Research Advisory Committee on Gulf War Veterans' Illnesses

April 7-8, 2008, Committee Meeting Minutes

Boston University School of Public Health
Boston, MA

DEPARTMENT of VETERANS AFFAIRS

Research Advisory Committee on Gulf War Veterans' Illnesses
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I hereby certify the following minutes as being an accurate record of what transpired at the April 7-8, 2008 meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses.

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/signed/
James H. Binns
Chairman
Research Advisory Committee on Gulf War Veterans' Illnesses

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Attendance Record

Members of the Committee
James H. Binns, Chairman
Carrolee Barlow
Floyd E. Bloom
Daniel J. Clauw
Beatrice A. Golomb
Joel C. Graves
Anthony Hardie
Marguerite L. Knox
William J. Meggs
James P. O'Callaghan
Lea Steele
Adam Such
Roberta F. White

Committee Staff
Callie Comtois
Kimberly Sullivan

Designated Federal Officer
William Goldberg

Guest Speakers
Ronald Bach
Allen A. Fienberg
Louis Fiore
Fred S. Gorelick
Avlin Imaeda
Neil Kowall
Ashok Tuteja
Richard J. Valente

Representative from the University of Texas Southwestern
Robert Haley

Abbreviations

AChE	Acetylcholinesterase
ALS	Amyotrophic Lateral Sclerosis
BU	Boston University
BUSPH	Boston University School of Public Health
CMI	Chronic Multisymptom Illness
CDC	Centers for Disease Control and Prevention
CDMRP	Congressionally Directed Medical Research Programs
CFS	Chronic Fatigue Syndrome
CRADO	Chief Research & Development Officer for the VA
DOD	Department of Defense
FY	Fiscal Year
GI	Gastrointestinal
GW	Gulf War
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
IOM	Institute of Medicine
IRB	Institutional Review Board
ITI	Intra-cellular Therapies, Inc.
LTC	Lieutenant Colonel
MCS	Multiple Chemical Sensitivity
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
NIH	National Institutes of Health
OEF	Operation Enduring Freedom
OIF	Operation Iraqi Freedom
PB	Pyridostigmine Bromide
PET	Positron Emission Tomography
PI	Principal Investigator
PTSD	Post Traumatic Stress Disorder
RAC, RAC-GWVI	Research Advisory Committee on Gulf War Veterans' Illnesses
TF	Tissue Factor
TF PCA	Tissue Factor Procoagulant Activity
U.K.	United Kingdom
UTSW	University of Texas Southwestern School of Medicine
VA	U.S. Department of Veterans Affairs
VISN	Veterans Integrated Services Network

Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses
April 7-8, 2008
BUSPH, Crosstown Center, 801 Massachusetts Ave., Room 462, Boston, MA
Agenda
Monday, April 7, 2008

8:00 – 8:30	**Informal gathering, coffee**	
8:30 – 8:35	**Welcome, introductory remarks**	**Mr. Jim Binns, Chairman** **Res Adv Cmte Gulf War Illnesses**
8:35 – 9:00	**Introduction of Boston University RAC scientific staff**	**Dr. Roberta White, Scientific Director** **Res Adv Cmte Gulf War Illnesses**
9:00 – 9:45	**Tissue factor and Gulf War associated chronic coagulopathies**	**Dr. Ronald Bach** **Minneapolis VA Medical Center**
9:45 – 10:00	**Break**	
10:00 – 10:45	**Bowel disorders in Gulf War veterans**	**Dr. Ashok Tuteja** **VA Medical Center, Salt Lake City**
10:45 -11:15	**Perspectives on gastrointestinal illness in Gulf War veterans**	**Dr. Fred Gorelick** **Dr. Avlin Imaeda** **VA Conn. Healthcare System, West Haven** **Yale University**
11:15-11:45	**Group discussion related to gastroenterological problems in Gulf War veterans**	
11:45 – 12:30	**Results of Rhode Island Persian Gulf War state survey of Gulf War veterans**	**Brig. General Richard Valente (ret.)** **Rhode Island Persian Gulf War Information and Relief Commission**
12:30 - 1:30	**Lunch**	
1:30 - 2:15	**ITI Gulf War research report**	**Dr. Allen Fienberg** **Intra-Cellular Therapies, Inc.**
2:15 - 3:00	**Update of recent Gulf War research**	**Dr. Beatrice Golomb** **Dr. Daniel Clauw** **Res Adv Cmte Gulf War Illnesses**
3:00 – 3:15	**Break**	
3:15 - 3:45	**Update of VA Gulf War research**	**Dr. William Goldberg** **VA Office of Research and Development**
3:45 – 4:30	**Update of VA Gulf War brain bank**	**Dr. Louis Fiore** **Dr. Neil Kowall** **VA Boston Healthcare System**
4:30 - 5:00	**Public Comment**	

Meeting of the Research Advisory Committee for Gulf War Veterans' Illnesses
April 7-8, 2008
BUSPH, Crosstown Center, 801 Massachusetts Ave., Room 462, Boston, MA
Agenda
Tuesday, April 8, 2008

8:00 – 8:30	**Informal gathering, coffee**	
8:30 – 9:30	**2008 Research Advisory Committee report discussion**	**Dr. Lea Steele** **Res Adv Cmte Gulf War Illnesses**
9:30 – 10:30	**University of Texas Southwestern Medical Center research program discussion**	**Mr. Jim Binns, Chairman** **Res Adv Cmte Gulf War Illnesses**
10:30 – 10:45	**Break**	
10:45 – 1:30	**University of Texas Southwestern Medical Center research program discussion**	**Mr. Jim Binns, Chairman** **Res Adv Cmte Gulf War Illnesses**
1:30 – 2:00	**Public Comment**	
2:00	**Adjourn**	

Day 1

The April 7-8, 2008 meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses was held in Room 462/462A of the Crosstown Center Building, at Boston University School of Public Health, 801 Massachusetts Avenue, Boston, MA.

Welcome, introductions, and opening remarks

James H. Binns, 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 8:30 am. He began by thanking Dr. Roberta White and her staff at Boston University for hosting the meeting, and setting up hotel accommodations in a location convenient to the meeting facilities. He welcomed the members of the Committee, BU faculty, the audience, any veterans in attendance, and all of the guest speakers.

Chairman Binns began by introducing two new members of the Committee. The first new member was Dr. Dedra Buchwald, who was unable to attend the meeting because of the late notice of the meeting's dates. Dr. Buchwald is from the University of Washington, and is an expert on CFS. She hopefully will be joining us at our next meeting.

The second new member is LTC Adam Such. He is currently the Executive Officer of 5th Special Forces Group at Fort Campbell. He graduated in 1989 from West Point and as a young Lieutenant served in the Gulf War as a Rifle Platoon Leader in northern Iraq. LTC Such has had a distinguished career in Special Forces since then – from January 2003 until the middle of 2006, he spent all but 8 months in OIF/OEF. He planned the infiltration of Special Forces into Baghdad in advance of conventional forces as Senior Commander of Special Operations Forces, in al-Anbar province. For most of the period, he served as the Operations Officer for the Joint Special Operations Command in Baghdad, with responsibility for all but northern Iraq, including over 1,000 of the Joint Special Operations Forces, the Iraqi Special Operations Forces, and a division or more of Iraqi military forces. Lieutenant Such brings with him an extensive background of knowledge about current military operations, and has also been recognized for his analytical abilities. The tactics being used in Iraq today were developed by his group in al-Anbar province, working with the local population and local leadership to pacify the area and engender cooperation. He was the leader of the Special Operations component of the Army Chief of Staff's review of OIF. He will be a great asset to the Committee, and believes strongly in the role the Committee plays in informing current military operations, as well as its importance to veterans. Chairman Binns thanked LTC Such for joining the Committee, and the rest of the Committee welcomed LTC Such with a round of applause.

Chairman Binns recognized the contributions of Dr. Lea Steele, who served as the Scientific Director for the four and a half years prior. He asked Dr. Steele to join him at the podium, where he informed the audience that although she was stepping down as the Scientific Director, Dr. Steele would continue to be a member of the Committee, work on the 2008 report, review the research program at UTSW, and serve on the CDMRP review board. He compared her role in

the Committee to the 100th anniversary of the first successful journey to the North Pole: if the Committee was a dogsled engaged in a scientific journey to the North Pole, Dr. Steele would have been the compass, map, blanket, and dogs. Dr. Steele has kept the Committee on a course that has made great progress, and kept true to scientific standards while doing so. Chairman Binns presented Dr. Steele with a framed copy of the 2004 Committee report, and asked each Committee Member to sign the cover so Dr. Steele could keep it as a memento of her time as Scientific Director. Chairman Binns thanked Dr. Steele.

Chairman Binns welcomed Dr. Roberta White, who has been involved with Gulf War related research since 1992, as the new Scientific Director of the Committee. Dr. White will be committing 40% of her time to the Committee, while maintaining her current role as the Chairman of the Department of Environmental Health at Boston University School of Public Health. He invited Dr. White to introduce the new Committee staff, as well as to describe her plans for a 'team approach' to studying Gulf War illness research and related topics.

Introduction of Boston University RAC scientific staff

Dr. Roberta White, Scientific Director
Chair, Department of Environmental Health, BUSPH

Dr. White noted that she was pleased to be taking on the role of Scientific Director, and stated that she has a strong interest in Gulf War research. She thanked the Committee and audience for coming to Boston, as she wanted everyone to meet the new staff and to see the RAC's new facilities. Dr. White introduced the Committee staff working through BUSPH (See Appendix – Presentation 1), and introduced some of the members of the internal scientific review committee. This group will be made up of Dr. White's colleagues at BU, and will serve to review relevant Gulf War research literature. Several members of this team have yet to be determined. Dr. White expressed her excitement at having this review committee become an additional resource for the RAC.

Chairman Binns thanked Dr. White. He stated that he has always had goal to have the RAC meetings take a multi-disciplinary approach to integrating information from various scientific fields; Dr. White's 'team approach' idea is a step to achieving that goal. Chairman Binns expressed his hope that this internal committee will aid in gaining answers to the questions the Committee has been trying to answer for the last six years.

Chairman Binns introduced Dr. Ronald Bach.

Tissue factor and Gulf War associated chronic coagulopathies

Ronald Bach, PhD
Research Health Scientist, VA Medical Center, Minneapolis, MN
Associate Professor, Dept. of Medicine, University of Minnesota School of Medicine

Dr. Bach gave an overview of the research project he and his colleagues at the VA Medical Center in Minneapolis, MN had recently completed. The study tested the hypothesis that tissue

factor (TF) procoagulant activity (PCA) in the blood of individuals with Gulf War illnesses is abnormal. (See Appendix – Presentation 2). The study further tested whether abnormal levels of TF PCA created a chronic hypercoagulable state.

During the presentation, Dr. Meggs, a member of the Committee, noted that only a certain percentage of GW veterans have coagulopathies, and asked if Dr. Bach would clarify if he was looking at a pool of ill veterans to see if any of them had coagulopathies. Dr. Bach responded that they were not relating any of their findings of TF PCA to coagulopathies, but they were comparing ill Gulf War veterans with CMI symptoms to other GW veterans, who may be ill, but do not have CMI symptoms. Dr. Meggs suggested that Dr. Bach's analysis might have washed out an effect by categorizing the groups the way he did. Dr. Bach responded that this was preliminary analysis, and that they would do more detailed groupings and analyses at a later date.

Dr. Meggs later asked if the data presented for the warfarin portion of the study was in terms of quantitative levels of TF, and not TF activity. Dr. Bach responded that they were quantifying levels of TF activity. The reason they were studying levels of TF activity, and not just the levels of TF in the blood, is that there is an inactive form of TF that is also in the blood. In measuring levels of TF only (and including the inactive form), they risk getting an incorrect reading of TF levels.

Dr. Golomb, a member of the Committee, then asked Dr. Bach to clarify the types of individuals constituting his control group for the warfarin study. Dr. Bach responded that these people were volunteers at the VA Hospital, and were not patients of the warfarin clinic. Some of these volunteers may have been veterans. Dr. Golomb noted that the study then does not permit disambiguation between 'veteran' vs. 'non-veteran' and 'warfarin' vs. 'non-warfarin' subjects. Dr. Bach agreed with Dr. Golomb's statement, and added that the purpose of the control group in the warfarin study was to find age and sex-matched groups. Dr. Golomb asked if the differences in TF PCA between participants in the 'warfarin' group vs. the 'control' group were from Dr. Bach's data collection, or from outside sources. Dr. Bach stated that they were results from studying his own data collection. He then proceeded with his presentation.

Chairman Binns thanked Dr. Bach for his presentation, and opened discussion to the Committee members and audience.

Dr. Clauw, a member of the Committee, stated that he was not aware of any case-control studies that suggested GW veterans had a higher rate of coagulopathies. Dr. Bach responded that the only study in the literature was the 2000 study he referred to at the beginning of his presentation – it was a fairly small study comparing levels of coagulation factors in GW veterans and controls. He noted that if coagulopathies were in fact higher in veterans with GW illness, researchers would expect to see more pulmonary emboli, for example, in this population. There is currently no published data on this subject; Dr. Bach suggested that it may be too early in the lifespan of this population to see any effects of chronic coagulopathies. Dr. Clauw further asked if Dr. Bach could clarify why the values for the GWV with illness and GWV without illness were different than that of the control group for the warfarin study. Dr. Bach noted that the data was originally presented in terms of picograms/milliliter of blood. When the slides for the GW data were shown, the data was presented in terms of picograms/10^6 cells, or /10^9 platelets.

Dr. Golomb asked if there was any literature on the monocyte to platelet ratio and the risk of deep venous thrombosis (DVT) or pulmonary embolism (PE) and is there a difference between platelet TF activity and monocyte TF in terms of PE/DVT risk? Dr. Bach stated that these relationships have never been studied. Dr. Golomb noted that we don't really know if an alteration in this ratio would be expected to induce a change, and Dr. Bach responded that his research is an 'outside the box' kind of study. When he first started this in 2000, few people believed there was TF in blood; now people acknowledge that TF does exist in blood. Studying TF in blood is an evolving area in research, and not much has been done to date. Dr. Golomb wondered if an answer was available regarding whether apoptosis has an effect on the monocyte to platelet ratio and offered apoptosis as alternate hypothesis to Dr. Bach's inflammation hypothesis. Dr. Bach agreed that this was another valid hypothesis. Dr. Golomb commented that if Dr. Bach was concerned about the issue of outliers in data, that he might consider a non-parametric test that involves ranking, like a rank-sum test, rather than a typical t-test. Dr. Bach thanked Dr. Golomb for her suggestion.

From the audience, Dr. Sherr, a member of the BUSPH faculty and internal review committee, asked the next question. He wondered how long after deployment the blood samples from the veterans were collected. Dr. Bach replied that the samples were all collected from GWV during the period of 2006-2008. Dr. Sherr suggested an easy way to test the hypothesis that the immune system is involved in coagulation: look for molecular signatures of cytokine production in monocytes. Dr. Bach stated that their lab is in the process of having those markers analyzed.

Dr. Meggs commented that there might be another hypothesis that might explain Dr. Bach's findings. He stated that there have been a number or chronic low-dose exposures to substances in the environment, and these exposures might by driving this inflammation. He suggested that the way to study this would be to use an environmental control unit where exposures could be controlled. Dr. Meggs said the he was talking to a physician who has treated thousands of patients in an environmental control unit, and posed a question to him - he asked if this physician could do only one study to look at only clinical syndrome, what would it be? The physician responded that he would study recurrent DVTs, and that he'd never seen a non-responder in his environmental control unit. Dr. Bach replied that this was an interesting point. The hypothesis in his study is that there is a hypercoagulation, and a person can achieve this state in several ways. A stimulus, such as a bacterial substance, sets off a cross talk between the immune system and the coagulation system, and it never quiets down, or a chronic exposure keeps the cascade in its active state.

Dr. Steele thanked Dr. Bach for presenting his interesting findings. She then asked how Dr. Bach went about defining Gulf War illness for his study. Dr. Bach informed the group that this area of his research was probably the least rigorous. Dr. Bach designed the study so that his study coordinator was the one to make the designation based on an interview with the individual, and looking at their medical records in the computer system. They based their criteria on the IOM report, as well as on the Committee's 2004 report. They diagnosed someone as having Gulf War Illness if they had at least 2 of the following: fibromyalgia, CFS, MCS, cognitive issues, and a few others. Dr. Bach's study coordinator made the designations without knowing the analyses that Dr. Bach would later do with the data. In this way, she remained blinded in making the groupings. He noted that his findings needed to be independently confirmed by

either a new definition of GW illness, or by an outside group who makes their own designation. Dr. Steele also wondered if a state of hypercoagulopathy in the body's periphery was any indication of brain functioning. Dr. Bach replied that microcirculation is affected any time there is a state of hypercoagulopathy, and multiple organs can be involved when there is an alteration in circulatory patterns. Any organ in the body can be affected. There's also a potential feedback of the brain involved in the immune system and the coagulation system. A direct feedback loop communicates with the immune system via specific chemical signals that are released to activate the immune system, so there's a potential for a positive feedback loop in that regard. Dr. Bach stated that he doesn't know much more mechanistically about the relationship. Dr. Steele mentioned that there are some imaging studies that show impaired blood flow in areas of the brain, specifically in relationship to pain and in general scans of people with GW illness and CMI, and that one could make a case that if there was impaired regional blood flow, it may reflect a thickened situation. Dr. Bach noted that there are two relevant responses to that. Having blood in a hypercoagulable state can impair blood flow, and stasis in blood flow can contribute to clotting. Clotting in the capillaries leads to a state where more clotting can occur. So, the relationship can be synergistic.

Chairman Binns brought the discussion to a close, and thanked Dr. Bach for his participation in the meeting.

At 9:52, Chairman Binns briefly stopped the meeting for a ten minute break.

At 10:02, the meeting reconvened.

Chairman Binns introduced Dr. Ashok Tuteja.

Bowel disorders in Gulf War veterans

Dr. Ashok Tuteja
Assistant Professor of Medicine, George E. Wahlen VA Medical Center, Utah
Adjunct Assistant Professor, Pharmacy Outcome Research Center, University of Utah

Dr. Tuteja spoke about the prevalence of bowel disorders in veterans of the GW. (See Appendix – Presentation 3). He noted that GW veterans often complain of gastrointestinal (GI) symptoms including abdominal pain, excessive gas, diarrhea, and constipation. He also presented findings from his current study, looking at the incidence of irritable bowel syndrome (IBS) in GW veterans both during and post-deployment.

Dr. Steele asked Dr. Tuteja to tell the Committee about his treatment trial. Dr. Tuteja stated that his group is currently still in the process of recruiting individuals for this study. They are looking at bacterial growth and treatment of IBS, but are not yet ready to present results of this research.

Dr. Golomb commented that she would caution against using the terminology 'loss of coping' or 'altered perception' to describe veterans' reactions to IBS because there is evidence of altered signaling – there's the same perception, but altered signaling. Phrasing it as 'loss of coping' or

'altered perception' gives the impression the patients are responsible for their illness. She also noted that it is possible the patients who come in have a bigger quality of life impact; they are in some way more affected by their symptoms, or have possible comorbidities that enhance the impact of the condition. If psychological stressors were a big factor, we would see large amounts of IBS in PTSD more than in GW veterans, and Dr. Golomb stated that she was not aware of any literature that suggested this scenario to be the case. In responding to Dr. Golomb's first point, Dr. Tuteja stressed that there is an abnormal brain-gut communication in IBS patients. Either the gut is abnormal, and it is perceived as so in the brain, or the gut is less inflamed and the brain perceives that, but there is a hypervigilance in the patients. There is abnormal signaling in these patients, and research has been done to determine why. Some research suggests the autonomic nervous system is altered, some that the corticotrophin levels are different, and others that the vagus nerve is somehow involved. Dr. Golomb suggested shying away from using the term 'perception' because it might suggest that the patients' problems are psychological instead of biological. Dr. Tuteja emphasized that IBS is not a psychological disorder; it is a real disorder with a genetic basis, an inflammation basis, and an altered brain basis. In looking at positron emission tomography (PET) scans of these patients, altered signaling in the amygdala is apparent. Now, scientists are looking at proteases as possible mechanisms for altering signaling. This is a real, biological disorder. Dr. Tuteja noted that in his presentation, he only touched briefly upon IBS – not everybody who develops a form of gastroenteritis will develop IBS. Most people who get gastroenteritis completely recover from their symptoms.

Dr. Meggs noticed that on one of Dr. Tuteja's slides, stress and inflammation were listed as possible causes of IBS, but toxins were not listed on the slide. Since Gulf War veterans had a very unique toxic exposure, Dr. Meggs inquired if anyone has researched the prevalence of IBS in this population and crossed it with their exposures to pyridostigmine bromide, organophosphates, insecticides, sarin, etc. He also noted that symptoms of a low-dose acute ingestion of an organophosphate resemble an attack of IBS. Dr. Tuteja responded that it sounds logical that these pesticides and toxins may cause IBS, but he is not aware of anyone that has studied this relationship.

From the audience, Dr. Gorelick, a speaker at the meeting, stated that Dr. Tuteja's presentation was clear, balanced, and very useful. He mentioned that there is a form of post-infectious diarrhea associated with an inflammatory response and usually dissipates in a couple of years, which is different from IBS. He asked what the durability of symptoms was for Dr. Tuteja's patients from the first GW – are their symptoms persisting over time, or do they seem to resolve? Dr. Tuteja replied that in looking at the non-veterans' study, about 50% of the people lose their symptoms in six years time. However, in looking at the data from the missionary study (discussed during Dr. Tuteja's presentation), the individuals still have symptoms after ten years. Dr. Tuteja's group of veterans is involved in a cross-sectional study, so it is not possible to tell how long they will continue to have symptoms. However, it has been more than ten years since the war, and the patients still show symptoms. Dr. Gorelick noted that some of the Gulf War veteran patients he has examined with these symptoms had an abnormality that one would see in children, and not usually in adults – nodular lymphoid hyperplasia in the terminal ileum or cecal area. Dr. Gorelick wondered if Dr. Tuteja ever had the same findings in examining any of his GW patients. Dr. Tuteja responded that they are performing small bowel biopsies and other

related colon biopsies on all of the diarrhea patients who are in the bacterial growth treatment study, and checking them for mast cells, eosinophils, and enterochromaffin cells. His lab is currently in the process of analyzing those samples.

