamate research, Crystal formation in muscle biopsies, Adjuvants in vaccines, the Anthrax vaccine, Nanobacteria related illness, Infectious disease, Genomic research with CFS, Cellular methylation, Chemical sensitivity, Sleep apnea, fibromyalgia, Gastroparesis, Dietary studies, EMP weapons in the Gulf War, Cerebral Perfusion, and Transcranial Color Coded Duplex Sonography.

Of which the PowerPoint can be found at:
<http://www.gulfink.org/rac/rac2006.ppt>

Thank you for your time and patients.

Sincerely
Kirt P. Love
Director, DSBR

Public Submission 2 – Denise Nichols

GULF WAR VETERANS CANCER DATA FROM 1991-1994 PRESENTED BY DENISE NICHOLS

A: THYROID CANCER MORTALITY DATA 1991-1994

- INCIDENCE: 26,000/YR OR 10 PER 100,000
- EXPECTED DEATH: 1500/YR OR 0.5/100,000

ACTUAL GULF WAR VETERAN DEATHS FROM THYROID CANCER BY YEAR

- 1991---0
- 1992 ---3 (2 <25 YEARS OLD)
- 1993 ---4 (1<25YRS; 1 25-34YRS; 2 35-44YRS)
- 1994---9 (4<25YRS; 3 25-34YRS; 3 35-44YRS)
- NOTE STATS EXPECTED IN 18-34 YR GROUP IS 0.1 PER 100,000 FOR 600,000 WOULD BE 0.6

RESULTS

- FOR THYROID CARCINOMA MORTALITY RATE FOR THE GULF WAR VETERANS LESS THAN 25 YR OLD IS 4.0. THIS IS 4 TIMES THE EXPECTED DEATH RATE FOR THIS CANCER IN THIS AGE GROUP.

DEATH RATE

- FOR <25 YR OLD : 25 OR LESS CASES/YR PER 300,000,000
- 4 ½ DEATHS WOULD BE EXPECTED IN POPULATION OF 30,000,000
- GULF WAR POPULATION 600,000
- ODDS RATIO 0.000011 STATISTICALLY SIGNIFICANT

B. TESTICULAR CARCINOMA FACTS

- RARE DISEASE 5,500 NEW CASES/YR
- OVERALL INCIDENCE 1.5-2.0/100,000
- RARE DISEASE < 2/100,000
- Highest Age Specific 20-34 yr
- DEATHS 0.2/100,000 or 1.2/600,000

MORTALITY TESTICULAR CANCER GULF WAR VETERANS

- 1991 2 (1 IN 18-24 YR; 1 IN 25-34 YR)
- 1992 16 (6 IN 18-24 YR; 5 IN 25-34 YR, 5 IN 35-44 YR)
- 1993 63 (18 IN 18-24 YR; 38 in 25-34 YR; 7 in 35-44 Yr)
- 1994 43 (11 in 18-24 YR; 32 in 25-34 Yr)
- Note: Expected Number of Deaths for yrs 1991-94 would be 30/600,000

Mortality Testicular Cancer Gulf War Veterans By Year

- 1993 shows 2.1 times the expected death rate
- 1994 shows 1.45 times the expected death rate.

Testicular Cancer Mortality By Age Gulf War Veterans

- 1991 1 in 25-34 age group expected 1.2
- 1992 5 in 25-34 age group 4 times expected
- 1993 total 16-- 5<25; 10 (25-34); 1 (35-49) 13 times expected
- 1994 total 11 still over 9 times expected

C. LEUKEMIA INFORMATION

- Over the last 30 years, research has shown that 95% of patients with leukemia have an acquired genetic defect. The defect is a translocation (one piece of genetic material moved to another piece of genetic material). The general divisions of leukemia are dependent upon the type of cells that are proliferating. The cell types are lymphocytes and myelocytes. Each type of leukemia, lymphocytic and myeloid is divided into acute and chronic. Acute lymphocytic leukemia is a disease of the young and old. 75% of cases of acute lymphocytic leukemia(ALL) occur in those younger than 15 years of age. Chronic lymphocytic leukemia (CLL) accounts for 10,000 new cases per year. The number of new cases of ALL are 4,000 per year. Thus 1,000 cases of ALL are among adults. A fascinating aspect of ALL disease is the age distribution in adults. It has been observed that of the adults, (1,000) per year, there is an age distribution. Of the occurrence of ALL.
 - In the Less than 25 age group, only 2% of 1,000 adults have ALL
 - In the 25 to 34 age group, 5% of 1000 adults.
 - In the 35 to 65 age group, 85% of 1,000 with ALL is seen
 - At 1,000 adult cases of ALL per year, ALL in adults becomes a RARE Disease 0.3 to 0.5 per 100,000 or 3.0 per 600,00.

Number of Cases of ALL for Persian Gulf Veterans

- 1992 1
- 1993 12
- 1994 13
- THUS FOR 1993 and 1994, THE NUMBER OF ALL IS FOUR (4) TIMES THAN EXPECTED.
NUMBER OF DEATHS
- From ALL and CML is more than expected. There are 15,000 deaths from ALL per year. For a population of 600,000, 3 deaths are expected.
- For ALL the years 1991 to 1994, the Persian gulf death rate is 3.0. For CML, the number of expected deaths is 850 per population or 2 per 600,000. In 1993, the number of deaths from CML was nine(9) and for 1994, the number of deaths was seven(7). Thus, in 1994 the number of deaths from CML is 3-4 times expected.
Myeloid Leukemia :Primarily a disease of the elderly. Myeloid leukemia is divided in acute and chronic. In chronic myeloid leukemia, 5,000 new cases are expected per year
 - Age distribution is
 - Less than 25 2% of 5,000 or 0.3/600,000
 - 25 to 34 age 10% of 5,000 or 1.0/600,000
 - 35 to 44 age 11% of 5,000 or 1.0/600,000
 - 45 and over 75% of 5,000
 - CML: One case per 600,000 is expected for adult CML in the less than 25 age group and one case per 600,000 is expected in adults with CML in the 25-34 age group.
Persian Gulf Group
 - 1992 11 cases (under 25), 6 cases (25 to 34), 2 cases (35 to 44)
 - 1993 7 cases (under 25), 10 cases (25-34), 3 cases (35-44)
 - 1994 2 cases (under 25). 0 cases(25-34), 0 cases(35 to 44)
- PERSIAN GULF INCIDENCE CML Results: The incidence of CML in the Persian Gulf Group is 6-10 TIMES THE EXPECTED RATE IN THE UNDER 44 AGE GROUP.