Mr. Hardie, a Committee member, inquired whether Dr. Tuteja has been finding that his Gulf War patients have been suffering from IBS that is stable, progressive, or relapsing-remitting. Dr. Tuteja noted that he is examining these patients at one specific point in time, and some of them report mixed findings, such as experiencing both diarrhea and constipation. Mr. Hardie also asked whether the patients' eating and drinking habits had any impact on their symptoms. Dr. Tuteja replied that his study did not assess that point.

Dr. Steele asked Dr. Tuteja about assessing the patients for bacterial overgrowth: was Dr. Tuteja's group also able to determine the type of overgrowth present? She also wondered if the bacterial overgrowth in IBS patients was responsible for the initiating infection. Dr. Tuteja responded that his group is currently analyzing the overgrowth, and is freezing and storing the stool samples. The bacterial overgrowth test they are running only determines if the sample is positive or negative. His hypothesis is that during deployment, the patients developed gastroenteritis and developed symptoms. Thus, bacterial overgrowth would have happened during deployment. The study will determine if the patients complaining of symptoms now and had their initial onset of symptoms during deployment are more likely to test positive for bacterial overgrowth than those who did not. Dr. Tuteja's group is testing people who deployed and now have IBS, people that deployed but do not have IBS, and people in the Guard who never deployed. Dr. Steele asked about the specific types of bacteria present in the samples. Dr. Tuteja noted that they may decide to later check the frozen stool samples for bacterial type. Dr. Steele further inquired whether a frank autonomic dysfunction has ever been shown to be associated with symptoms of IBS. Dr. Tuteja answered by saying that there is a significant amount of research done on the autonomic nervous system and IBS. Researchers have looked at the heart rate variability, vagal nerve response, skin temperature, and epinephrine/norepinephrine levels, but Dr. Tuteja was not definitely sure of the results of those studies.

Dr. O'Callaghan, a member of the Committee, recalled that Dr. Tuteja had mentioned that there were higher levels of enterochromaffin cells, TNF-α, and IR1-β. These markers are clear indicators of an inflammatory response, yet steroids as a treatment do not work. Dr. O'Callaghan wondered if the endpoints were found in the same cases, and why the steroids do not work. Dr. Tuteja replied that there was a study done in Nottingham, England that tested steroids. They started with 300 subjects in the study, and ended with 18. Most of the people dropped out because of the intense side effects of steroids. In analyzing the data, they found no change in patients' symptoms, but the cells in the rectum improved. Dr. Tuteja emphasized that there is not only one cause of IBS. Inflammation might be one of the causes, but is not the only one. One of the best treatments for inflammation is prednisone, but it does not work – there must be other causes that come into play, including genetic factors, trauma, and stressors.

Rev. Graves suggested that Dr. Tuteja consider narrowing the definition of combat for his study. He noted that this definition could be that veterans were involved in shooting; Dr. Tuteja's subjects might be assuming that because they were in the combat theatre of operations, they qualify as being in combat. Dr. Tuteja agreed. Rev. Graves stated that while he was serving in

the Gulf War, he noticed many people who were suffering from IBS symptoms. The troops, including Rev. Graves, were given containers to carry their water around. Some of the containers were previously used to hold jet fuel, and when drinking from the containers troops could tell that it was harmful. Other water troops were given (in clear plastic bottles) was stored for months in direct sunlight, and when opened to drink, emitted a very strong chemical odor. At the time, Rev. Graves wondered if the water was damaging the troops' guts. In addition, for about two weeks the troops were in an oil ring that contaminated everything: clothes, food, and water. The ring was a cocktail of toxins and other nasty components. The troops were also exposed to organophosphates, sarin, possibly VX (a chemical nerve agent), carbamates, repellents, solvents, and CARC (a type of paint) and all of those may have synergistically affected the pathway of the brain, or insulted the intestine. The troops were also constantly breathing in ultra-fine particles on the battlefield from the oil well fires, depleted uranium, burning vehicles (including the munitions on the vehicle). Rev. Graves thought these exposures may have occurred at such a low dose that they might not show up on a test, but wondered if the particles became lodged in the gut, and disrupted normal activity that makes a person healthy. He hoped that Dr. Tuteja's biopsies might shed some light on these phenomena. Dr. Tuteja agreed with Rev. Graves' points, and said that his study will hopefully show an association between deployment to the Gulf and experiencing these IBS symptoms.

Dr. Golomb asked if any research has looked at a relationship, pro or con, between receiving antibiotics for a gut infection, and then subsequently developing IBS. Dr. Tuteja replied that there are studies demonstrating that people on antibiotics are more likely to develop IBS. IBS could be the result of too much bad bacteria, or a lack of good bacteria – researchers are still not sure.

From the audience, Gen. Valente, a meeting speaker, asked if there was a similar database of Vietnam veterans with IBS. In looking at this data, Dr. Tuteja might be able to tease out the effect of combat stressors vs. environmental stressors on developing the symptoms of IBS. Gen. Valente is aware of several Vietnam veterans who suffer from IBS, and have also been to combat. Dr. Tuteja stated that this was a good idea, but was not aware of any such database.

Chairman Binns commented that there was time built in to the schedule following the next presentation for discussion on gastrointestinal issues in GW veterans, and wanted to wait until that time to continue the comments and questions. Chairman Binns thanked Dr. Tuteja for his presentation.

Chairman Binns introduced Dr. Imaeda and Dr. Gorelick.

Perspectives on gastrointestinal illness in Gulf War veterans

Dr. Avlin Imaeda
Assistant Professor of Medicine, Yale University School of Medicine
Clinical Practitioner, VA Medical Center, West Haven, CT
Dr. Fred Gorelick
Professor of Medicine and Cell Biology, Yale University School of Medicine
Staff Physician, VA Medical Center, West Haven, CT

Dr. Imaeda began the presentation (See Appendix – Presentation 4) by introducing several cases of veterans suffering from IBS. She then explained various aspects of IBS, including possible etiologies, genetic implications, and the role of the central nervous system and the enteric nervous system in the syndrome's manifestation. Dr. Gorelick concluded the presentation by giving an overview of the many potential mechanisms by which a person might develop IBS, as well as supplying a list of prospective research to be done for IBS.

Following the presentation, Dr. Tuteja spoke from the audience to answer a previous question that was posed to him by Rev Graves. In reference to narrowing the definition of combat for his study, Dr. Tuteja noted that his research group was unable to obtain approval to include the combat severity scale on their mail-in study questionnaire. However, for the diagnostic testing and treatment study, they are able to use the combat severity scale for their analysis.

Dr. Gorelick asked Dr. Tuteja if he has seen any patients returning from the most recent Gulf War who exhibit the following characteristics: young males, severe esophageal reflux, unresponsive to medical therapy, and present with recurrent bouts of nausea and vomiting. Dr. Tuteja replied that his group has collected data from recent Gulf War returnees, and looked at functional dyspepsia, nausea and vomiting. He said those symptoms are common in the group, but he has not yet begun to analyze the data.

Dr. Bloom, a Committee member, mentioned that he found the veterans' unresponsiveness to Loperamide as a treatment to be surprising. He wondered if either Dr. Imaeda or Dr. Gorelick had found any changes in bowel biopsies of intestinal-opiate systems, either an overproduction or underrepresentation of the receptors that might make the patients tolerant to the external opiate agonist. Dr. Imaeda noted that Dr. Bloom made an interesting point, but they have not had the chance to perform the test. Dr. Gorelick commented that most of the biopsies taken from their patients are of the mucosa, and not always deep enough to gain all the information. He advocated establishing a tissue bank so these types of tissues may be retrieved and studied at a later date to answer these questions. Standard biopsies taken do not allow a deep investigation into the possible pathways involved in those responses.

Dr. Steele added to the discussion by mentioning that Dr. Clauw was probably not surprised that the opiate agonist treatment was not working in these patients, based on his familiarity with the drug and its use for pain management. Dr. Clauw agreed that in patients with fibromyalgia or IBS, opioids do not work well, and never have. He stated that there is evidence in PET imaging and cerebrospinal fluid (CSF) neurotransmitter studies suggesting the opioidergic systems are hyperactive in fibromyalgia. It makes sense that if a person is already releasing enough endorphins and they are given an exogenous opioid, there's no place for the opioid to bind. Dr. Steele wondered if in treating patients with fibromyalgia and administering a substance that alters serotonin and norepinephrine levels, the GI symptoms also improve. Dr. Clauw said the GI symptoms do improve, and occur on a par with the pain symptoms. However, the drugs being used to treat IBS are targeting different serotonin receptors than those used in treating fibromyalgia and general pain. Dr. Steele asked if treatment trials generally assess GI symptoms in patients with fibromyalgia. Dr. Clauw noted that the trials asses for a comorbidity of GI

symptoms with fibromyalgia, but have not yet been completed specifically in patients with IBS. When people with fibromyalgia get better on these drugs, everything gets better - IBS included.

Dr. Golomb commented on the case Dr. Imaeda presented of the veteran who responded to a self-treatment of ginger to quell his nausea. Dr. Golomb mentioned a double-blind, randomized control trial looking at ginger as a possible treatment for nausea. The study showed an efficacy with minimal side effects. She is also aware of a veteran patient who was being treated for GI problems, and upon taking ginger had his nausea resolved, but still had other symptoms. Dr. Gorelick concurred that ginger helps with nausea, but for reasons researchers do not understand. He stated that they have never actually tried that as a treatment for any of his patients at the VA, but it was a good idea.

Dr. Steele inquired if there were any studies of people who had primary brain pathologies and subsequently developed GI pathologies as a result of a disruption of regulatory processes. Dr. Gorelick answered that there were many examples of this being the case. One of the most dramatic is in people with Parkinson's disease; aside from the side effects of their medications, they have significant changes in GI functioning. The same is true for people with Multiple Sclerosis. Dr. Tuteja commented that he is not aware of any studies showing this relationship to be the case, but noted that in treating patients with mental retardation and patients in nursing homes, he has seen numerous cases of GI problems.

Dr. Meggs asked if there was any overlap between IBS and inflammatory bowel disease (IBD) and if the overlap was ever seen in GW patients. Dr. Tuteja responded that there was overlap of the two, but IBS was more common. One proposed hypothesis is that IBD and IBS are on opposite ends of a spectrum; IBD patients have more inflammation, while the IBS patients have more psychological disorders. In the middle of the spectrum is post-infective IBS where patients have less inflammation, and a more functional, biological disorder. Dr. Imaeda added that she sees a large amount of IBD in the older veterans at her clinic at the VA, but not typically in GW veterans.

Dr. Steele questioned if Dr. Gorelick knew of any connection between organophosphate induced delayed neurotoxicity (OPIDN) and pancreatitis or pancreatic disease. Dr. Gorelick responded that primary control of the pancreas (the part controlling digestive products) occurs through cholinergic pathways. One response of the pancreas that is similar to the brain is excitatory neurotoxicity. Putting too much of a normal ligand on a neuronal cell causes it to die, and doing the same to a pancreatic cell elicits the same response causing a disease known as pancreatitis. Pancreatitis is a severe inflammatory response of the pancreas that has an appreciable level of lethality. In one instance, research found that developing pancreatitis was not uncommon for workers in the San Joaquin Valley, who were spraying pesticides containing cholinesterase inhibitors. It was thought that their exposures resulted in an over-stimulation of the pancreas, and this situation can be reproduced in an animal model. There are studies just being published whose data suggest the mechanisms of alcohol induced pancreatitis include sensitization and upregulation of cholinergic pathways. Dr. Gorelick offered two considerations in the case of GW veterans' exposures to toxins: 1) he would not be surprised if anyone in the group developed pancreatitis, and imagined that many of their abdominal complaints might actually be acute pancreatitis and 2) since GW veterans' symptoms seem to be long-lasting, there might be a

chronic cholinergic stimulation, predisposing individuals to another form of pancreatitis called chronic pancreatitis. In this situation, the pancreas develops a chronic inflammatory response and is gradually destroyed. Dr. Gorelick has thought about ways to study this in GW veterans, but it is difficult to do in a rational way; the tests they use to determine pancreatic function are invasive. It remains a possibility to study this, but pancreatitis has not become an obvious problem in the group of GW veterans he treats.

Dr. Barlow, a Committee member, discovered that Dr. Gorelick also has an interest in researching Alzheimer's disease proteins. She mentioned that many Alzheimer's patients are on AChE inhibitors, which work through different mechanisms than organophosphates, and wondered if pancreatitis was common in Alzheimer's patients as well. Dr. Gorelick thought Dr. Barlow's question was insightful. He did not recall the relationship ever being recorded, and offered that Dr. Barlow's question should be looked at. Dr. Barlow suggested that looking at it might give some clues as to the specificity of pesticides and organophosphates. Dr. Steele further inquired about general GI symptoms in Alzheimer's patients. Dr. Imaeda replied that she sees diarrhea only as a result of the patients' medication.

Dr. Golomb asked if there were any studies done showing opposite direction effects following withdrawal of AChE inhibitors and if there might be reduced secretion of pancreatic enzymes leading to problems with assimilation and lowering of B12 and carotene levels. Dr. Gorelick noted that this was another insightful question. He mentioned studies, conducted by Dr. Kevin Tracey, which identified characteristics of macrophages and established a direct relationship between the nervous system and inflammatory responses. His studies have been primarily acute studies looking at the elaboration of inflammatory mediators. Dr. Gorelick believes that Dr. Golomb's question would be interesting in two contexts: 1) the long term effects of AChE inhibitors and 2) what happens when the AChE inhibitors are taken away.

Chairman Binns remarked that the RAC has received communication from the Blastocystis Research Foundation related to an ongoing study in Oregon. The study has found a blastocystis infection in a Gulf War veteran. Chairman Binns wondered if Dr. Gorelick or Dr. Imaeda ever tested for that type of infection in their patients. If they had not, Chairman Binns inquired if testing for it or other parasites would be a useful diagnostic tool. Dr. Imaeda replied that they check for parasites in all of the patients that present with either diarrhea or constipation. However, they have not found much blastocystis. Dr. Tuteja said that he is aware of this Blastocystis group, and he and members of the group communicate frequently. He said his own group is checking for parasites as well, but have not found this infection to be prominent. Dr. Gorelick added that parasites are very difficult to identify in stool; even if a person is well trained, they might easily miss finding any parasites. Similar to the need to identify bacteria in the stool, a molecular approach to identifying viruses and parasites needs to be established to decrease the difficulty of detection.

Chairman Binns thanked Drs. Imaeda, Gorelick, and Tuteja for participating in the Committee's meeting.

Chairman Binns introduced Brigadier General Valente (retired).

Results of Rhode Island Persian Gulf War state survey of Gulf War veterans

Brigadier General Richard Valente (retired)
Member of the Rhode Island Persian Gulf War Commission

Gen. Valente spoke about the Rhode Island Persian Gulf War Commission, which was established in 1998 (See Appendix – Presentation 5). The goals of the Commission include locating Gulf War veterans within Rhode Island, creating a registry of these veterans, and tracking their health changes over time. The Department of Defense estimates that approximately 6,500 veterans of the GW were from RI; the Commission has identified 3,500 of those people, and successfully located 241 to date. Gen. Valente presented an overview of the results of a mail-in health survey completed by the 241 veterans.

Following the presentation, Dr. Golomb thanked Gen. Valente for his work. She admitted that his work meant a great deal to veterans and researchers to have somebody from the military as an ally for such an important issue. Gen. Valente thanked Dr. Golomb and informed the Committee of the makeup of the rest of the RI Gulf War Commission. Most of the Commission members have military experience – there is a Vietnam veteran, a Desert Storm veteran, and several other retired military individuals. Gen. Valente is attempting to recruit another member who is a Colonel in the Army Reserve, and works at a combat support hospital. He noted that the bottom line is they are soldiers who are trying to continuously support other soldiers.

From the audience, Dr. Tuteja mentioned that significant numbers of veterans are not seeking care from the VA hospitals. Thus researchers' results may be skewed as all informative data may not be gathered. He wondered how researchers might go about solving this problem. Gen. Valente noted that one of the agencies the Commission reports to is the State Agency in the Department of Health and Human Services. One of the issues these departments deal with is deciding if the state is covering health care costs that should normally be covered on a Federal level. Also, private practitioners might not be familiar with military medicine. He suggested that the information (about the low rates of veterans using VA hospitals for health care) should be made public, and better outreach programs need to be established for the veterans. Gen. Valente concurred that the point Dr. Tuteja raised is an important issue.

Mr. Hardie asked Gen. Valente to elaborate on the process the Commission used, sorting through veterans' DD 214s, to identify Rhode Island's Gulf War veterans. Gen. Valente stated that he, along with the rest of the Commission, would gather at the VA offices to sort through all of the veterans' DD 214s looking for the phrase 'Southwest Asia Medal'. Based on that criterion, the veterans qualified for the registry. Gen. Valente noted that this was a painstaking process, as all of the documents were filed only alphabetically and not by conflict as well.

An audience member, Ms. Denise Nichols, commented on Dr. Tuteja's previous question. She was not surprised that so many veterans seek care outside of the VA because upon returning from the war and seeking care at the VA, they were referred to psychological services. She believes that veterans saw this referral as a 'slap in the face', and decided to seek help elsewhere. Ms. Nichols agrees that the Committee has made advances against this notion, but the veterans are still hesitant to go to the VA because of OIF/OEF. Gen. Valente responded that there's no doubt that the VA has a problem, and explained one way in which the Commission has tried to

help solve it. The Commission was able to have a bus donated for their use; they then located veterans, and bussed them to the VA to have physicals and to be added to the registry.

Mr. Edward Bryan, an audience member and a Massachusetts Gulf War veteran, referred to slides in Gen. Valente's presentation alluding to chemical alarms sounding. He asked if Gen. Valente knew how much time had to elapse between the chemical alarms sounding and the veterans receiving permission to take their protective gear (i.e. masks) off. Gen. Valente replied that he was not sure of the exact regulations. He knew that one person in the group was responsible for giving the 'all clear' notice, and then the gear could be removed. Mr. Bryan believed that a section in the 1990 manual for the military required 45 minutes before the protective gear could be removed.

Ms. Marguerite Knox, a Committee member, informed Gen. Valente that she is currently a member of the South Carolina Army National Guard as a nurse practitioner. She performs health assessments of military personnel before they are deployed, as well as after they return from combat. She noted that soldiers coming back now have had their briefings done by the VA, but have not received proper treatment and have not been assigned primary care providers. Ms. Knox said that many returnees are suffering from PTSD, and if they have Tri-Care through the Guard she encourages them to seek medical treatment from their family physician or another available physician outside the VA. Gen. Valente stated that he understands that when faced with no treatment options at the VA, a person has to go with the most logical choice and seek treatment from somewhere else. Ms. Knox commented that Gen. Valente was doing a great thing by bringing the VA back to his state, and hopefully the Commission's data will help make further changes to serve the RI soldiers.

Chairman Binns thanked Gen. Valente for his presentation, and for the work the Commission is doing in Rhode Island.

Chairman Binns concluded the morning session at 12:40 for lunch, and instructed the Committee and audience to return at 1:45 pm.

The Committee and audience reconvened for the afternoon session at 1:48 pm.

Chairman Binns introduced Dr. Fienberg.

ITI Gulf War research report

Dr. Allen Fienberg
Vice President of Business Development, Intra-cellular Therapies, Inc. (ITI)

Dr. Fienberg presented a progress report (See Appendix – Presentation 6) on the program funded by the Department of Defense (in 2004) at ITI. The main goals of this program include researching the effect of nerve agent exposure on cellular signaling, and developing an antidote and/or treatment for nerve agent exposure. Dr. Fienberg also presented research and findings from other studies at ITI that are relevant to Gulf War Illness research.

During the presentation, Dr. Barlow asked which type of tissue was being used for the sub-convulsant sarin exposure study. Dr. Fienberg replied that the results presented were from striatum tissue. They checked other brain regions as well, but the results were not as pronounced as in the striatum.

Later in the presentation, Dr. Golomb inquired if the low-level sarin doses that the mice were exposed to were enough to produce clinical symptoms. Dr. Fienberg replied that the exposure does not produce symptoms.

Dr. Steele followed that question by asking about the percentage of the LD50. Dr. Fienberg said that he wasn't sure of the exact percentage, but thought it was between 1/10 and 1/20, and probably closer to 1/20.

While Dr. Fienberg was presenting the findings of the low-level sarin exposure study, Dr. Golomb asked if there was any information about baseline levels of T75 in the rats. Dr. Fienberg replied that baseline levels were not known, so comparisons were made relative to the acute exposure information. He noted that the experiment needs to be redone looking at baseline levels and that when the experiment was originally performed, the researchers were not able to check baseline levels.

Dr. Steele asked if Dr. Fienberg could reiterate the scenario for a sub-chronic delay exposure for the rats. Dr. Feinberg detailed the exposure as lasting for 5 hours. The rats were subsequently euthanized 8 weeks later for data collection.

Following the presentation, Dr. Meggs asked about the nature of the antidotes ITI was developing. He wondered if giving the antidote acutely would prevent the delayed development of encephalopathy, and also if the antidote would have any efficacy once the encephalopathy has developed. Dr. Fienberg stated that the antidotes would be for an acute exposure, and the researchers do not yet know if they would help in preventing any delayed changes.

From the audience, Dr. Gorelick asked if researchers had found any effect on any other predicted dark targets during the delayed scenario in the sarin exposure study. Dr. Fienberg replied that they did not find any other changes. Dr. Gorelick also wondered how the T75 generates a phenotype. Dr. Fienberg stated that the mechanism is not clear. The pathway changes under different doses at different time points. He suggested that researchers at ITI need to conduct the experiment several more times to be conclusive about not seeing downstream effects. Change in CDK5 in other systems has a clear effect on synaptic plasticity and learning and memory. Dr. Gorelick asked if Dr. Fienberg's group had ever looked at P11. Dr. Fienberg replied that they had not. Dr. Gorelick wondered if a neurologic phenotype resulted from the delayed exposure. Dr. Fienberg replied that the researchers did not look at that aspect, but did look at levels of AChE in the brain and blood and found a clear inhibition of the enzyme. This inhibition was not persistent.

Dr. Steele asked Dr. Fienberg to explain, in non-neurology terms, what a persistent increase in DARP-32 phosphorylation would look like in a human. Dr. Fienberg replied that the DARP-32 protein and its pathway is clearly involved in the way the brain engages in learning something

new, or in remembering the process of something learned before. Their research so far has not found structural changes, but rather has found changes in signaling and making permanent changes in the brain. Many of the aspects cited as effects of Gulf War Illness - like PTSD and depression - can be thought of as altered signaling that results in persistent memories or inability to learn different ways. Dr. Fienberg's group is seeing changes in signaling proteins related to molecular switches. Subsequent experiments would be to create a mouse lacking CDK5, and as an adult expose it to nerve agents. Then, behavioral deficits arising could be characterized and compared to other mice. One could also test whether the cognitive deficits induced with a nerve agent could be restored with a CDK5 inhibitor.

Chairman Binns thanked Dr. Fienberg for his presentation.

Chairman Binns introduced the next agenda item: presentations from Committee members. Both Dr. Golomb and Dr. Clauw would be presenting findings from their recently published literature, pertaining to Gulf War research.

Update of recent Gulf War research

Dr. Beatrice Golomb
Associate Professor of Internal Medicine, University of California, San Diego
Dr. Daniel Clauw
Professor of Internal Medicine, University of Michigan
Director, Chronic Pain and Fatigue Research Center, University of Michigan

Dr. Golomb presented her information first. She described the findings of her review paper entitled "Acetylcholinesterase inhibitors and Gulf War illness" (*Proceedings of the National Academy of Sciences* 2008; 105(11):4295-4300). She also presented findings from other scientists' recently published literature pertaining to Gulf War Illness research (See Appendix – Presentation 7). These publications included a study of CFS prevalence in Gulf War veterans from the United Kingdom (Ismail 2007), a study of guinea pigs' responses to soman exposures (Johnson 2008), a review paper about the metabolism of chemicals (Hodgson 2007), and a study of the effects of chronic dichlorvos exposure in the rat brain (Kaur 2007).

After the presentation, Rev. Graves mentioned that Dr. Golomb spoke about detoxification. However, Rev. Graves was under the impression that AChE inhibition caused permanent damage, and wondered how the detoxification came into play. Dr. Golomb explained that when she talked about detoxification, she meant changing the form of the chemical agent to a form less toxic to the body. Some damage may have already occurred, but the agent could be converted to a less toxic form. This way, the ongoing damage may be stopped. Rev. Graves asked if the implication of this detoxification was that the progression of ALS might be stopped. Dr. Golomb replied that this is not the case; one theory of conditions like ALS is that an imbalance in pro-oxidant to oxidant forces occurred at some point, possibly due to a genetically vulnerable background. This imbalance lead to mitochondrial DNA damage, which may self-perpetuate once induced - even if the substance that triggered the process, which doesn't have to be an organophosphate, is reduced. Once the process is triggered, it cannot be reversed through detoxification.

Dr. Barlow asked what researchers can do with the information presented in both Dr. Golomb's and Dr. Fienberg's presentations to understand why an exposure to an agent one, two, or five times can cause changes in the long run. She noted that in Dr. Fienberg's presentation, there was a change in the CDK5 phosphorylation that occurred in the sub-chronic delay exposure scenario. Dr. Barlow inquired if CDK5 is involved in the DNA damage pathway – if DNA damage occurs as a result of an exposure, does that lead to a hyperphosphorylation of CDK5, causing it to remain for a long time? Alternatively, Dr. Barlow wondered if an anatomical cellular change, by losing or damaging cells, changes the brain in a fundamental way that is no longer at the molecular level, but at a cellular-physiological level. Therefore, the CDK5 change is a different cellular substrate component, not necessarily something that happened at the molecular level. Dr. Barlow clarified her statements by asking if this is a persistent molecular event, or if a molecular event causes a physical change to the character of the brain. Dr. Golomb responded that her answer would be 'yes' to both. Once the process of oxidative stress is initiated, mitochondrial DNA becomes damaged; damaged mitochondrial DNA leads to more reactive oxygen. Oxidative free radicals are produced mainly in the mitochondria, and more free radicals are produced when the mitochondria are injured. This is because the 13 proteins coded on the mitochondria are all involved in respiratory chain function - damaged mitochondria leads to reduced energetics and increased reactive oxygen species, which can feedback to cause more mitochondrial DNA damage. There's a chronic effect of reactive oxygen species that damages proteins and lipids. Dr. Golomb noted that a second process occurs where sufficient oxidative injury causes furthering of mitochondrial DNA injury, occurring at between 10 and 1,000 times more frequently than nuclear DNA injury because of the proximity to the free radicals, leading to a process that has latency in the development of symptoms. Loss of cellularity can occur by both apoptosis and necrosis. When cell energy demands exceed supply, and injured mitochondrial supply goes down, necrosis can occur. When oxidative stress reaches a significant level, the cell can kill itself through apoptosis.

Dr. Barlow responded that she has an issue with the oxidative stress theory: it would have to be in response to a single event/exposure. There is not much evidence to show how the process actually occurs. She asked Dr. Fienberg about the relationship between changes in CDK5 phosphorylation and any of the pathways that Dr. Golomb mentioned. Both Dr. Golomb and Dr. Fienberg have seen muscarinic changes (in M1 & M3), and Dr. Barlow wondered how they related. Dr. Fienberg replied that they have found changes in M1. He noted that his group was not able to look at structural changes after the dosing in the animals. However, work done by researchers at the Institute for Chemical Defense (ICD) showed that after high dose exposures to sarin, distinct hippocampal cell damage occurs. Dr. Fienberg was unsure if the ICD repeated the experiment with low dose exposures to sarin. Dr. Barlow asked Dr. Fienberg to speculate as to why CDK5 would be hyperphosphorylated so long after a low dose exposure, and if there were certain cell types that do not express it. Dr. Fienberg stated that not every cell type in the brain expresses the protein. This protein is involved in structural changes of the cortex. A knockout of CDK5 is lethal – animals are born with abnormalities in structural and cortical lamination. In the adult, it is not clear what the protein is involved in. If an animal is exposed to something it is not normally exposed to, it is reasonable that some process could induce structural changes. Dr. Golomb added that the typical profile, even with known mitochondrial genetic mutations, is

variable and often has a long latency to onset of symptoms as the process progresses differently in different organs, which are variably sensitive to loss of cellularity.

Rev. Graves asked for a clarification of the difference between apoptosis and necrosis in cells. Dr. Golomb explained that necrosis happens when a cell doesn't have enough energy to live, for example. Conversely, apoptosis occurs when the body kills the cells.

Chairman Binns thanked Dr. Golomb.

Dr. Clauw then presented findings from recent studies of fibromyalgia. These studies included two recently published literature articles for which he was co-author. Dr. Clauw spoke about potential mechanisms for the development of fibromyalgia, reducing pain in fibromyalgia, and brain imaging in patients with fibromyalgia (See Appendix – Presentation 8).

Chairman Binns asked about the use of acupuncture as a means to control the pain experienced by a patient with fibromyalgia (referring to one of Dr. Clauw's published papers investigating Glutamate levels). Dr. Clauw responded that investigators used acupuncture as a non-pharmacological way to predictably engender an improvement in pain and in pain threshold, so that the longitudinal studies were possible. There are some data showing sham acupuncture differing slightly from active acupuncture, but it wasn't the purpose of this study to test that relationship.

Dr. Golomb remembered Dr. Clauw had mentioned that a range of environmental exposures is linked to fibromyalgia. She noted that even though there may be a final common pathway, it does not mean that a certain toxic exposure is not related to the excess risk of these conditions seen in Gulf War veterans. Dr. Clauw agreed with Dr. Golomb. He added that there is a list of drugs that are known to trigger fibromyalgia; however, organophosphates have not yet been shown to trigger the disease. He would still agree with Dr. Golomb that the evidence is accumulating about organophosphates in the setting of the first GW. However, organophosphates were not the only exposure the veterans had. This is similar to fibromyalgia in that a number of different things can trigger the disease.

Dr. Steele followed Dr. Golomb's comments by asking what types of drugs trigger fibromyalgia. Dr. Clauw replied that there are some cases of people who are taking HMG CoA reductase inhibitors and develop severe myalgias and arthralgias – when they are taken off the drugs, these myalgias and arthralgias do not go away. Epstein-Barr virus and other infections can also trigger fibromyalgia; there are a number of different triggers of the onset, and once those triggers are removed, fibromyalgia does not go away. Dr. Golomb added that in the case of statins, multiple studies have determined 1) mitochondrial mechanisms are involved and 2) there are mitochondrial genetic predispositions, especially in people who have genetically low coenzyme Q10 (coQ10) or in people who have a range of other mitochondrial mutations that were previously clinically silent. Upon withdrawal of coQ10, which provides mitochondrial antioxidation, the antioxidant/oxidant balance is shifted, and causes the process to be demonstrated in this vulnerable group. There are also biopsy studies showing evidence of this process following withdrawal, including partial reversal of the mitochondrial pathology.

Dr. Tuteja was curious if changes in opioid receptors seen on the PET scans (referring to findings from one of Dr. Clauw's studies) were a cause or a result of the problem. Dr. Clauw hypothesized that the changes were a result. The scans showed a hyperactivity of the opioidergic system – which would cause decreased pain, rather than increased pain. Results of the opioid binding studies plus the cerebrospinal fluid levels of enkephalins and endorphins (twice as high in patients with fibromyalgia than in controls) leads Dr. Clauw to hypothesize that endogenous opioids are being bound – there's not a receptor open for an exogenous PET ligand. The opioid system seems to be functioning as it should, releasing endorphins and enkephalins, but does not seem to be enough to reduce pain.

Dr. Bloom wondered if Dr. Clauw's Institutional Review Board (IRB) would allow treating fibromyalgia patients with malaxon. Dr. Clauw stated that they have previously proposed the study through National Institute of Health (NIH) grants. Low-dose malaxon is an intriguing way to attempt to interrupt the loop, and in previous trials has not been found to cause worse pain. Dr. Clauw's IRB would allow the treatment, but their research group has not yet been funded for the study. Chairman Binns added that there is a study of low-dose naltrexone currently occurring at Stanford.

Chairman Binns thanked Dr. Clauw for his presentation.

At 3:20, Chairman Binns called a recess in the meeting for a 15-minute break.

The meeting began again at 3:38 pm.

Chairman Binns introduced Dr. Goldberg, the Committee's Designated Federal Officer.

Update of VA Gulf War research

Dr. William Goldberg
Gulf War Research Portfolio Manager, VA Office of Research and Development

Dr. Goldberg stated that he would have liked to present the annual report to Congress, but it was not yet fully completed and approved. Hopefully by the next meeting, he will be able to present the full report and hand out hard copies. He noted that since the data was public and had to do with federal funding, he could speak briefly about the financial portfolio. The Department of Defense (DOD) FY '07 expenditure for Gulf War research was approximately $3.4 million. The Department of Health and Human Services (HHS) is still funding about half a million dollars. The VA committed $21.7 million to Gulf War research; $15 million of that was contracted for the research program at the University of Texas Southwestern Medical School (UTSW), headed by Dr. Robert Haley. The total expenditure for GW research for FY '07 was approximately $25 million.

The other matter Dr. Goldberg spoke about was the VA's announcement for a new program designated to clinical trials (See Appendix – Handout 1). The program aims at linking small clinical trials with single-site investigators to the Cooperative Studies Coordinating Centers. The coordinating centers have large amounts of resources and design biostatistics to better write and

prepare small clinical trial proposals. In this way, the VA can better identify and fund more small trials aiming at developing new treatments for veterans' issues, including GW veterans. Dr. Goldberg hopes that through this new program, investigators will be able to develop protocols designed to treat GW veterans.

Dr. Goldberg welcomed questions from the Committee and audience.

Dr. Steele asked if funded Gulf War research will be posted on the website http://clinicaltrials.gov so veterans can easily find the studies and related information. Dr. Goldberg stated that by law, every clinical trial funded by the VA or NIH has to be registered with this website. Trials that are not posted before they begin will not have their data published by medical journals. Dr. Steele mentioned that last year Dr. Goldberg had informed the group that any Gulf War studies recruiting human subjects also had to be on the site. Dr. Goldberg replied that they were still trying to get all of those studies registered with the site. The website is a requirement for all interventional studies, but optional for other human studies that recruit patients but do not study interventional treatments. Dr. Barlow further inquired if the studies posted on the website will be left up after they have been completed. Dr. Goldberg responded that there is no reason that they shouldn't be able to leave the studies on the website as there is a date that each study ends. Rev. Graves suggested that the site have a link to a page entitled 'previous trials'. Dr. Goldberg said he will talk to the people in charge of registering the studies on the website to make sure the studies remain on the site after their completion.

Dr. Golomb wondered if in these studies, a VA employee has to be at 5/8 time in order to be a principal investigator. Dr. Goldberg responded that there is a mechanism by which clinicians that are less than 5/8 can request a waiver to have the requirement lifted. Dr. Golomb was advised that the VA was becoming more stringent in upholding the 5/8 rule for principal investigators. Dr. Goldberg noted that the eligibility requirements are very clear in order to be a 5/8 VA employee. However, there is also a set of rules allowing a waiver from this requirement. Local VA has no authority to approve this waiver – it must go through Central Office.

Chairman Binns asked if there was an amount budgeted for the new clinical trials program. Dr. Goldberg replied that there was no amount budgeted because there is no way to know what types of proposals will come in. They are willing to examine all incoming proposals and develop the budget accordingly. If resources need to be shifted for the program, it will be done to the best of the VA's ability. Dr. Goldberg added that every VA research office received notice of the new clinical trials program. Notice of the program came to Dr. Goldberg through his director, Dr. Tim O'Leary, who is also the Acting Deputy Chief Research & Development Officer (CRADO).

Chairman Binns thanked Dr. Goldberg for the information he shared with the Committee.

Chairman Binns asked Dr. Steele to inform the Committee about DOD's plan for funding Gulf War related research for FY '09.

Dr. Steele noted that in FY '06, DOD designated $5 million for GW research through a new Congressionally Directed Medical Research Program (CDMRP). The program focused on identifying treatments, diagnostic procedures, and underlying mechanisms for GW illness.

Projects were subsequently funded, but it initially was unclear what these specific projects were. Now, a list is posted on the RAC website (http://www1.va.gov/RAC-GWVI/) under the link called 'Recently Funded Gulf War Research'. In FY '07, there was no money designated for this research program from DOD. Conversely, in FY '08, $10 million was available for this CDMRP research program. In reviewing proposals for the funding, DOD has a two-tiered process. The first tier is a scientific merit review conducted by scientists with areas of expertise relevant to the proposals. The second tier is an integration review conducted by a panel of people who decide whether the proposal is in line with the program's objectives. A number of the RAC members have been involved in the second tier review for the CDMRP program. For FY '08 funding Mr. Hardie will be on the panel, and Dr. Steele will be chairing the panel. The process has begun to release a funding announcement and a request for proposals, but it is still early in the process. Most of the goals of the funding announcement are determined by the Congressional language that allocated the money to DOD. Dr. Steele noted that the discussions of the panel are strictly confidential, but she could share that the funding announcement will be out by mid-May, it is for $10 million, and there are a variety of mechanisms that will be allowed. Investigators will be required to submit a pre-proposal; the pre-proposals will be reviewed and then select investigators will be invited to submit full proposals. On the RAC website, there is a link to the website for the CDMRP research program for GW veterans. Individuals can also go to the DOD website and find an abstract for every GW related research study that has ever been done. A lot of research has been completed, but not all findings were published. Looking at this website will give an indication as to the types of research the DOD has previously funded.

Chairman Binns thanked Dr. Steele for her brief presentation as well as her service to the DOD review panel. He also thanked Mr. Hardie for his service to the panel.

Chairman Binns introduced Dr. Fiore and Dr. Kowall.

Update of VA Gulf War brain bank

Dr. Louis Fiore
Director, VA Clinical Trials Coordinating Center, VA Boston Healthcare System
Chief of General Internal Medicine, VA Boston Healthcare System
Dr. Neil Kowall
Chief of Neurology, VA Boston Healthcare System
Director, VA New England Geriatric Research Education and Clinical Center

Drs. Fiore and Kowall spoke about the eventual establishment of a Gulf War veteran brain bank (See Appendix – Presentation 9). The brain bank was established in May of 2006. Since that time, a facility was designated, equipment was obtained, staff was hired, and brains from patients once suffering from ALS have begun to be collected. However, to date no brains from Gulf War veteran patients have been collected. Establishing this brain bank will allow for stores of tissues to be distributed and studied by various interested study investigators.

During their presentation, Rev. Graves asked if the brain bank had an established relationship with the VA hospitals so that they would be notified when a patient is dying. Dr. Fiore explained the process by which his group has decided to obtain the brain tissue. In brief, they will be

combing through regional databases of patient files to make a note of the patients who might qualify to donate their brain to the bank. They will then contact these patients and ask for their consent as well as their families' consent.

After Dr. Fiore explained the process, Rev. Graves had a suggestion. He mentioned that when a patient dies in the ER, by law the tissue bank must immediately be notified and the patient's family is asked if they would be willing to donate the patient's organs. He suggested that the CRADO of the VA establish a procedure for the emergency rooms: when a Gulf War veteran is about to die, or has just died, contact the brain bank to let them know about the patient. Rev. Graves noted that Dr. Fiore's method for finding potential donor seemed labor and manpower intensive, and may not be as easy or practical as getting the CRADO involved. From the Committee table, Dr. Goldberg explained that unfortunately the CRADO cannot make this edict. This is a medical care/medical center issue that the Secretary of the VA would have to review and implement. Dr. Goldberg added that the text-mining technology that Dr. Fiore spoke about using to identify potential brain bank donors is very good at this identification. However, he agreed that having the ER call when a patient dies would be a fairly immediate way to notify the brain bank of a potential donor.

During his presentation, Dr. Fiore posed a question to the Committee: is there a Gulf War veteran registry list that his group can use to cross reference with potential donors for the brain bank? Dr. Steele responded that there is a Gulf War registry for veterans, and most of these veterans have a type of multisymptom illness. When joining the registry, veterans are given a questionnaire that includes items about the different exposure types experienced in theatre. There are about 100,000 veterans on the VA registry, and the data is housed in Austin. There is also a comparable DOD registry for Gulf War veterans, but Dr. Fiore might not have access to this database.

Dr. Steele noted that the Committee had previously suggested the VA establish some data-mining protocols to develop a database from VA clinical data. Doing this would facilitate determining which veterans have GW illness, and identifying what their symptoms are, what their diagnoses are, who is treating them, if they are getting better, etc. The Committee has been repeatedly told that it is impossible to use VA clinical records to identify these data. Dr. Steele suggested that before Dr. Fiore's group relies only on data mining, they have a trial run to ensure they get the results they are looking for. Rev. Graves mentioned that various places, such as Seattle, have Gulf War clinics - contacting these places might help to narrow the types of patients the brain bank is targeting for donation. Dr. Steele noted that the problem with this idea is that people do not die from Gulf War related illnesses, and the brain bank would still need to be notified if the patient was about to die or had just died.

Also during the presentation, Ms. Knox noted that Gulf War veteran patients have surgery every day in the clinics; the tissue they have removed is normally discarded. She wondered if the tissue bank could collect this tissue and distribute it to investigators studying various aspects of GW illnesses. Dr. Fiore stated that he appreciated Ms. Knox's comment, and that they have only begun to establish the brain bank so far. The next step would be to reach out to VA clinics and other VA facilities via a website to allow them to notify the bank when other bodily tissue could be donated.

Following their presentation, Drs. Fiore and Kowall invited questions and comments from the Committee and audience.

Dr. Steele mentioned that a newsletter is distributed to all the veterans on the Gulf War registry. She suggested advertising the existence of the brain bank through the newsletter so veterans and their families would be aware of a possible donation. Dr. Fiore replied that Dr. Steele's idea was wonderful - and the option should be given to the veterans.

Mr. Hardie inquired about the length of time after a patient dies before their brain can be harvested for donation. Dr. Fiore responded that for the central nervous system and spinal cord, a beautiful sample is harvested less than 10 hours after death and a very useful sample is collected between 10 and 20 hours after death; at more than 20 hours after death, various factors come into play (such as whether the body was housed in a cold room) that determine if the tissue can be harvested. Mr. Hardie also asked if a patient has to die in a VA medical center in order to donate to the brain bank. Dr. Fiore replied that this does not have to be the case. Their group has tapped into various sources nationwide that provide people who can get to a donor within 2 hours of the notification that the donor has died. These people harvest the tissue and ship it through private couriers to the appropriate location, where the tissue is immediately processed. Mr. Hardie further asked about the consenting process for the donation: if the patient has consented to donate before his or her death, does that give the brain bank the legal right to harvest the tissue in all cases? Dr. Kowall replied that the law differs by state. After a person's death in Massachusetts, the next of kin becomes responsible for the body - no matter what the patient consented to while still alive. The brain bank is not able to rely solely on pre-death consent; the family must also be in accordance with the deceased's wishes. On a related note, a patient does not necessarily need to consent to the donation of his or her tissue while still alive; the family can donate the tissue following their death.

Dr. White commented that Dr. Brown, the president of BU, has just begun a content oriented interdisciplinary initiative; Dr. White will be heading one of the groups, as will Dr. Kowall. Dr. White wanted to know if Dr. Kowall planned to include any Gulf War issues or brain bank details as part of his working group. Dr. Kowall stated that at this point he had not planned to include either issue because his involvement in the BU program is still at an early stage. In the future, this could be a topic of discussion.

Dr. Goldberg added that the VA has begun to discuss with DOD about the Gulf War registry, and about the ALS tissue bank. The two agencies are debating making a concerted effort to determine what the main priorities should be for research conducted on the collected tissues. The VA will continue to maintain the ALS registry, and is speaking to DOD about combining the registry with the information they have available, especially for active duty personnel. Dr. Goldberg also mentioned that Dr. O'Leary has initiated a new program at the VA in pharmacogenomics. Within that program will be a genome-wide association study for ALS, and they are hoping to get DOD involved as well. VA is also hoping to gain a better understanding of sporadic ALS vs. familial ALS through this study.

Ms. Knox added that she has learned medical records from the VA and DOD might soon be linked via computer so that both agencies have access to military personnel's medical history. Dr. Goldberg noted that the merging process is still ongoing and is an active effort by both agencies.

Dr. Steele asked if either Dr. Fiore or Dr. Kowall could estimate the length of time that a patient has to live following a diagnosis of ALS, and if this timeframe was different for GW veterans. Dr. Fiore stated that he was not sure if there were differences in the timeframe. Dr. Goldberg noted that in general, death occurs approximately 2 years after the diagnosis of ALS. Dr. Golomb added that a recent study of patients with ALS and hyperlipidemia showed the median time range to live following diagnosis of ALS to be 5 years. She also mentioned that an interesting effect of lithium is that it increases cholesterol levels, showing a potential mechanism. Dr. Steele asked if Dr. Goldberg had seen the data from Gulf War veterans with ALS. Dr. Goldberg had not seen this database, but did not think that anyone maintaining the registry had noticed a difference in survival time for GW veterans.

Dr. Steele inquired if any Gulf War veterans have sporadically contacted the brain bank to donate their tissue upon the time of their death. Dr. Fiore noted that the mission of the tissue bank they manage is to collect brain and spinal cord tissue. They have been contacted about the donation of other types of tissue, but not yet for the central nervous system.

From the audience, Ms. Nichols suggested contacting coroners, in addition to the contacts Dr. Fiore and his group were planning to make, to inform them about the brain bank. She also suggested letting Service Officers know about the possibility of donation and preparing flyers so they could pass the information on to veterans they work with.

Mrs. Angie Newbold, an audience member and the mother of a Gulf War veteran with multiple sclerosis (MS), spoke to Drs. Fiore and Kowall. She said that this topic is very emotional for her. Mrs. Newbold observed that there were numerous research studies of Gulf War veterans on both the east and the west coasts, but the information learned does not seem to be making its way to the small VA facilities elsewhere in the country. In Nebraska, where Mrs. Newbold and her family are from, providers do not appear to be knowledgeable about Gulf War research and illnesses. For example, her son had an appointment with the psychology department to again change his medications because they do not work well for him. He asked the psychologist if any of his symptoms could be due to Gulf War related issues; the psychologist replied that she was unfamiliar with the illness, and did not have any information about it. Mrs. Newbold connected this experience with the fact that it would be extremely hard for her son, any other veteran, or any veterans' family members to consider making a tissue donation to research when they feel their medical needs are not being sufficiently met by the VA. She suggested that reaching out to all VA hospitals and training them to recognize, or at least acknowledge, Gulf War illness would make veterans and their families more willing to donate. Overcoming the frustration that many GW veterans have experienced in receiving treatment is the first step in getting them to donate to the brain bank or other related research interests. Dr. Fiore thanked Mrs. Newbold for her comments. He stated that in contacting patients who might be potential donors to the brain bank, he often hears the same concerns that Mrs. Newbold mentioned. His group and others are

working hard to attempt to rectify the feelings of GW veterans, and they understand veterans' frustration.

Dr. Golomb noted that it was not the charge of the Committee to inform doctors at the VA about Gulf War Illnesses; however, the Committee has previously discussed distributing some type of educational material to facilities and physicians. The materials previously distributed to physicians suggested that GW illness was psychologically related; now that researchers know this is not the case, nothing has replaced the old educational media. Dr. Golomb mentioned an email she had recently received from a veteran who was seeking treatment for GW illness; doctors used the words 'psychogenic' and 'malingering' to describe his condition. Though there are currently no good treatment options for the illness, Dr. Golomb suggested providing material to reeducate VA clinicians about diagnosing GW illness. Dr. Goldberg responded that the VA is in the process of constructing an additional Gulf War Advisory Committee. This committee will be in charge of monitoring health care, benefits, and other issues related to service in the first Gulf War. The VA is currently in the process of nominating people to serve on this committee. Chairman Binns noted that the Committee has recommended the VA update educational materials; however, this has not yet been accomplished. He has been in contact with Dr. Deyton, an official at the VA who is interested in correcting the inaccurate guidelines surrounding the care of ill Gulf War veterans.

From the audience, Gen. Valente added to this discussion. He shared a story with the Committee about a Retired Brigadier General with whom he was friends. This gentleman had served in 4 separate conflicts, and imparted the following advice to Gen Valente: 1) a General is responsible for teaching his unit about the history of the unit and 2) a General should always take care of his soldiers. Gen. Valente said that telling this story was a way to emphasize that the VA should be advised to 'take care of your soldiers'.

Chairman Binns thanked Drs. Fiore and Kowall for their informative presentation.

Chairman Binns invited members of the audience wishing to make a public comment to speak at the microphone.

Public Comment – Day 1

Mr. Erwin Steffen, who is the husband of Mrs. Newbold and the stepfather of her veteran son suffering from MS, spoke to the Committee. He expressed concern for his stepson, who is trying to seek appropriate treatment for his MS within the VA. His stepson has had lesions on his brain for years, but has not started treatments until recently. Mr. Steffen also noted his frustration with the methodology by which his stepson has to acquire the appropriate treatment.

Chairman Binns thanked Mr. Steffen for his comments.

Ms. Alison Johnson, Chair of the Chemical Sensitivity Foundation, wished to address the Committee. She noted her willingness to provide copies of her book and DVD detailing Gulf War Syndrome to anybody that wanted more information. Ms. Johnson expressed her concern at the lack of public knowledge about Multiple Chemical Sensitivity. She asked the Committee to

make a statement to the public that would validate MCS as a real condition. Ms. Johnson shared the experience of a Gulf War veteran named Tara. While in the service, Tara became sick from breathing paint fumes while painting vehicles. Since then, she has suffered from MCS. In her recent job as a prison nurse, Tara had problems convincing some of her coworkers of her condition. She is now working at a VA, and still encounters people who do not believe she has a real condition. Another instance that Ms. Johnson wanted to share with the Committee was of a woman living in California. The woman and her son both suffered from MCS; upon mentioning to a new doctor that they had the condition, the woman was reported to child protective services and was threatened to have her child removed from her custody. Ms. Johnson noted that she knows of many other cases in which people suffering from MCS have not been recognized as having a real illness. In addition, Ms. Johnson pointed out that she has recently produced a 15 minute documentary about MCS. The public can view this documentary on her website for free. She again expressed her hope that the Committee can make an effort to validate MCS as a real disorder.

Chairman Binns thanked Ms. Johnson for her remarks.

Ms. Denise Nichols thanked the Committee members for their efforts in deciphering Gulf War illness. She suggested that the Committee make every aspect of the meetings public after they occurred. By videotaping the meetings and posting the speakers' presentation slides online, people who were unable to attend the meeting can be informed about the meetings' proceedings. Also, clinicians would be able to view the meetings and thus might become better aware of Gulf War illness.

Chairman Binns thanked Ms. Nichols for her comments.

Chairman Binns asked the Committee to review the recommendations that would be made for the UTSW research program during Tuesday's meeting. He also reminded the Committee members to sign the memento 2004 report for Dr. Steele.

Chairman Binns concluded the day's meeting at 5:11 pm.

Day 2

Chairman Binns began the second day of the meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses at 8:31 am. The meeting was again held in Room 462/462A of the Crosstown Center Building, at Boston University School of Public Health, 801 Massachusetts Avenue, Boston, MA. Dr. Daniel Clauw was not in attendance.

Chairman Binns asked Dr. Steele to begin her portion of the meeting's discussion.

2008 Research Advisory Committee report discussion

Dr. Lea Steele
Past Scientific Director, Research Advisory Committee on Gulf War Veterans Illnesses

Dr. Steele handed out a new draft report of the 2008 Committee Report to the members of the Committee. She asked the Committee to read through the draft and to let her know if they had any suggestions for changes or any comments about the text. She expects the report to be completed within the next few months. The completed report will contain five sections with Committee recommendations at the end of each section. For this portion of the meeting, Dr. Steele wanted to discuss alterations in wording of the recommendations made since the previous draft had been given to the Committee.

The first recommendation Dr. Steele discussed with the Committee came at the end of the section on epidemiological research studies. The highest priority for the Committee is identifying treatments for GW illness. The newly added recommendation to achieve this priority reads: "conducting research to evaluate novel therapies based on scientific results presented in this report". If someone has an idea of the pathophysiology behind of GW illness, and there are novel therapies to treat those pathophysiologies, research should be conducted accordingly. Dr. Bloom was concerned that this recommendation does not point specifically to the scientific results that might be noteworthy for novel therapies to be derived. Dr. Steele responded that in this section of text, studies about the hypothalamic-pituitary-adrenal (HPA) axis in GW illness, immune parameters, and other categories of biological processes are presented. Dr. Bloom suggested that the recommendation instead read: "conducting research to evaluate novel therapies based on scientific results". Dr. Steele suggested: "conducting research to evaluate novel therapies based on scientific results as they emerge". Chairman Binns added that this statement should connect with results presented in the report. Dr. Steele said that she would think about how to word it properly to connect to the report as well as with future research findings. Chairman Binns stated that the way it was worded currently may be too strong; Dr. Golomb added that the wording might also be too limiting.

The second point Dr. Steele wanted to discuss was the current longitudinal study being done by a Dr. Han Kang of the VA Environmental Agents Service. In the 1990's Dr. Kang studied a group of 11,000 GW deployed veterans and 10,000 non deployed veterans. At about the same time the Committee began, Dr. Kang began follow-up studies with his original study population. However, none of the data from his research has been published. One recommendation Dr. Steele included for Dr. Kang's research is having this data published as soon as possible. Also,

the recommendation asks Dr. Kang to identify and analyze data from subgroups within the 'deployed' and 'non-deployed' categories of veterans. Dr. Steele asked the Committee if they had any reservations about this recommendation; nobody did. A second suggestion for Dr. Kang's research is that his group continues to conduct the longitudinal surveys at regular intervals. Dr. Steele asked the Committee to help define what the 'regular interval' should be. Dr. Meggs thought the Committee could specify an interval of 5-10 years for a follow-up study. The problem with waiting 10 years for follow-up is the possibility of losing track of the study participants during that time. Five years would be optimal, but one always runs into the issue of finding funding for the research. Dr. Golomb agreed with Dr. Meggs' comments. Dr. Steele and the Committee agreed to set the interval between the collections of new data for the longitudinal study at 5 years.

Another area of recommendations Dr. Steele wanted to discuss pertained to federal funding for Gulf War related research. The recommendations followed the section in the report identifying 1) funding levels from year to year for various agencies supporting GW research 2) different types of funded studies, and whether they fit in with the goals of GW research and 3) other programs at VA that are not specifically research, i.e. benefits and healthcare. In previous reports, the Committee suggested that the government allocate $45 million annually for Gulf War research. However, to reflect inflation rates the Committee now recommends $60 million be allocated annually for GW research; $40 million would go to DOD (to the CDMRP program), and $20 million would go to VA ($15 million of which is for the UTSW program). Chairman Binns added that the Committee should make a special note in this recommendation that the funding for the program at UTSW is $15 million annually through FY '10.

Dr. Steele noted that this Committee is lucky to have the opportunity to oversee all the numerous studies dedicated to Gulf War veterans' health concerns. Chairman Binns commented that he is responsible for the delay in getting this report finished; Dr. Steele has been working very hard to complete it and is often interrupted by other tasks Chairman Binns asks her to manage.

Dr. Meggs mentioned that one of the Committee's first recommendations as a Committee was to broaden funding for GW research to agencies in addition to the VA. Now with the CDMRP, this recommendation has been achieved. Looking back on the last six years, funding has changed for the better.

Dr. Steele asked the Committee to send their comments and suggestions to her via email or phone call. She emphasized that no member should hesitate in expressing their opinion about anything in the report. This way, it will reflect the views of all the Committee members.

Dr. Golomb wondered if there should be any additional funds targeted though NIH in addition to funds coming from military institutions. Chairman Binns answered that he would be open to discussion on the topic. He noted that NIH was once thought to be the 'great white knight' because it did not have any military affiliation. However, now with the DOD CDMRP program established, this discrimination may have been eliminated. If CDMRP should fail, the Committee could recommend establishing a relationship with NIH for more funding. Dr. Meggs added that he believes there is now a mechanism for investigators to submit proposals coming from any quarter; until a time when the process no longer remains objective, the Committee

should give the CDMRP program a chance. Chairman Binns mentioned that there are programs at NIH that deal with related issues, and wondered if the language of the Committee's recommendation is strong enough in terms of coordinating with other research institutions or other governmental research. Dr. Steele noted that the Committee has previously discussed this issue in a vague way. She also informed the Committee that there was a recommendation in another section of the report suggesting that agencies conducting research in similar areas of GW related illness coordinate with each other.

Dr. Steele asked if any other member of the Committee had any other comments or questions.

Chairman Binns thanked Dr. Steele for leading the discussion on the 2008 Committee report.

University of Texas Southwestern Medical Center research program discussion

Mr. James Binns
Chairman, Research Advisory Committee on Gulf War Veterans' Illnesses

Chairman Binns lead the discussion about the Committee's recommendations for the research program at the University of Texas. For each recommendation the Committee presented, Dr. Robert Haley - the head of the research program at UTSW - was asked to comment.

General Recommendations

After giving background information (See Appendix - Presentation 10) about the UTSW research program, Chairman Binns presented General Recommendation # 1: UTSW should "establish an 'expert panel' of outside experts (including some Committee members) who are knowledgeable in GW illness research. This panel should identify key research issues and provide guidance on the types and characteristics of studies to be included in the plan, as envisioned in the 2004 Committee Report".

Chairman Binns stated that it is not an obligation for any of the Committee members to serve on this 'expert panel'. However, it would be greatly appreciated if some Committee members could share their expertise with Dr. Haley and his group if they were asked. In addition to GW illness expertise, the panel should consist of members who have fields of expertise in other areas. Chairman Binns believes that this recommendation is the 'cornerstone' for all the other recommendations. Dr. Haley agreed that this recommendation is a good idea and that his group will pursue it.

Chairman Binns read General Recommendation # 2: "The Committee recommends that UTSW also utilize the expert panel as liaison to the UTSW merit review group, to provide guidance on the degree to which proposed studies address key issues relevant to the health of Gulf War veterans". Chairman Binns further explained that as individual studies are proposed, the panel will review the degree to which the proposal is in line with the goals of GW research. Currently, the UTSW program has a panel that reviews study proposals for their scientific merit; this panel is not made up of members that have expertise in GW illnesses. The proposed 'expert panel' would work in conjunction with the merit review group. Dr. Steele added that a proposed study

can be supported by 'outstanding science' and produce 'important results', but the study may not be answering the proper questions about GW illness.

Dr. Haley responded that this recommendation is complicated by the contract set up at UTSW. The wording of the contract very clearly states that review of proposals should be carried out by a Merit Review Committee. Right now, Dr. Haley has no interaction with the Merit Review Committee. Dr. Gilman, the Deputy Program Director for the GW research program at UTSW, is the liaison between Dr. Haley and the University. The University is at a large financial risk in hosting the program, so is invested in the review process for the program. Dr. Perry Adams, the Vice President for Research Administration at UTSW, is the liaison between Dr. Haley and Dr. Gilman. Dr. Haley said that he will discuss this recommendation with Dr. Adams upon his return to Texas because he does not directly have input into the merit review process.

Dr. O'Callaghan noted that using the expert panel as a liaison will not change the function of the Merit Review Committee. The proposals will still be reviewed on a scientific basis. Dr. Haley replied that he does not see any conceptual problem with this recommendation, but the Merit Review Committee might not be accepting of advice from another committee. Drs. Meggs, Steele and Haley discussed the possibility of changing the wording of the recommendation. Dr. O'Callaghan noted that in implementing this recommendation, Dr. Perry's role at UTSW would be expanded slightly to communicate with the review committee on an advisory basis.

Dr. Goldberg added that the research program at UTSW was established through a legally binding contract between the VA and UTSW. Under the conditions of the contract, VA is prohibited from any decision-making processes involved in the research design. The only input the VA can make is determining if projects approved by UTSW fit the program's original statement of work. The contract states that approval for research projects comes from within the University. Chairman Binns noted that the RAC is not the VA, and is simply making recommendations that may or may not be implemented by the Gulf War research program at UTSW. Dr. Bloom suggested an alternate way to word the recommendation; Drs. Steele, Haley, and Meggs concurred.

Dr. Haley further explained the terms of the UTSW contract with VA to the Committee. The University fronts the money needed to conduct the research program; the VA evaluates the money spent and subsequently reimburses the University. Dr. Adams' role is to oversee the research and ensure 'good science' is being conducted so that the VA will ultimately reimburse the University. Dr. Haley expressed concern that the University might not be willing to have a panel from outside the University directing research decisions when they are handling such a large financial obligation. Dr. Haley and Dr. Steele discussed the role of the Contract Officer in determining the relevance of research conducted. Dr. Haley mentioned that he does not think it will be a problem to eventually implement this recommendation, but wanted to make the Committee aware that he might incur some opposition from the University. Dr. Haley and the Committee members decided to keep the recommendation's wording as it originally was presented.

Chairman Binns read General Recommendation # 3: "The Committee welcomes UTSW's practice to seek the input of knowledgeable outside scientists regarding individual studies. The

Committee recommends that UTSW continue to contract out studies or functions that outside research teams are best able to provide." Dr. White stated that the way the recommendation was written sounded bland, but some of the Committee members were disappointed there was not more outside contracting for research projects.

Dr. Haley agreed that contracting outside investigators is a major commitment of UTSW. Currently, projects are being formulated in conjunction with six other universities, including Johns Hopkins University and the University of Florida. Dr. Haley noted that the Information Security changes taking place within the VA system slow his ability to set up outside contracts. Setting up security clearances with each outside institution takes time; however, investigators at the UTSW research program are eager to expand their research to outside sources.

Chairman Binns read General Recommendation # 4: "The Committee notes with approval that UTSW envisions adding other research components to the plan in the future, including clinical translation of its research results and the conduct of a genome study. The Committee recommends that UTSW add a clinical translation component now. The lead PI should ensure that potential applications of UTSW research to treatments and diagnostic tests are considered at every stage of the program." Chairman Binns added that while there might not be a plan now at UTSW for either a clinical trial or a pilot study of a treatment protocol, it might benefit the program to have a senior investigator begin planning for the future proposals and studies. Dr. Meggs questioned the ability of the UTSW program to implement a clinical translation component 'now' when they have nothing yet to translate. Dr. White mentioned that the reason the recommendation was worded as such was to emphasize that similar types of research programs at other institutions have a clinical translation component in place from the start. A 'translation team' would be identifying possible outcomes of ongoing research and consequently would plan for future projects, including translational clinical trials, changes in the direction of the research proposals, etc. Dr. Steele added that this 'translation team' would also direct and advise future clinical research by suggesting methodologies that have shown to be beneficial in other clinical trials documented in the literature. Dr. White further suggested that there be one single PI in charge of this aspect of the program's research.

Dr. Haley agreed that this point is important. He noted that the program does not have a specific group that holds this title, but this type of future thinking has been underway since the beginning of the program. In establishing the research contract between the University and the Dallas VA, one of the stipulations was that UTSW establish a clinical translation center. After several months of planning, Dr. Haley and his team built a model Gulf War clinic, where they planned to implement eventual findings from their ongoing studies. The clinic included a health services research component and a section to run clinical trials. At the beginning of the research program, Dr. Haley's group purchased a 3T Magnetic Resonance Imaging scanner to also house at this clinic. Also, Dr. Haley began working with Dr. John Hart, a cognitive neurologist who has established Vietnam veterans' and Gulf War veterans' clinics at the Little Rock VA. Dr. Hart is responsible for directing future research within the UTSW program. In developing ongoing research projects, the research teams have discussed the potential clinical or diagnostic applications that could stem from projects' outcomes. Because future research planning is already occurring for the Gulf War research program, Dr. Haley questioned whether he needs to reorganize the system and establish a 'visible team' to handle this issue.

Dr. Steele wondered if there would be scientists with pharmacological expertise housed within this clinic as well. Dr. Haley stated that there would be. Dr. Barlow inquired if the recommendation was stemming from the Committee's concern that there was not a researcher with pharmacological expertise included in the list of the research program's current researchers, or that there was not anyone with clinical expertise within the list. After listening to the discussion, Dr. Barlow concluded that the point of the recommendation seemed to suggest acquiring expertise in the area of translating research findings, from animal trials for example, into studies that would become an effective clinical trial. Dr. White replied that this recommendation was simply a conceptual way to approach this type of research in a large center with many concurrent research programs. A team should be appointed to be knowledgeable about the ongoing research studies and to integrate those findings into future related clinical translational studies.

Dr. Bloom noted that in speaking with Dr. Haley, he has become aware of Dr. Haley's group's ideas for future research. This recommendation gives the group a means to formally develop the clinical translational component. Dr. Meggs expressed his view that the way the recommendation was worded seemed like an insult because it implied that some component of the UTSW research program was missing – clinical translation. He suggested the Committee define exactly what was meant by 'clinical translation component'. Dr. Barlow commented that she believes the point of the recommendation was to have some concrete evidence of a global plan in place for the direction of the UTSW research program. Chairman Binns added that this recommendation implies that organizational grounds need to be established for managing the $15 million per year.

Dr. Haley suggested changing the wording of the recommendation; he and the Committee extensively discussed the change. The Committee decided to reword the second half of the recommendation so that the need to add a 'project manager' to be in charge of the future research oversight was evident.

Chairman Binns read General Recommendation # 5: "The Committee recommends that UTSW add a genetics/genomics component to the plan now. The plan should address other physiologic and pathophysiological processes potentially associated with Gulf War illness, including associations between Gulf War illness and central neuroinflammatory processes, immunological measures, and overlaps between systems (e.g., neuroimmune/autonomic processes potentially affected by toxic exposures)."

Dr. Haley asked that the word 'add' in the recommendation be changed to 'expand'. Chairman Binns complied with the request. He added that the recommendation's purpose is to achieve a formal way of addressing a genetics/genomics component of the Gulf War research program. Dr. Haley concurred with the recommendation.

Chairman Binns presented General Recommendation # 6: "The Committee recommends that UTSW incorporate the overall research plan, and the structures and studies developed to execute the plan, into a program document comparable to the format utilized for a major NIH research center. This program document would include information on the objectives and hypotheses of

the program and of each study, the design of each study, and how individual studies and components relate to one another. It would also describe the program's management structure, including provisions to ensure the program is managed as a coherent whole."

Dr. Barlow asked if Dr. Haley had the opportunity to have a program manager working solely for him without any influence from the VA or UTSW, and if that person could prepare the document proposed. Dr. Haley replied that he did have that person, but he/she would not be responsible for preparing the document. Dr. Haley added that he had recently composed a draft of this document; it is now circulating, for comments/alterations, among individuals involved in the research program. He will eventually send it to the RAC to ask for the Committee's input. Dr. Barlow suggested hiring an individual whose only job would be to prepare documents for the research program; this would allow Dr. Haley to spend time task-managing, strategizing, and evaluating research. Chairman Binns agreed with Dr. Barlow's statements and further stated that in the business world even the chief executive officer needs a chief operating officer. Dr. Haley explained that his program is well delegated and organized so that their work can be accomplished. Dr. Steele added that this recommendation was not only about creating a program document, but also advised UTSW to describe and formalize the management structure of the program.

Chairman Binns addressed General Recommendation # 7: "The Committee applauds UTSW's commitment to managing the program on an industrial model, reviewing all components at the end of pilot and successive phases in light of new developments, and eliminating, modifying, or adding studies as indicated. The Committee also notes with approval that UTSW has charged all lead PIs on the program management team with the ongoing responsibility to review existing and emerging external research relevant to their areas of interest, particularly research that may contribute to identifying diagnostic tests and treatments. The Committee recommends that formal procedures to carry out these commitments be detailed in the program document."

Dr. Haley completely agreed with this recommendation. He also mentioned his research group's eagerness to collaborate with the RAC in the future about this point.

Chairman Binns read General Recommendation # 8: "The Committee notes that UTSW and VA discussed a clinical/research collaboration, and that UTSW developed plans for a Gulf War Illness Treatment Clinic at the Dallas VAMC and committed the first $3 million of its research funding to an Advanced Neuroimaging Center there. The Committee recommends that VA and UTSW undertake a clinical/research collaboration to develop a model for evaluating and treating ill Gulf War veterans within VA facilities, which might logically include clinical research utilizing the 3T MRI system provided to the Dallas VAMC with UTSW research program funding."

Dr. Haley informed the Committee that he and his research group are working with the Dallas VA to potentially establish and build up the area of the VA currently dedicated for use of the MRI machine.

Chairman Binns presented General Recommendation #9: "The Committee notes that VA has no mechanism for providing funding to non-VA organizations as grants and that UTSW and VA

have had to develop contract formats for this complex program from scratch. The Committee appreciates both parties' efforts to arrive at constructive solutions and recommends that VA Central Office provide guidance to the Dallas VA Contracting Office and its VISN leadership to support the UTSW program's ability to operate with the flexibility and speed necessary to make the program successful."

Dr. Goldberg mentioned that VA Central Office used to have all authority in the VA; many years ago, the VA decentralized. Now, all authority for the medical centers lies within the VISN. Central Office deals with deciding which research projects to fund, but does not have the authority to direct research efforts within a medical center. Chairman Binns suggested deleting the words 'Central Office' from the recommendation. Dr. Goldberg concurred.

Neither Dr. Haley nor any Committee members had any further comments about this recommendation.

Chairman Binns addressed General Recommendation # 10: "The Committee recognizes that the hopes of 175,000 Gulf War veterans who suffer from chronic multisymptom illness rest on this program. It is not an ordinary research project where an investigator is funded to pursue a particular thesis, while other investigators are also funded to pursue alternative theses. The Committee recognizes the considerable effort that has been invested to date by UTSW and VA, and that some of these recommendations envision reconsideration of decisions already made. The Committee encourages UTSW and VA to optimize this vital program for success."

Dr. Haley requested that two words, 'and resources', be added after the phrase 'considerable effort' in the third sentence. This way, the University and VA will be recognized for the large sum of money contributed to the research program thus far. Chairman Binns agreed to Dr. Haley's request.

Chairman Binns mentioned his appreciation of the willingness that both UTSW and VA have shown in reviewing and applying the recommendations of the Committee.

Chairman Binns temporarily stopped the discussion of the UTSW research program recommendations for a short break. He asked everyone to return to the meeting room in 15 minutes.

Chairman Binns called the meeting back to order at 10:41 am. He asked Dr. Steele to lead the discussion on the next set of recommendations for UTSW.

Case Definitions Recommendations

Dr. Steele read Case Definition Recommendation # 1: "UTSW plans for developing a "new" Gulf War illness case definition utilizing symptom data collected in the national survey be modified as follows. The revised approach should use a rational method, other than factor analysis, to identify a case definition based on the pattern of symptoms that best characterizes the chronic ill health of Gulf War veterans since the Gulf War. At minimum, this approach should identify the pattern of symptom expression that most clearly distinguishes the chronic symptoms

affecting Gulf War veterans from ambient symptoms affecting individuals who did not serve in the Gulf War. The case definition should also be "portable", that is, straightforward enough so as to be usable in other research and clinical settings. Additional efforts to identify clinically meaningful illness subgroups would also be highly valuable."

Dr. Steele added that Dr. Haley had previously developed a 'Haley Case Definition' of Gulf War syndrome using a unique factor analysis approach; subsequently, other researchers attempting to replicate a definition of GW syndrome using Dr. Haley's factor analysis criteria have been unable to do so. Using new data collected at UTSW through their national GW survey and a system other than factor analysis, Dr. Haley should consider reformulating an optimal GW Syndrome case definition. Dr. Steele further pointed out that one of the criteria in the new definition should be specific enough to distinguish ill Gulf War veterans from other ill veterans who did not serve in the Gulf. Also, a 'portable' definition of Gulf War syndrome will allow other researchers studying GW veteran populations to effectively apply Dr. Haley's definition to their own data, and possibly allow for a clinical diagnosis.

Dr. Steele invited comments from the Committee and from Dr. Haley.

Dr. Haley mentioned that it is an 'interesting conundrum' to have service in the Gulf War as a criterion for Gulf War syndrome. Developing a case definition of GW syndrome, and then testing it on data to determine how well it distinguishes deployed vs. non-deployed veterans is the traditional way to approach the problem. However, in developing a case definition of GW syndrome that includes a criterion to distinguish deployed vs. non-deployed veterans, one cannot apply the definition to the data to validate how well it distinguishes deployed vs. non-deployed veterans. Dr. Golomb suggested validating the case definition using a split-halves sample. Dr. Haley noted that this a complicated case definition to establish; if it were not such a complicated set of symptoms, research would not still be discussing it 17 years after the end of the war. He stated that the CDC definition of GW illness is not of good quality, and that someone who fell and hurt their knee qualifies as having joint pain under this definition. Most clinical trials of GW veterans have based their findings on the CDC definition. Using factor analysis as an approach to developing his initial 'Haley' definition was a way for Dr. Haley to mathematically formulate criteria for the syndrome.

Dr. Haley stated that his research group is currently conducting a pilot study to develop a new case definition. They are using a split-thirds method, and will be using factor analysis to define one of the thirds. Also, they will be testing cluster analysis to develop a definition; Dr. Haley was hesitant to use this approach however, as there is no mathematical output that can be applied to other similar situations (thus, it is not portable). Using cluster analysis will allow the researchers to determine if any symptoms/health concerns cluster together, and then develop a beginning point for a case definition and an approach to running the factor analysis. Dr. Golomb mentioned that there are modeling approaches used to distinguish between two groups. For example, establishing a neural network and using a training sample, a validation sample, and a test sample to run the program might be an effective modeling method. Dr. Steele added that by relying solely on a mathematical approach to defining the syndrome, you miss the 'human, common sense' factor that allows for a rational definition. Dr Meggs recalled a study conducted in 1968 by industrial hygienists at University of California Berkley. Researchers studied people

who had been poisoned by organophosphates to determine if they were experiencing any long-term sequelae. Findings varied from person to person within the study; people suffered from chemical sensitivity, cognitive dysfunctions, chronic asthma, rhinosinusitis, IBS, and some people had a combination of these conditions. These findings are similar to that of Gulf War veterans in that there is biological variability, overlap with other illnesses, diagnosable medical illnesses induced by exposures, and a spectrum of manifestations. He suggested that Dr. Haley's definition might include the following two criteria 1) a veteran was in the Gulf War theatre and had an exposure and 2) a veteran developed one or more specific chronic illnesses associated with GW syndrome. Dr. Steele added that investigators studying fibromyalgia and MCS have been faced with difficulties as well in trying to determine a specific definition of the syndromes.

Dr. Haley responded to Dr. Meggs' comments. He noted that he does not expect GW illness to be only one syndrome, and may be composed of several drastically different syndromes. Dr. Haley said they need to tease out the different syndromes and then proceed. Dr. Haley and Dr. Steele discussed teasing out different syndromes from the deployed group vs. the non-deployed group. Dr. Meggs shared another relevant study with the Committee: Dr. Steenland conducted neuropsychological testing on people with a known exposure and compared the results to neuropsychological testing results from people with no known exposures. The study found that although cognitive dysfunction occurred in the non-exposed group, there was a higher odds ratio for cognitive dysfunction occurring in the exposed group. So, Dr. Meggs concluded that in an individual person, you can never be sure from where a dysfunction might stem; there are overlaps of different factors that might cause the same clusters of symptoms. Therefore, teasing out subgroups, whether or not done by factor analysis, is imperative to study the dysfunctions' pathologies; at some point, mechanisms are bound to show. Dr. Golomb noted that the differences found in the subgroups will most likely be the difference initially identified. Drs. Haley and Steele discussed the process by which Dr. Haley should categorize his study's GW veteran subgroups.

Dr. Barlow commented that utilizing mathematical approaches, such as factor analysis or neural networks, is a great exploratory tool when used in conjunction with biological data. However, there is still a separate issue in trying to help clinicians and therapists with patients suffering from GW syndrome. Developing criteria for Gulf War syndrome for this purpose might require a more 'rational' method – something driven by experience as well as statistical analysis. Dr. Steele added that using this 'rational' method should not only be used for clinical value, but also in research. Rev. Graves expressed his thoughts on the matter: a collaborative team should be identified to discuss the different angles of each statistical method, as well as adding a common sense approach to choosing the best option. One person cannot solve this complex issue.

Dr. Haley shared the importance of developing the case definition of GW illness from the large survey study. The definition will be used in his group's future studies, i.e. neuroimaging, genomics, and RNA expression. Objective markers resulting from these studies will be used to refine the definition; eventually a 'disease' definition will be identified for clinicians and therapists. Initially, Dr. Haley and his group will develop several different definitions based on the questionnaire data, and will collaborate with individuals, including members of the RAC, in determining the best one.

Mr. Hardie emphasized the need to define exactly what a 'Gulf War veteran' is. He recently took part in a study that categorized Gulf War veteran as someone who served in the military during the period spanning from the first Gulf War until the present. Mr. Hardie expressed his concern that studies like this are not useful in identifying real GW veterans' issues, and noted that identifying a 'Gulf War veteran' definition is crucial to overall definition of Gulf War syndrome.

Chairman Binns wondered if using the Kansas definition of GW illness as a point of reference would be useful. Many investigators have used the Kansas definition in analyzing their study data, and so far no one has found a reason to discredit this definition. Dr. Steele noted that the questions asked in Dr. Haley's GW veterans health survey may not specifically allow for a 'Kansa definition' analysis. He will be optimizing a definition based on the data he collected, methods used for the Kansas definition, and the CDC definition. Dr. Haley added that asking questions in one situation might yield different answers when asked in a different situation. Questions worded in Dr. Haley's questionnaire were slightly different than in the Kansas definition, and might yield different results than the Kansas study.

Chairman Binns mentioned that the Committee was not attempting to solve this problem at the present time. The recommendation was made as a suggestion for future data analysis, and Dr. Haley and his group ultimately had the final decision. Dr. Meggs pointed out that researchers might find that GW syndrome is not GW-specific, and is a combination of symptoms and syndromes resulting from certain specific exposures. Others in the Committee agreed.

Chairman Binns asked Dr. Steele to proceed with the next recommendation.

Dr. Steele addressed Case Definition Recommendation # 2: "That UTSW administer symptom questionnaires to clinical study participants to clearly identify those who, at the time of intake, meet defining criteria for the newly-identified GW illness case definition, the CDC CMI case definition, and the Kansas GW illness case definition. This will allow all clinical measures to be evaluated in relation to differently defined illness outcomes, allow comparisons between those case definitions and the Haley syndromes, and allow findings from the UTSW program to be compared to those from previous clinical studies."

Dr. Steele added that since the original questionnaire Dr. Haley's study centered on did not contain the same types of questions as the Kansas and CDC questionnaires, subsequent questionnaires used for clinical trials should contain those questions. This way, a better comparison can be made using both the CDC and the Kansas definition and an optimal case definition can be derived. Dr. Haley noted that as study participants - including those in control groups - come in for the clinical trials they will be given new questionnaires.

Sampling Study Recommendations

Dr. Steele read Sampling Study Recommendation # 1: "That UTSW collect serum and DNA samples from all consenting survey participants (approximately 10,000 veterans), to permit a broader range of comparisons between sick and healthy veterans, as well as different exposure

subgroups." Dr. Steele noted that as of right now, UTSW's program is only planning to collect blood from 2,000 study participants.

Dr. Haley agreed that this was an important suggestion. However, it is not possible under the terms of the established contract. Now, Dr. Haley and his group are conducting power calculations to determine an ideal sample size for blood collection. In addition, his group is making an effort to add another blood collection tube to the study protocol to allow for RNA analysis (enabling investigators to look at the efficacy of an individual's DNA). Dr. Golomb argued that increasing the sample size will allow for more power in analyzing less prevalent conditions, such as ALS. Dr. Haley mentioned that the blood samples will be taken from all the sick people, and only from a random sampling of the well people. Dr. Barlow wondered if the analysis will be based on whole genome scanning; Dr. Haley stated that it would be. Dr. Barlow mentioned that in almost all cases of whole genome scanning, a large population is ideal. Dr. Haley noted that his study contains a fixed number of cases; the only way to obtain more cases would be to expand the study. Dr. Barlow countered that Dr. Haley's group will be using the whole genome scanning results in conjunction with the survey questionnaire data in order to define a diagnostic tool. Power calculations will become difficult when limited by a defined number of cases; the point of the genome association study is to ask what the best case definition is. Thus, researchers would not know a priori what the 'cases' were. Dr. Haley expressed his confusion at this notion. In all the genomic studies he has read, researchers started with a definition of the 'case'. Dr. Golomb noted that in those situations, conditions are already known. Dr. Barlow added that those situations work for people who have known cardiovascular disease because they had a heart attack, or are known to have ALS, but do not work for conditions such as bipolar disorder, schizoaffective disorder, and depression. Disorders that are neurocognitive in nature do not follow standard approaches to genomic association studies. Conversely, using a combination of genomics, genetics, and endophenotyping allows for a better analysis. Dr. Barlow suggested that Dr. Haley work with researchers at the forefront of psychiatric and subtle neurocognitive screenings to develop more firm diagnostic categories to perform the endophenotyping. She added that GW syndrome seems like a perfect candidate for endophenotyping. Dr. Haley thanked Dr. Barlow for her helpful comments.

Dr. Bloom subsequently stated that in having a larger sample size, analyzing the DNA might reveal a protective factor. For example, genomic differences between veterans who developed sequelae and those who did not after the Khamisiyah exposure might become evident. Dr. Barlow concurred; for diagnostic purposes, and for developing hypotheses to better stratify what is occurring, a large sample size is needed. She suggested that Dr. Haley look at the website for the Broad Institute at Massachusetts Institute of Technology (MIT) to get ideas for the genomic association study.

Dr. Steele addressed Sampling Study Recommendation # 2: "That UTSW substantially expand the number of subjects to be evaluated clinically. Calculated estimates of the precise sample size needed to address study questions of interest require data from the survey, the genetics/genomics study, and the pilot phase of the clinical study. Back-of-the envelope estimates, based on effect sizes expected for neuroimaging and neurocognitive studies, suggest that a clinical sample of 400 subjects would be required." Dr. Steele mentioned that a subset of the survey's study participants will undergo all of the other studies as well, i.e. neuroimaging, clinical, blood work,

and autonomic testing. This subset is planned to be made up of 80 people: 20 people with each of Dr. Haley's 3 syndromes, and 20 people for control. The Committee recommends that this subset become larger to include 400 people. This way multiple hypotheses can be tested, such as exposed vs. non-exposed veterans, or Dr. Haley's definition of GW illness vs. the CDC definition of GW illness. Increasing the sample size will increase the power for various subgroup analyses.

Chairman Binns invited discussion on this recommendation; there were no further comments. He asked Dr. Barlow to lead the discussion of the next grouping of recommendations.

Serum DNA Bank Recommendations

Dr. Barlow read Serum DNA Bank Recommendation # 1: "That UTSW add a large scale, unbiased genetic/genomics study as a major program arm now, utilizing the expanded number of serum samples previously recommended." She invited discussion of this recommendation; no one had comments.

Dr. Barlow addressed Serum DNA Bank Recommendation # 2: "That identified experts, with appropriate expertise in the design and execution of genomic and genetic studies, be contracted to design the plan for experimental design, data collection, statistical data analysis, and the details required to structure the experiments and sampling to achieve the appropriate deliverables." Dr. Barlow added that commercial companies may be able to complete the genomic analyses at a lower cost than in an academic setting. Offloading the generation of the data and early statistical analyses to a commercial company would be beneficial; subsequent analyses should occur in an academic setting.

Dr. Barlow presented Serum DNA Bank Recommendation # 3: "That RNA be collected from a substantial fraction of survey participants using appropriate specialized techniques, as determined by the plan previously described." Dr. Barlow noted that the Committee had already discussed this point in a previous section. RNA analysis has recently become a factor in genomic studies. Combining genetic experiments with expression QTL mapping allows for better diagnostics in affected and non-affected patients.

Dr. Barlow read Serum DNA Bank Recommendation # 4: "That the study results be utilized to inform the selection of the optimal neuroimaging evaluations to be included in the full clinical study and the selection of veteran subgroups to be included in the clinical sample, even if that results in a hiatus between the completion of the neuroimaging pilot studies and the full clinical study." She also presented Serum DNA Bank Recommendation # 5: "That costs of this study be paid for through savings achieved through elimination of other studies in the program."

Chairman Binns invited comments on recommendations 1-3. Dr. Bloom supplied the Committee with an analogy: in looking at the genomes of everyone in the room, they would be 99.9% the same. However, everyone's protein structure is quite different. Understanding how the 'blueprints' for each person leads to different attributes in different cells also includes learning how individual people have different vulnerabilities to various diseases. The serum bank will be a unique resource for biomedical research and using the serum bank to identify factors for GW

illness will lead to important insights. Combining these results with the neuroimaging results gives a cutting edge view of interpreting behavioral symptoms in the real world.

Dr. Haley agreed with the points made in the first three recommendations.

Chairman Binns invited discussion about recommendation # 4.

Dr. White disagreed with the statement as it was written. In her experience with gene-environment interaction studies, she has learned that sometimes the genomic study adds to information gathered in exposure-outcome studies, and sometimes it does not. With this in mind, Dr. White did not feel that the selection for the neuroimaging studies has to wait for the completion of the genomic portion of the study. Combining case definitions with information learned from exposure and genomic analyses will give a strategy for the selection for neuroimaging. She would argue not to hold up the neuroimaging, but rather to think at a high level about the information that might be coming from gene-environment interaction studies. Dr. Bloom argued that the recommendation was not to wait on everything; the extension of the twin study and the Seabees study will control for the genetics for the neuroimaging. Researchers do not first have to know what the genes are; in comparing monozygotic twins to each other, the genes are already built in. Dr. Steele wondered 1) if there is confidence that something will come out of the genomic study, and 2) how long it takes to know if something will come out of the genomic study to help drive the neuroimaging study choices. Dr. Bloom stated that if there is a sufficient amount of twins in the twin study, a protocol for neuroimaging can be developed.

Chairman Binns asked for clarification from Dr. Bloom. If the twin studies show enough power, a neuroimaging protocol can be developed. However, if the twin study does not have enough power, they would need to wait for the completion of the other genomic study to develop an ideal neuroimaging protocol. Dr. Bloom stated that this was correct. Dr. Golomb asked if the twin study consisted of monozygotic twins that are discordant in exposure and in outcome. Dr. Bloom answered 'yes'. He noted that if there were monozygotic twins who were concordant for exposure, they would be a wonderful control. At this point, it is not possible to make a binding decision, but all that is learned from the pilot studies will help to make the decision for neuroimaging study selection. Dr. Barlow noted that there is a large cost for operating the neuroimaging portion of the study, and optimizing the best candidates - whether from the twin study data or from the final genomic data - for the neuroimaging studies is key.

Dr. Haley commented that Dr. White captured his sentiments on the issue. He and his group do not want to stop the neuroimaging to wait for the results of the genomic study. Collecting data from the pilot studies and analyzing it will hopefully drive the neuroimaging projects. Dr. O'Callaghan believed that if the twin study provides sufficient results, the neuroimaging should go forward. Dr. Golomb added that in the twin study, they should include twins that are concordant in exposure, but discordant in outcomes if there are any available.

Dr. Steele wanted to clarify a point Dr. Barlow made earlier. In the event the twin study does not provide the information necessary to go forward with the neuroimaging studies and researchers need to rely on the results of the whole genomic study, there would not be a significant delay in developing the neuroimaging studies. This is because the data will be continuously coming in

for the genomic study, and can be continuously analyzed to determine the best course of action for the neuroimaging studies. Dr. Barlow replied that this was the case; the data will be collected and analyzed in real time to gather information. Dr. Haley said that his group will move forward with their pilot studies during the next year and at the end will have more information than now to determine neuroimaging projects. Chairman Binns commented that there is a possibility of a delay if both the twin study and the genomic study do not give an informative result. In that case, researchers would rely on environmental information to move forward.

Chairman Binns mentioned that everyone seemed to be in agreement of recommendation # 5. Subsequently, no one had any comments.

Dr. Barlow excused herself from the rest of the meeting as she needed to leave for the airport. Chairman Binns thanked Dr. Barlow for her invaluable contribution to the development of the recommendations for the research program at UTSW.

Chairman Binns allowed for a 5 minute break in the meeting.

The meeting reconvened at 12:15 pm. Chairman Binns asked Drs. Bloom and White to present the next section of recommendations.

Neuroimaging and Neuropsychological Project Recommendations

Dr. White presented Neuroimaging and Neuropsychological Project Recommendation # 1: "That UTSW prioritize the neuroimaging studies from the onset, and consider eliminating those with lowest priority." She further added that certain proposed neuroimaging studies are based on case definitions that have yet to be evaluated by the survey data. Thus, some of these studies may be eventually eliminated depending on the results of the survey data. Also, advice given by the eventual 'expert panel', should aid in the prioritizing of the neuroimaging projects.

Dr. White presented Neuroimaging and Neuropsychological Project Recommendation # 2: "That UTSW conduct its planned pilot protocols on the remaining studies in a subgroup of Seabees evaluated in previous UTSW research, and proceed with an appropriately sized twin sample." Dr. White explained that these pilot studies will be used to determine the feasibility, sensitivity, and reproducibility of magnetic resonance spectroscopy (MRS). The pilot studies will further drive the prioritization of the neuroimaging studies.

Dr. White then read Neuroimaging and Neuropsychological Project Recommendation # 3: "That, in addition to the data collected in the survey and pilot phase of the neuroimaging program, UTSW also utilize data collected in the genetics/genomics study to determine the optimal group of neuroimaging and neuropsychological studies to be used in the larger clinical sample." Dr. White commented that UTSW should also use a developed case definition and information gathered from exposure groupings.

Dr. Bloom mentioned that if the twin study is successful, the genetics/genomics data will not be necessary to prioritize the neuroimaging studies. Dr. Steele noted that if the twin study is unsuccessful, UTSW will need to rely on the genetics/genomics study mentioned in the third

point. Chairman Binns pointed out that the recommendations should be thought of as 'steps in order'; the first recommendation should happen before the second, and the second should occur before the third. Dr. Haley agreed with the recommendations, and thought they were the most important ones suggested by the Committee.

Chairman Binns asked Dr. Steele to present the next recommendations.

Other Clinical Evaluation Recommendations

Dr. Steele addressed Other Clinical Evaluations Recommendation # 1: "That UTSW evaluate all previously-identified indicators of objective differences between Gulf War illness cases and controls. This evaluation should include consultation with investigators who identified these differences and other experts to determine optimal testing procedures to ensure that previously-identified findings are adequately retested and/or further characterized. In particular, tests should be added to assess the association of GW illness with mycoplasma and leishmania infections, squalene antibodies, immune parameters, and coagulopathies." Dr. Steele mentioned that researchers at UTSW should consult with other researchers who have previously conducted similar tests. This way, the protocols designed by UTSW will be in line with other research, and similar findings might be obtained.

Dr. Golomb mentioned that squalene antibodies should be evaluated for recipients of the anthrax vaccine as well. If alterations in levels of squalene antibodies are seen as effects of the anthrax vaccine, failing to separate those who received the vaccine and those who did not during the analysis would wash out the result.

Dr. Haley completely agreed with the recommendation. Performing the squalene antibodies test is a certainty; the test will be conducted by an outside contracted laboratory. Mycoplasma studies are planned; Dr. Haley is in the process of identifying a capable investigator who knows how to properly handle the serum samples for this test. For the immune parameters area of study, Dr. Haley and his colleagues are searching for the optimal parameters to study. Dr. Haley wondered of the Committee could provide him with contacts so that he can identify the best means to study leishmania. Dr. Steele said that she would give him names following the meeting.

Dr. Steele presented Other Clinical Evaluations Recommendation # 2: "That UTSW consult with researchers with expertise in other multisymptom conditions to identify clinical tests most likely to be informative in Gulf War illness." She added that markers found in other related multisymptom illnesses, such as fibromyalgia, may be seen in ill Gulf War veterans as well. Consulting researchers with expertise in other multisymptom illnesses will benefit the direction of the clinical aspect of the program at UTSW.

Dr. Haley noted that his program already has an expert in fibromyalgia who will be evaluating study participants. He stated that his group is hesitant to run spinal tap studies this early in the program because they might not obtain enough study participants. He wondered if it would be possible to study substance P (a marker of pain sensitivity) levels in blood serum as opposed to in the cerebrospinal fluid (CSF). Dr. Steele said that she was not sure. Dr. Haley considered

studying serum cytokines, but found that would not be an effective measure for GW illness. Dr. Golomb mentioned that there are studies that show altered cytokine levels in GW veterans, and that it might be worthwhile for Dr. Haley and his group to perform this assay. Dr. Steele added that Dr. Haley might examine the presence of the herpes virus (as a trigger for developing multisymptom illnesses) in study participants suffering from multisymptom illnesses.

Ms. Knox asked Dr. Steele to explain who will be monitoring Dr. Haley's pursuit of consultations with researchers. Dr. Steele replied that she hopes that Dr. Haley will report back to the Committee to describe the steps he has taken to ensure his studies are conducted under optimal circumstances. This includes consulting with various scientific experts. Chairman Binns added that Dr. Haley has asked the Committee to make these formal recommendations for his research program, and that he has mentioned at various times during the discussion that he will be consulting with the Committee before making any final decisions regarding study protocol. Dr. Steele mentioned that from the beginning of his program, Dr. Haley has consulted the Committee about the most productive biological marker studies to focus on. Dr. Golomb pointed out that Dr. Haley is currently conducting autonomic nervous system testing as well.

Chairman Binns asked that if any Committee members had specific recommendations for potential biomarker studies, they should consult with Dr. Haley directly.

Chairman Binns asked if anyone wished to discuss Other Clinical Evaluation Recommendation # 3: "That UTSW contract out specific clinical tests to laboratories most experienced in testing for the abnormalities targeted for evaluation." All Committee members and Dr. Haley accepted the recommendation as it was written.

Dr. Haley asked for the Committee's view on sleep studies. His group has run sleep studies, and has not seen any difference in sleep patterns in Gulf War veterans. Dr. Steele suggested that Dr. Haley talk to other researchers that have conducted sleep studies, as some have found differences (in studying other multisymptom illnesses) and some have not. Dr. Golomb added that in one of her statin studies, participants reported a significant disturbance in sleep while on the medication. However, when given sleep related issue questionnaires, the difference was not significant between statin and placebo users. Thus, there is a different mechanism involved in the sleep disturbances of statin users. Dr. Steele suggested utilizing specifically designed questions on a questionnaire to address the sleep issue with GW veterans, avoiding having to run sleep studies. Dr. Haley hypothesized that the disturbances might not be due to changes in the sleep cycle, but rather a lack of parasympathetic activity at night. Dr. Golomb added that 'rest' may not be occurring properly during sleep.

Chairman Binns suggested Dr. Haley consult with other researchers in the field on the matter. He invited Dr. O'Callaghan to present the next grouping of recommendations.

Preclinical Studies Recommendations

Dr. O'Callaghan read Preclinical Studies Recommendation # 1: "That UTSW add a comprehensive neuropathological evaluation of exposed animals to the preclinical arm. This evaluation should include sensitive, state-of-the-art measures to evaluate neural degeneration,

astrogliosis, and microglial activation to determine if the exposure models are associated with underlying neural damage that is not obvious by traditional histopathology. These studies should be conducted by an experienced outside contractor."

Dr. Steele commented that this was a good recommendation because in evaluating exposure models, researchers should also be looking at the tissue of the models. Dr. O'Callaghan noted that there is a unified central exposure paradigm envisioned to make a cross-comparison on outcome measures for the preclinical studies. Results found on the preclinical studies can eventually inform the human clinical studies. There is also data to gather through trials in animals and in culture that researchers cannot gather from humans. Making the most of this model requires state-of-the-art neuropathology. Dr. Haley agreed that this point was imperative and that his group is working to make the studies happen.

Dr. O'Callaghan presented Preclinical Studies Recommendation # 2: "That UTSW eliminate the mouse neuroimaging studies. These studies could later be reconsidered if results from the human neuroimaging studies or animal neuropathology studies suggest priority research questions that are best addressed by mouse imaging studies, despite spatial resolution and other limitations associated with these methods."

Dr. Haley responded to the recommendation. He said he and his colleagues will discuss suspending this study; they began the study based on recommendations made in the RAC's 2004 report expressing the need for studies to connect animal models of GW illness to human GW illness. Dr. O'Callaghan added that the neuropathology analyses would function at level of accuracy beyond what the most sophisticated mouse neuroimaging could do. Dr. Golomb countered Dr. O'Callaghan's statement by approaching the neuroimaging from another angle. She mentioned that there are few neuropathology studies done in humans, but there are many neuroimaging studies. Thus, comparing neuroimaging results from mice and humans would be easier than comparing neuropathology results. Dr. O'Callaghan argued that researchers could compare the results of human neuroimaging to sophisticated neuropathology seen in mice; this would allow for predictions and potential explanations of the neuroimaging results in humans. Dr. Steele added that after researchers have the neuroimaging in the human and the neuropathology in the mouse models, they can try to explain findings; however, they do not yet know if this process will work.

Dr. Haley noted that this recommendation is one he could not completely comment on. He and his colleagues need to reevaluate the planned neuroimaging studies to determine which ones are worthwhile to begin before the results of the neuropathology studies are known. Once the neuropathology studies have been completed, they will again reevaluate the planned neuroimaging studies.

Dr. O'Callaghan read Preclinical Studies Recommendation # 3: "That UTSW establish criteria for assessing a "positive" outcome from the findings obtained in the first year's animal studies that is unified across all projects." Dr. O'Callaghan mentioned that a "positive" outcome from the animal studies will direct future human studies, so defining what a "positive" outcome is remain crucial.

Dr. Haley clarified that he needs to revisit each projects' protocol to ensure that an endpoint for each is clearly stated. Dr. O'Callaghan added that the preclinical investigators need to have a feel for what results are expected and would be of the most relevance to obtain additional funding to move on with the study.

Dr. O'Callaghan then addressed Preclinical Studies Recommendation # 4: "That the neurotoxin dosing protocol be expanded to include individual and combined effects of DEET and permethrin, and that delayed and persistent effects of exposures be assessed six months and 12 months after exposures." Dr. O'Callaghan noted that adding studies of agents that have already been shown through epidemiological studies to have a combined effect would be worthwhile.

Dr. Haley agreed with the recommendation.

Dr. O'Callaghan spoke about Preclinical Studies Recommendation # 5: "That studies in the preclinical arm of the UTSW program be limited to those that specifically explore effects of Gulf War exposures in relation to abnormalities associated with Gulf War illness, reflecting previous research in the field. This would require that some currently proposed studies, while of excellent scientific design, should be eliminated, including studies focused on aging, developmental neurotoxicity, fear conditioning and PTSD, vaccine effects previously shown not to be a concern, ALS, and cancer."

Dr. Haley wanted to go through each focus listed to inform the Committee of the current research plans at UTSW. Aging studies were designed based on results of Dr. Haley's 'Syndrome 2' study; increasing in age lead to a higher prevalence of people with 'Syndrome 2'. In the developmental neurotoxicity study, researchers are investigating the effects of sarin, pesticides, or PB on the developing mouse fetus. However, it has been pointed out that very few women were pregnant during their time in the Gulf War - Dr. Haley remarked that this study would probably be one to reevaluate. They might instead investigate the effects of those agents on maternal and paternal germinal cells. Dr. Haley mentioned that the fear and conditioning study protocols for his program are not designed to evaluate stress as a cause of GW illness. Instead, the study is designed to evaluate if a neurotoxic exposure alters the fear response in the brain so that a person would present with symptoms that resemble PTSD. Dr. Steele argued that although there is a percentage of GW veterans with PTSD, this percentage is small and less than percentages of PTSD observed following other military conflicts. Therefore, conducting PTSD studies should not be a priority. Dr. Golomb asked for clarification of the specifics of the protocol. Dr. Haley explained that there is a mouse model that elicits the fear conditioning response, which is the equivalent of PTSD in a mouse. Other mice will be exposed to sarin, PB, and other agents to determine if they develop symptoms that resemble those of the mice with PTSD following attenuated fear conditioning. Dr. Golomb stated that she did not have a problem with this protocol becoming a study, and it is an interesting question to be answered. However, she did agree that conducting the study specifically in the Gulf War setting might not be a priority.

Dr. Steele added that there was not an 'expert panel' functioning at the beginning of the research program at UTSW. Therefore, there was no official outlined mission of the program. Dr. Steele wondered if the mission was to study Gulf War illness, or to study illnesses prevalent in Gulf

War veterans. Regardless, she stated that PTSD is not an illness that is prevalent in GW veterans. Dr. Golomb mentioned that there is an entire agency at NIH whose focus is aging. Also, there is a large investment already in PTSD research because of the current war. Rev. Graves furthered the discussion by talking about some current studies showing that in the current group of Gulf veterans, what presents as PTSD is actually traumatic brain injury (TBI). He noted that it would be interesting to discover that what is termed 'PTSD' in veterans of the first GW is actually a result of chemical exposures such as sarin and organophosphates. Dr. White added that there is a large amount of research showing that sarin changes emotional responsivity, and may cause anxiety disorders, and residual depressive disorders. These changes are not PTSD; the definition of PTSD has two components - a stressful situation, and a stressful reaction. In a sarin exposure, there is no stressful situation, so it would be a misnomer to identify sarin exposure with developing PTSD. Dr. Golomb clarified that Dr. Haley's study was designed to identify if a less stressful situation following an exposure would cause PTSD. Dr. Golomb mentioned that there are funding agencies outside of Gulf War research that might be interested in the protocol, and that she would agree that Dr. Haley's program might not be the proper venue for the study.

Dr. Haley challenged the portion of the recommendation pointing to vaccine effects shown to not to be of concern to Gulf War veterans. He stated that he was not aware of any such vaccines. Dr. Haley's group will be working with a member of the National Academy of Sciences to develop a study protocol to evaluate the 'Rook effect' – that the multitude of vaccines given to GW veterans led to an immune response that altered cytokines in the blood causing GW veterans to feel as if they constantly have the flu. The protocol involves monitoring the immune system in an animal model given vaccines and exposed to sarin and other neurotoxicants. Dr. Steele argued that this protocol might not answer key questions. For example, did GW veterans have a shift in their levels of Th1 and Th2 (cytokines)? Animal models are not needed to answer this question; blood from GW veterans could be tested. Dr. Steele pointed out that other studies investigating the effects of vaccines and exposures found results in the brain, and wondered why there was no brain component to this protocol. Dr. Haley replied that there is a separate protocol for vaccine effects with neuroimaging. Dr. Steele further questioned the study protocol because other similar studies have shown no peripheral effect of multiple vaccine exposures. Drs. Steele and Haley discussed the study protocol further, including studying immune parameters in the periphery of humans.

Chairman Binns asked for other comments from the Committee. Since no other members had comments, Chairman Binns read an email from Dr. Melling, a Committee member who was unable to attend the meeting. Dr. Melling (an expert from the United Kingdom) noted that the major difference between GW veterans' research in the U.S. and in the U.K. was in measuring immunological parameters. He expressed his willingness to work with Dr. Haley in establishing a research protocol for the UTSW program that is comparable to protocols of studies done on GW veterans in the U.K. Conducting this study may provide information about GW veterans that the U.K. studies were unable to gather.

Dr. Golomb expressed her concern at Dr. Haley's lack of interest in studying human immune parameters found in the periphery. She explained the importance of performing studies in humans parallel to those done in animals. Dr. Haley pointed out that he was not against

conducting immune parameter studies in humans; his group has not yet come to an agreement on which parameters to study. He invited ideas from the Committee on this issue.

Dr. O'Callaghan wished to revisit one of Dr. Steele's previous points. He pointed out that Dr. Haley's proposed studies are scientifically sound, and are good projects. However, they do not necessarily fit under the 'umbrella' of understanding the pathophysiology of GW illness.

Dr. Haley understood this point. Next, he wanted to go over the planned studies for ALS and cancer at UTSW. To study ALS, Dr. Haley was working with an investigator who designed an animal model of ALS. The researcher would expose the animals to sarin, PB, and other chemical agents to determine if the time it takes for each animal to develop ALS is hastened by the exposure. If a positive result is obtained, it might explain why GW veterans have a 4 times higher risk of developing ALS than others their age. As a reason to study cancer development, Dr. Haley looks toward Dr. Kang's study, which found a 2-fold increase in brain cancer (almost 90% of which were glial brain cancers) in GW veterans. A researcher from Harvard will be working with Dr. Haley on a protocol for UTSW. This researcher's hypothesis is that cholinergic receptors are involved in producing glioma-type brain cancers. In genetic studies, glioma-type brain cancers have a strong environmental component to their development – in the environment of the GW, veterans were exposed to pesticides, sarin, and other chemical affecting the cholinergic pathway. Dr. Haley explained that this researcher will be exposing an animal model to pesticides and sarin to determine if the rate at which they develop glial-type cancers is increased/accelerated. Dr. Haley added that he and his group feel that the overall mission of the research project is to study GW illnesses; all of their task orders refer to illnesses. In addition, he believes that ALS is of major concern to GW veterans and their families, and the protocol developed at UTSW will address their concerns.

Chairman Binns asked for comments from all Committee members about the proposed protocols. Dr. Steele reiterated her original point: Dr. Haley needs to evaluate each proposal to determine if it addresses the mission of the research. Are they funding projects dealing with GW illness, or with illnesses of GW veterans? She added that answering this question will allow Dr. Haley to make final decisions about the projects funded in the future. Dr. White expressed her agreement with Dr. Steele's point. Chairman Binns noted that for the CDMRP program, the Committee defined GW illness as a multisymptom illness; ALS and cancer are not part of the definition. Traditionally in the VA, ALS research is separate from GW research even though some veterans have developed ALS. In studying ALS specifically in GW veterans, the protocol would not be considered GW illness research, but rather would be under a different category.

Rev. Graves said the way Dr. Haley explained the study protocols to the Committee portrayed GW related illnesses as targets of the research, and were not only for ALS and cancer. He explained that this might be good information to know, and did not see a problem with the protocol being funded. Mr. Hardie expressed his awe at the limitations on the number of projects able to be funded from $75 million dollars of research money. With this in mind, he agreed with Rev. Graves' comments. Running the ALS and cancer studies while the money is currently being devoted to GW research makes sense. If this type of research is not being conducted elsewhere, through VA or an ALS research program, the UTSW might be an appropriate venue for it. Ms. Knox inquired about the cost of running the animal studies, knowing how expensive

the neuroimaging portion of the program is. Dr. Haley replied that the animal studies would require about $300,000 each in funding. Ms. Knox pointed out that the issue of increased prevalence of ALS in GW veterans has not yet been answered, and needs to be. She would be in favor of Dr. Haley funding the ALS research protocol. Dr. Golomb mentioned that there are currently funded studies investigating the role of paroxonase in ALS, and others investigating a connection between paroxonase and organophosphates. She expressed her understanding of both sides of the argument: the proposed project was an important study for GW veterans, but the program at UTSW should be focusing more on multisymptom illness research.

LTC Adam Such, a Committee member, took a programmatic approach to evaluating his thoughts. If there is a limited amount of funding available, and there are other potential funding mechanisms through other agencies, deciding to include the studies in the UTSW program should be delayed. If not able to be funded elsewhere, the studies can later be reevaluated for funding through UTSW. Chairman Binns noted that if NIH were to fund a similar study, they would probably not investigate the same compounds that would be relevant to GW veterans. If Dr. Haley can design the study in a way that would determine the chemical compounds that might lead to the development of ALS, he should proceed and fund it. Rev. Graves added that if the exposures Dr. Haley was proposing to study are found to even lead to lesions on the brain, as the veteran son of the family in the audience from Nebraska had experienced, the study would be worthwhile.

Chairman Binns noted that the Committee seemed to be evenly divided on the issue. He suggested obtaining Committee members' opinions on the topic of aging protocols. Dr. Steele noted that from what she understood about the study, it did not seem to be investigating a main component of GW illness. Having aging veterans with Dr. Haley's 'Syndrome 2' as the only justification for the study is not enough. Dr. White stated that she was uncomfortable with asking each Committee member for their opinion on each of the study protocols. She suggested rewording the recommendation to include phrases such as 'review, 'substantiate', or 'consider eliminating', to reflect the Committee's belief that Dr. Haley should eliminate some irrelevant studies. Dr. O'Callaghan mentioned that the studies being discussed have already been approved, and a line needs to be drawn for the recommendation. Dr. Golomb wanted to specifically address the aging study. She pointed out that every person, regardless if they were a GW veteran, is aging. In any study of most diseases, age is always a risk factor of developing the disease. For this reason, the National Institute on Aging (NIA) was established to study the effects of aging. Of all Dr. Haley's studies discussed, Dr. Golomb believed the one on aging was least appropriate to continue. Ms. Knox agreed with Dr. Golomb.

Dr. Bloom continued the discussion. He noted Dr. Haley's need to develop some 'bread and butter' studies in order to gain the interest of study participants. It will be far into the future before any results from the human studies come to light. So, if Dr. Haley and his group are able to demonstrate no effect of sarin on the development of ALS or cancer in a mouse model, it will be a minor victory. The studies will establish an end to a question, or foster a new question that can be answered by another outside investigator. These studies should not take more than a year to complete. Dr. Bloom agreed with Dr. White that the recommendation should be reworded so Dr. Haley will reevaluate the studies in accordance with the UTSW research plan to justify these

pilot studies. LTC Such agreed with Dr. Bloom: in running these small studies, researchers could learn a great deal.

Chairman Binns considered the rewording of the recommendation # 5. Dr. Haley agreed that the recommendation should be reworded.

Mr. Hardie inquired about the components of the program that would be investigating specific exposures other than sarin and vaccines. Dr. Haley replied that the Merit Review Committee at UTSW approved preclinical (animal) studies for sarin, chlorpyrifos, and PB. After the completion of the studies, designed to conclude within 1 year, the results will be analyzed to determine if expanding exposure studies to other chemicals is feasible. Mr. Hardie wanted to clarify if the program would ever be considering chemical warfare agents other than sarin. Dr. Haley responded that they would not be.

Ms. Knox expressed her admiration towards the Committee. She commended that there have been many brilliant minds serving on the Committee over the years, and realized that the Committee is now at a great point in its journey. Dr. Haley added that it is nice to finally be discussing the types of studies to do rather than discussing whether the studies would ever be done. Chairman Binns thanked Ms. Knox for her comments, and thanked the Committee members who have put so much time into its functioning. He asked for any other comments from the Committee.

Chairman Binns invited members of the audience to make any comments.

Public Comment – Day 2

Mr. Edward Bryan wanted to address several points of interest. First, he wondered if the Committee would consider stem cell research and treatment for ALS, white matter disorders, and MS as a priority. Secondly, Mr. Bryan commented on Mr. Hardie's remarks about the need to properly define 'GW veteran'. He mentioned that any veteran who received the Southwest Asia Service Medal considers themselves to be a GW veteran. Mr. Bryan then handed out a letter from himself, addressed to Dr. Kimberly Sullivan - the Scientific Coordinator for the RAC - as his written statement for the record (See Appendix – Handout 2).

Mrs. Angie Newbold addressed Dr. Haley and his research program at UTSW. Dr. Haley had mentioned at the beginning of the day that the University was at large financial risk by housing the research project, and was also taking a risk with the program because it might not be favorable with the public opinion. Mrs. Newbold, as the mother of a veteran and a contributor to the public opinion, advised Dr. Haley to take the risk, and if that means having to be monitored by an outside organization (such as the RAC) then he should welcome the advice. Mrs. Newbold asked Dr. Haley where the veteran study participants would be recruited because there are vast amounts of veterans located all over the country. In addition, there are many veterans who do not seek medical care within the VA, so researchers might neglect to gather valuable information from those veterans. She added that one way to seek the cooperation of veterans for these studies is to reach out to their wives, mothers, etc. She understood that there would be limitations in their involvement because of Health Insurance Portability and Accountability Act (HIPAA)

laws, but that she and other individuals would be willing to help involve their veteran relatives in these studies. Dr. Haley commended Mrs. Newbold's insight. He mentioned that there is a list of every person who served in the GW stored at a DOD facility. The DOD has allowed Dr. Haley's program to obtain a random sampling of the people on the list, regardless if they have ever sought medical treatment. Dr. Haley's group has mailed each of these veterans a survey; based on the results of the survey, veterans will be invited to participate in subsequent studies through UTSW.

Mr. Erwin Steffen thanked the Committee members for the hard work and effort they put into overseeing GW research. Chairman Binns thanked Mr. Steffen for his attendance.

Ms. Alison Johnson thanked the Committee as well. Though she has been attending meetings since the RAC was first established, this one was probably her last because it is becoming increasingly expensive to travel. Ms. Johnson informed the Committee that she has been studying MCS since she developed the condition 34 years ago. To no avail, she has been to every major clinic and seen every major doctor, including in Germany, for treatments for MCS. Similar to her experiences with MCS research, Ms. Johnson believes that 10 years from now, there will still be no cure for GW syndrome. She expressed her conviction that GW syndrome will not be unraveled without first unraveling MCS. Ms. Johnson added that in 2004 the *Archives of Environmental Health* included a publication that detailed a national phone survey. The survey's results showed 2.5% of the population had been diagnosed with MCS. Therefore it is not as rare of a condition as the media portrays it to be. Ms. Johnson's book about MCS, entitled 'Amputated Lives: Coping with Chemical Sensitivity' will be available this summer.

Ms. Denise Nichols again expressed her desire to have the RAC meetings videotaped and placed online. This way, researchers and clinicians can gain some valuable information about the current state of GW research. Ms. Nichols asked Dr. Haley to comply with the Committee's recommendation to consult with other researchers to ensure he is conducting the best possible research with the money he has been allotted. She also emphasized the need to take care of the soldiers. Ms. Nichols provided a written comment for the meeting record (See Appendix – Handout 3).

Chairman Binns invited final comments.

Mr. Hardie addressed Mr. Bryan's comments about the Southwest Asia Medal. He mentioned that many veterans who have received this award do not realize how broad the criteria are for inclusion. Service between August 2, 1990 and November 30, 1995 is considered as the qualification for the award - this could include veterans who served in Egypt, Oman, Israel, and other countries that were outside areas of concern for exposures. So, Mr. Hardie would disagree with utilizing the Medal as a definition of a 'GW veteran'.

Chairman Binns invited members of the audience to remain after the meeting for a discussion with several Committee members about any specific issues that were of concern to them.

Chairman Binns adjourned the meeting at 1:50 pm.

Appendix A
Presentation 1 - Roberta White

Research Advisory Committee Gulf War Illnesses

Scientific Office
Boston University
School of Public Health

RAC-GWI Scientific Committee Staff

- Roberta F. White, PhD, ABPP/cn
  - Scientific director
  - Professor and Chair
  - Department of Environmental Health
  - BUSPH
- Kimberly Sullivan, PhD
  - Research Assistant Professor of Environmental Health

RAC-GWI Scientific Committee Staff

- Callie Comtois
  - Research Assistant
- Ilga Wohlrab, MPH
  - Environmental Health Department Administrator

RAC-GWI Scientific Committee Bedford VA Staff

- PB (Ben) Cipolloni, MD
  - ACOS for Research
- Elaine Gibson
  - Contracts officer
- Joseph Squiciarrini
  - Research administrator

BUSPH Discussion Group on GW-related Illnesses

- Background at VA and BUSPH
- Purpose
- Meeting plan

Discussion Group Members

- ***George Annas, JD*** — Biomedical ethics, law

Professor and Chair
Department of Health Law, Ethics
and Human Rights
Boston University School of Public Health

- ***Peter R. Bergethon, MD, PhD*** — Neurology, physical sciences, NIRS, aging

Assistant Professor
Department of Anatomy and the
Neurobiology of Aging
Spivack Neuroscience Center
Boston University School ofMedicine

Discussion Group Members

- ***Richard Clapp, ScD*** Environmental epidemiology, cancer

Professor of Environmental Health
Boston University School of Public Health
(Boston Environmental Hazards Center, Project PI)

- ***Kristin J. Heaton, PhD*** Military health, behavioral research, sarin exposure

Research Assistant Professor
Department of Environmental Health
Boston University School of Public Health
(Boston Environmental Hazards Center)
USARIEM, Natick, MA

Discussion Group Members

- ***Timothy C. Heeren, PhD*** Biostatistics

Professor
Department of Biostatistics
Boston University School of Public Health
(Boston Environmental Hazards Center, Center statistician)

- ***Ronald Killiany, PhD*** Neuroimaging, neuropsychology

Associate Professor
Department of Anatomy and the Neurobiology of Aging
Spivack Neuroscience Center
Boston University School of Medicine

Discussion Group Members

- ***Maxine H. Krengel, PhD*** — Neuropsychology, TBI, pesticides

Assistant Professor (Neuropsychology)
Department of Neurology
Boston University School of Medicine
(Boston Environmental Hazards Center)
Staff Psychologist
VA Boston Healthcare System

- ***Richard Myers, PhD*** — Neurogenetics, Parkinson's, Alzheimer's, pesticides

Professor (Neurogenetics)
Department of Neurology
Boston University School of Medicine

Discussion Group Members

- ***David M. Ozonoff, MD, MPH*** — Medical epidemiology, spatial & mathematical modeling

Chair Emeritus and Professor
Department of Environmental Health
Boston University School of Public Health
(Boston Environmental Hazards Center, Medical Director)

- ***Lewis Pepper, MD, MPH*** — Occupational health, occupational toxicology

Assistant Professor
Department of Environmental Health
Boston University School of Public Health
(Boston Environmental Hazards Center)

Discussion Group Members

- ***Susan P. Proctor, DSc*** Military health, occupational toxicology

Associate Professor
Department of Environmental Health
Boston University School of Public Health
(Boston Environmental Hazards Center, Associate Director)
USARIEM, Natick, MA

- ***David Sherr, PhD*** Molecular biology, immunology, immunotoxicology

Professor
Department of Environmental Health
Boston University School of Public Health
(Boston Environmental Hazards Center, Project PI)

Discussion Group Members

- ***Jennifer J. Vasterling, PhD*** PTSD, clinical psychology

Chief, Psychology Service
VA Boston Healthcare System
Professor
Department of Psychiatry
Boston University School of Medicine
(Boston Environmental Hazards Center, New Orleans collaborator)

- ***Veronica M. Vieira, DSc*** GIS, spatial analysis

Assistant Professor
Department of Environmental Health
Boston University School of Public Health

Discussion Group Members

- ***Pending:***
  - Neuroinflammation
  - Pulmonary
  - Pharmacology
  - Exposure assessment

Presentation 2 - Ronald Bach

TISSUE FACTOR AND GULF WAR-ASSOCIATED CHRONIC COAGULOPATHIES

Minneapolis VAMC

Ronald R. Bach, Ph.D.
(Principal Investigator)
&
Billie Slater, M.A.
(Study Coordinator)

Funding for Pilot Study

VHA ORD Request for Proposals, Fall, 2005:

"RESEARCH DIRECTED TO UNDERSTANDING ILLNESSES AFFECTING GULF WAR VETERANS"

Activation of the coagulation system in Gulf War Illness: a potential pathophysiologic link with chronic fatigue syndrome

A laboratory approach to diagnosis

K. L. Hannan, D. E. Berg, W. Baumzweiger, H. H. Harrison, L. H. Berg, R. Ramirez and D.Nichols

Blood Coagulation and Fibrinolysis 2000, 11:673-678

Hypothesis

Tissue factor (TF)
procoagulant activity (PCA)
in the blood of individuals with
Gulf War Veterans' Illnesses (GWVI)
is abnormal.

This change creates a chronic hypercoagulable state.

Experimental Test

Quantify TF PCA in the blood of GWVI (+) and GWVI (-) volunteers.

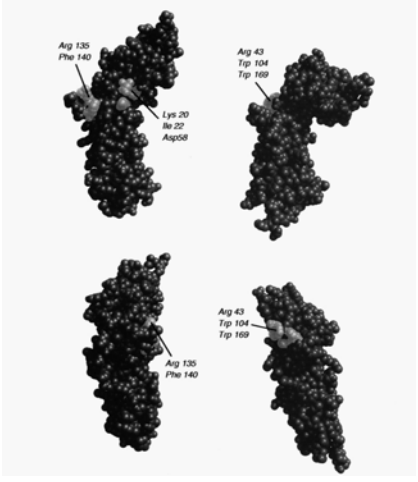
Is there a difference?

YES or NO

Tissue Factor

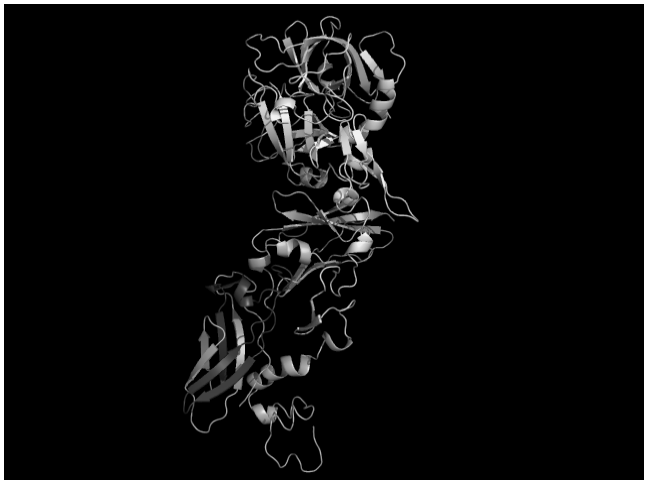
EXTRACELLULAR DOMAIN

TF and hGHR



TF-FVIIa ON THE CELL SURFACE

BASED ON THE CRYSTAL STRUCTURE OF THE sTF-FVIIa COMPLEX
Banner et al., Nature, 380:41-46, 1996.



TF PCA is Encrypted on the Surface of Unperturbed Cells

- TF exists on the surface of a healthy cell in an inactive (encrypted) state.
- Cell death (apoptotic or necrotic) triggers the rapid expression of TF PCA.
- Platelet activation results in the decryption of platelet TF PCA.
- Calcium influx induced by the addition of a calcium ionophore is the way we decrypt TF PCA in our experiments.

Decryption of TF PCA on HL-60 Cells

Preliminary data omitted
from meeting minutes

TF Summary

- Function:
  Biological Initiator of Blood Coagulation
- Origin:
  Cytokine Receptor → Clotting Factor
- Location:
  Extravascular >99.9%
  Intravascular <0.01%
- Regulation:
  Location / Encryption / Inhibitors

Bacterial Endotoxin Stimulates TF Gene Expression in Human Blood

Preliminary data omitted
from meeting minutes

In vivo

In vitro

TF PCA Before and After Total Knee Arthroplasty

Preliminary data omitted
from meeting minutes

TKA Study

Preliminary data omitted
from meeting minutes

Blood Levels of TF PCA in 51 Normal Subjects and 114 Veteran Patients on Warfarin

Preliminary data omitted
from meeting minutes

Total TF PCA in Blood

Preliminary data omitted
from meeting minutes

Monocyte TF PCA

Preliminary data omitted
from meeting minutes

Platelet TF PCA

Preliminary data omitted
from meeting minutes

Platelet TF PCA / Monocyte TF PCA

Preliminary data omitted
from meeting minutes

Platelet TF PCA vs. Platelet Count

Preliminary data omitted
from meeting minutes

Conclusions

- Individuals with GWVI have abnormal intravascular TF PCA.
- The observed changes in intravascular TF PCA are consistent with a chronic hypercoagulable state.

New Hypothesis

- The etiology of at least some Gulf War Veterans' Illnesses is chronic inflammation produced by autostimulatory crosstalk between the immune and coagulation systems.

Future Directions-Basic Science

- Independent confirmation of the pilot study results.
- Reassay the initial study subjects.
- Examine stored plasmas for 92 additional markers of inflammation/coagulation.

Future Directions-Therapeutics

- Five way to block the expression of intravascular TF PCA:

1. Platelet inhibitors
2. Inhibitors of TF gene expression
3. Specific inhibitors of TF PCA
4. Systemic anticoagulants
5. Anti-inflammatory drugs

Presentation 3 - Ashok Tuteja

Bowel Disorders in Gulf War Veterans

Ashok K. Tuteja
M.D., M.R.C.P. (U.K.), M.P.H.

Division of Gastroenterology
George E. Wahlen V.A. Medical Center & University of Utah
Salt Lake City, UT

Gulf War Illnesses

Overall

↓

Bowel Disorders in Gulf War Veterans

↓

Irritable Bowel Syndrome

High Prevalence of Bowel Disorders in Persian Gulf Veterans (8 '90-6 '1991)

- 700,000 military personnel deployed
- 25% reported chronic health complaints
- Among the most reported symptoms:
  - Chronic diarrhea
  - Abdominal pain relieved with defecation
  - Bloating

Arch Intern Med 1995; 155:262-8.

Chronic GI Symptoms in PGV

	PGV % N=57	Control % N=44
Abdominal Pain	70	9
Abdominal pain relieved with BM	47	16
Loose or >3 BM/day	74	18
Incomplete rectal evacuation	60	7
Mucus with stool	19	0
Excessive gas	74	23

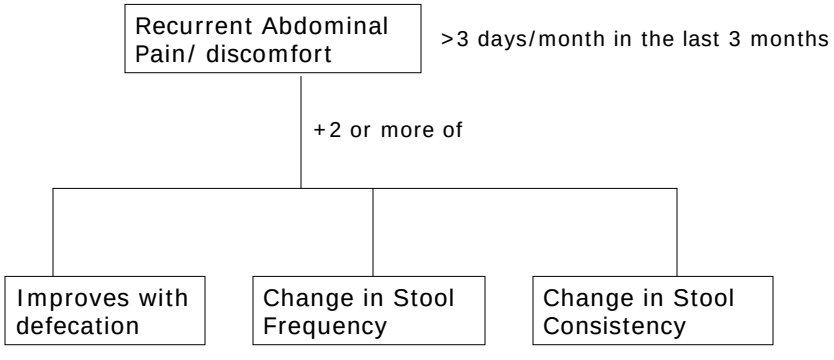
Sostek MB et al. Am J Gastro 1996: 2494

Diarrhea During Desert Shield

- 57% of troops had at least one episode of diarrhea within 2 months of arriving
- A bacterial pathogen was identified in 50%

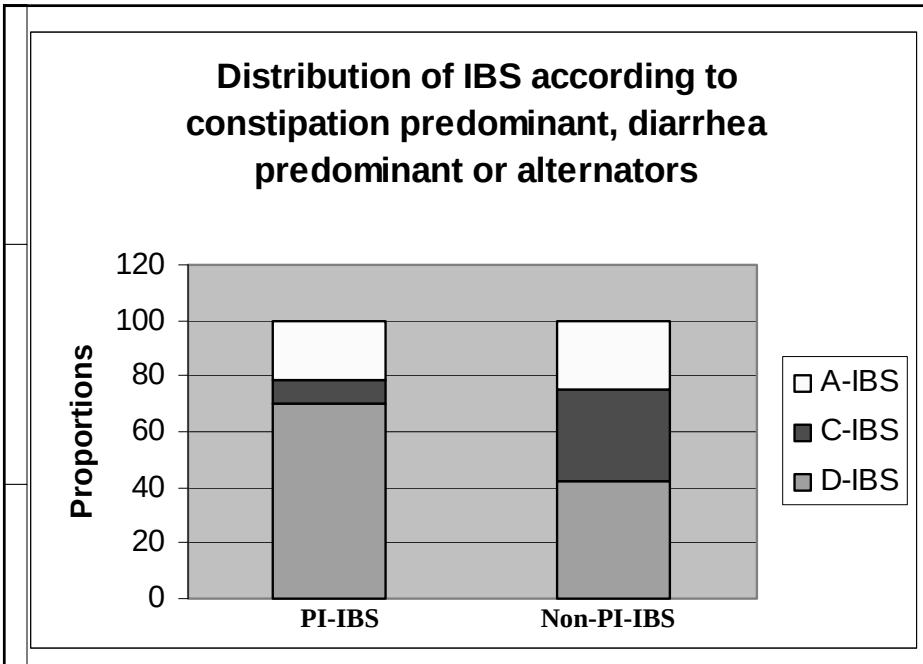
Hyams et al. NEJM: 1991;1423

IBS- Rome III

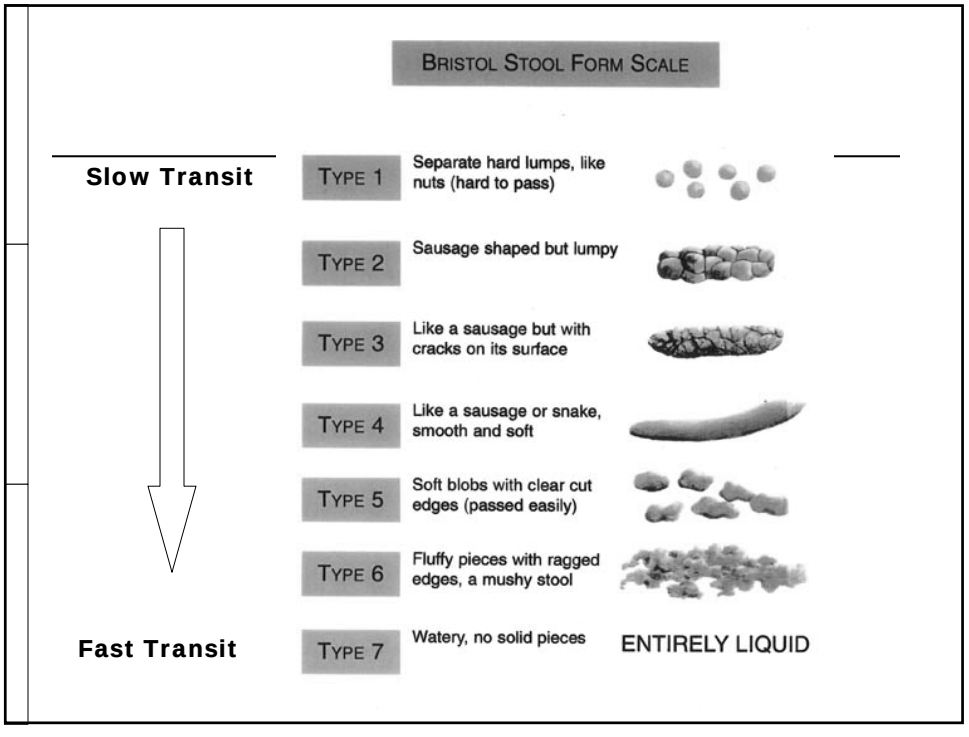


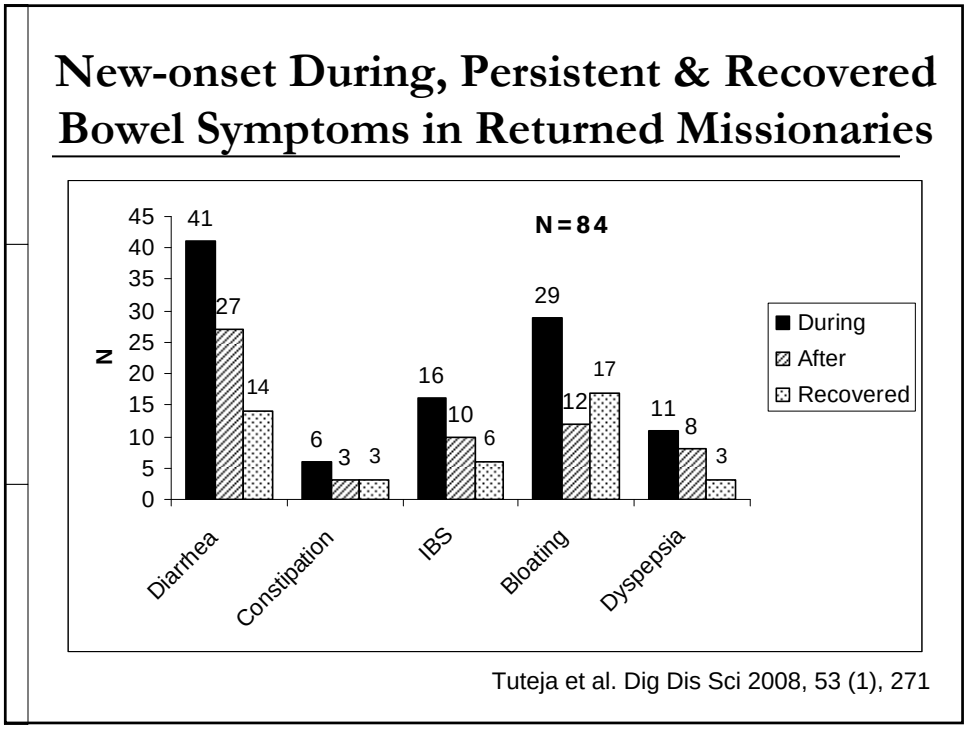
Irritable Bowel Syndrome

- 5-20% of general population.
- 20- 50% referrals to gastroenterology clinics.
- 2.4- 3.5 million physician visits / year
- reduces QOL.
- estimated cost of IBS= $8 billions/yrs.



Dunlop SP. Am J Gastro 2003; 1578


BRISTOL STOOL FORM SCALE
Slow Transit
TYPE 1
Separate hard lumps, like nuts (hard to pass)
TYPE 2
Sausage shaped but lumpy
TYPE 3
Like a sausage but with cracks on its surface
TYPE 4
Like a sausage or snake, smooth and soft
TYPE 5
Soft blobs with clear cut edges (passed easily)
TYPE 6
Fluffy pieces with ragged edges, a mushy stool
Fast Transit
TYPE 7
Watery, no solid pieces
ENTIRELY LIQUID

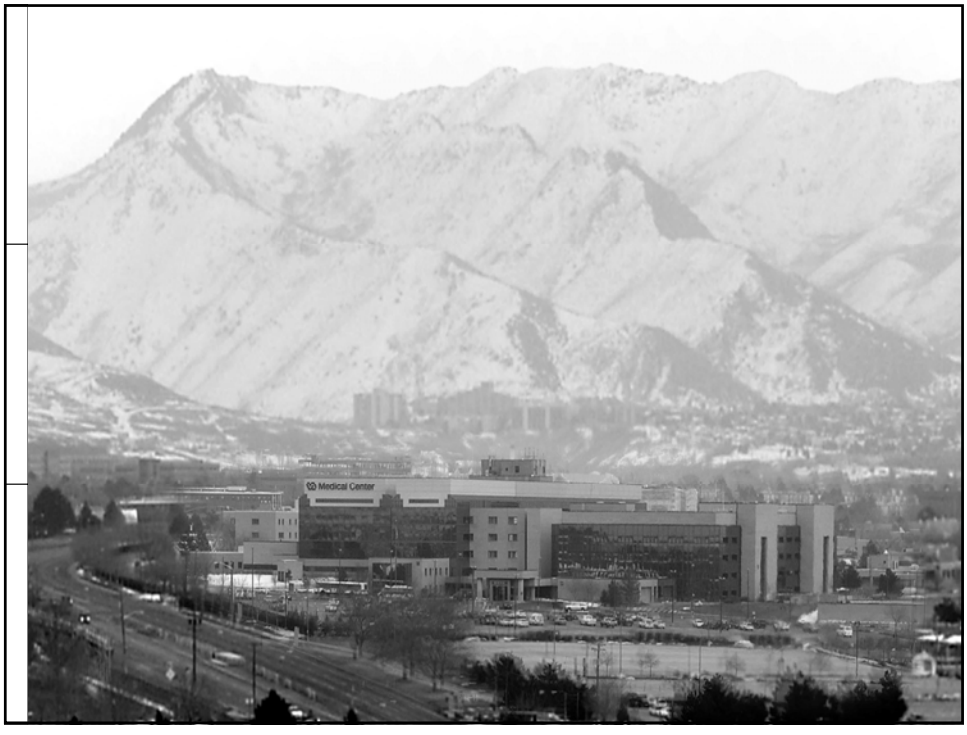

New-onset During, Persistent & Recovered Bowel Symptoms in Returned Missionaries
N=84
N
45
40
35
30
25
20
15
10
5
0
41
27
14
6
3
3
16
10
6
29
12
17
11
8
3
During
After
Recovered
Diarrhea
Constipation
IBS
Bloating
Dyspepsia
Tuteja et al. Dig Dis Sci 2008, 53 (1), 271

Objective of the Study

1. Prevalence of bowel disorders before, during, and after deployment
2. Effect of deployment on prevalence of functional bowel disorders
3. Effect of combat on IBS
4. Effect of IBS on QOL
5. Effect of Psychological factors on IBS

Methods

- Two center study:
  - George E. Wahlen VAMC, Salt Lake City, UT
  - Malcom Randall VAMC, Gainesville, FL
- Three Part Study:
  - Epidemiology/ Diagnosis/ Treatment
- Measurements
  - Demographics-Deployment
  - Bowel Symptoms (BDQ-Rome III)
  - Quality of life (IBS-QOL)
  - Psychological assessments (BSI-18)
  - Combat Exposure



Bowel Disorders in Gulf War Veterans

- Data available from 335 veterans, 318 deployed
- Gender: Men=92%
- Age: median 48 yrs (range 30- 76)
- Marital Status
  - Single 11% Married 73%
  - Divorced 12% Widowed 1%
- Education:
  - High school/less 18%
  - Some college 51%
  - College 16%
  - Beyond college 15%
- Employment:
  - Retired 26% Part time 5% Not working/student 3%
  - Disability 15% Full time 49% Others 3%

- **Duration of deployment (%)**
  - ≤ 6 months 41
  - 7- 12 months 48
  - > 12 months 11
- **Duration of combat (%)**
  - Never 8
  - ≤ 1 month 9
  - 2-6 month 55
  - 7-12 months 24
  - >12 months 4

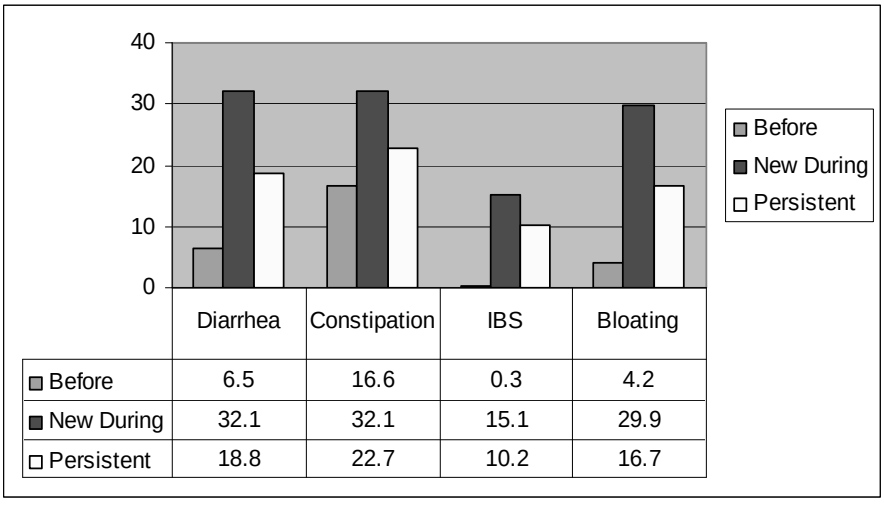
Prevalence of Bowel Disorders Before/During/Post Deployment

	Before Deploy	During Deploy	P	During Deploy	Post-Deploy	P
Diarrhea	**6.8**	**39.3**	**<0.01**	**39.3**	**35.4**	**<0.01**
Constipation	**16.7**	**49.6**	**<0.01**	**49.6**	**54.0**	**<0.01**
IBS	**0.3**	**15.5**	**0.02**	**15.5**	**40.7**	**<0.01**
Bloating	**4.1**	**34.9**	**0.01**	**34.9**	**46.6**	**<0.01**
Non-IBS-D	**6.5**	**23.7**	**0.01**	**23.7**	**12.5**	**<0.01**
Non-IBS-C	**16.3**	**33.3**	**<0.01**	**33.3**	**24.4**	**<0.01**

Prevalence of IBS-Subtypes (%)

	Before deploy N=1	During deployment N=44	Post deployment N=127
IBS-D	-	8.5	17.3
IBS-C	0.3	2.3	10.1
IBS-M	-	2.0	4.7
IBS-U	-	1.8	8.3

Bowel Symptoms Before, New During and Persistent Post-Deployment



	Diarrhea	Constipation	IBS	Bloating
Before	6.5	16.6	0.3	4.2
New During	32.1	32.1	15.1	29.9
Persistent	18.8	22.7	10.2	16.7

Effect of Deployment and Combat on Bowel Symptoms

	IBS (N=127)	Diarrhea (N=112)	Constipation (N=169)
Deployment <6 months (N=127) 7-12 months (N=150) >12 months (N=34)	1 1.50 (0.92- 2.44) 0.11 1.37 (0.63- 3.00) 0.43	1 1.21 (0.73- 2.00) 0.45 1.93 (0.89- 4.18) 0.09	1 1.71 (1.06- 2.78) 0.03 1.12 (0.52- 2.39) 0.77
Combat Never (N=25) <1 month (N=28) 2-6 month (N=172) 7-12 month (N=74) > 12 month (N=14)	1 3.17 (0.96- 10.48) 0.06 2.30 (0.88- 6.05) 0.09 2.26 (0.81- 6.34) 0.12 1.98 (0.47- 8.40) 0.35	1 2.75 (0.79- 9.55) 0.11 2.43 (0.87- 6.79) 2.43 2.21 (0.74- 6.59) 0.15 2.22 (0.51- 9.65) 0.29	1 1.96 (0.62- 6.19) 0.25 2.85 (1.17- 6.97) 0.02 2.64 (1.01- 6.88) 0.05 2.12 (0.55- 8.14) 0.27

IBS During and after Deployment

- 28% improved after deployment
- 70% developed new onset IBS post deployment
- The prevalence of IBS during and post deployment
  - not associated with age
  - marital status
  - education

IBS and QOL

- IBS post deployment associated with reduced QOL on all subscales of IBS-QOL (all P<0.01)
- 23% of subjects with IBS post-deployment unemployed

Psychological Factors and IBS

	During Deployment IBS Mean score		Post-Deployment IBS Mean score	
	IBS	No-IBS	IBS	No-IBS
Anxiety	10.88	6.10	8.71	5.37
Depression	9.59	6.10	8.27	5.43
Somatization	12.39	6.97	10.18	5.96
GSI	32.77	19.17	27.11	16.76

Raw mean score=0-24, GSI=0-72)

Conclusion-1

Conclusion-I

- Functional bowel disorders increases during deployment and the high prevalence persists post-deployment.
- Duration of deployment and combat are directly associated with prevalence of all bowel disorders post-deployment

Conclusion-II

- IBS is common in Persian Gulf Veterans and is associated with reduced QOL.
- Most of the subjects who develop IBS during deployment continue to have symptoms after deployment.
- The increased prevalence of IBS post deployment is not explained but raises the possibility of residual stress post-deployment.

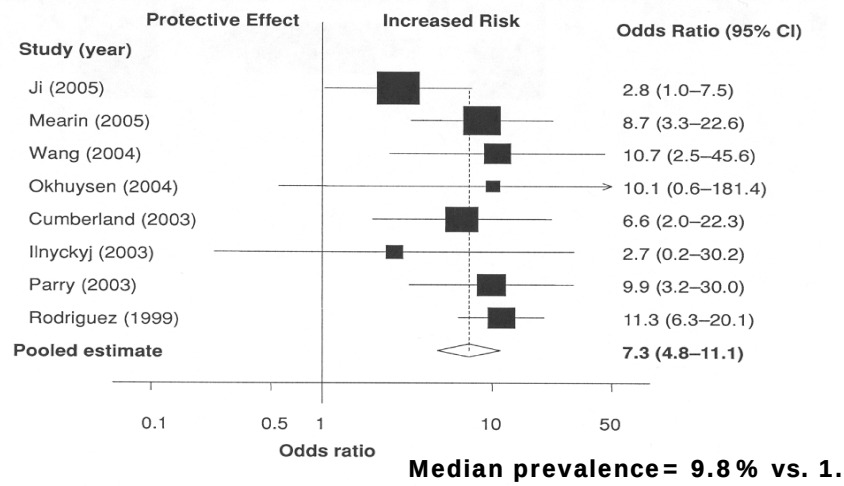
Mechanisms of IBS

Post-infective IBS

- Acute onset of IBS symptoms *after* an acute episode of gastroenteritis, characterized by at least two of the following features:
  - Fever
  - Vomiting
  - Diarrhea ,with or without a positive stool culture

Spiller et al. Gastroenterol 2003;124:1662

Gastroenteritis increases risk of PI-IBS



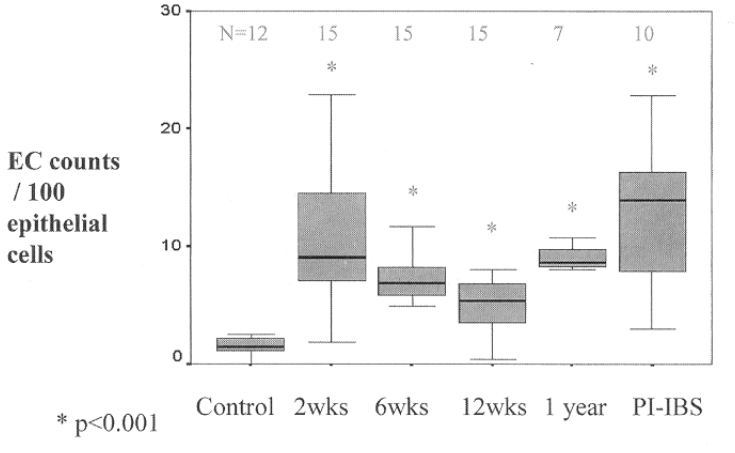
Median prevalence= 9.8% vs. 1.2%

* size of boxes denotes inverse-variance weight of each study

Mechanism #1

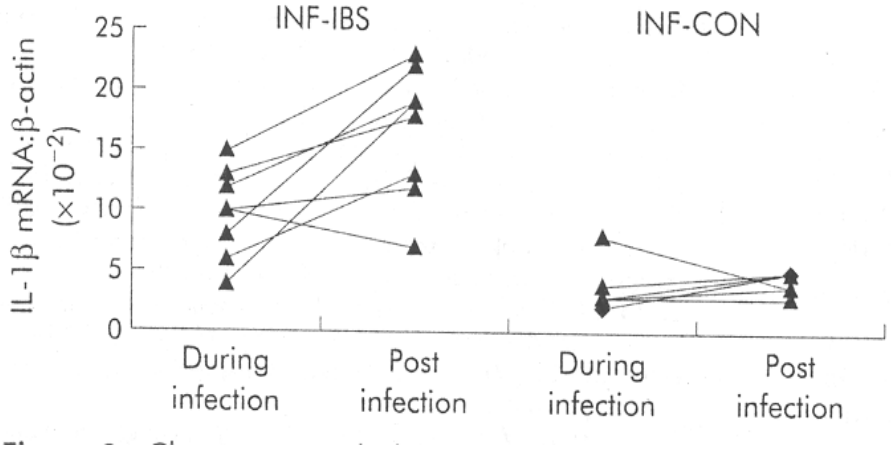
- Acute Infection leads to sub-clinical mucosal inflammation

Enterochromaffin Cells in IBS



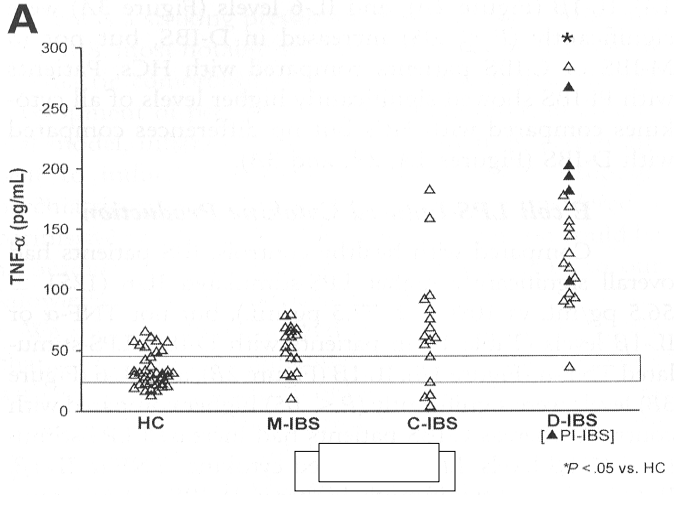
* p<0.001

Changes in Interleukin 1β mRNA Expression in PI-IBS and Infection Controls



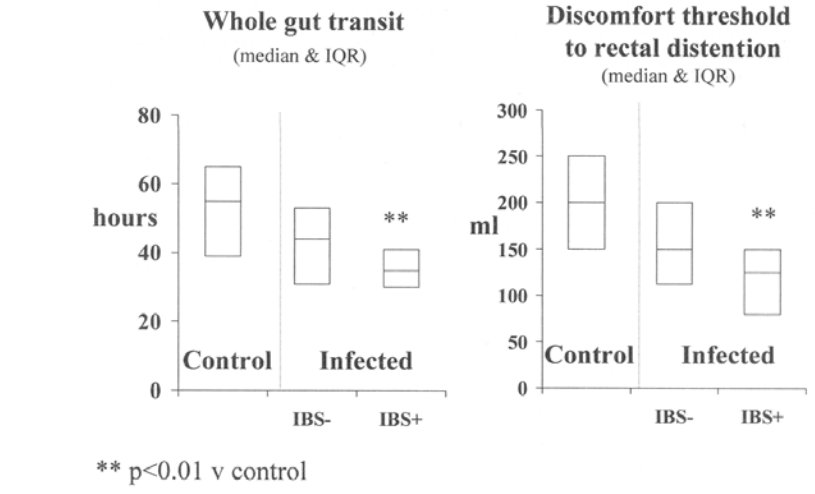
Gwee et al. Gut 2003, 523

TNF-α Production by PBMC from Healthy Controls and IBS (55 patients=D20,C17,M18)



Liebrgts et al. Gastroenterol 2007; 132:913

Physiologic Changes in PI-IBS



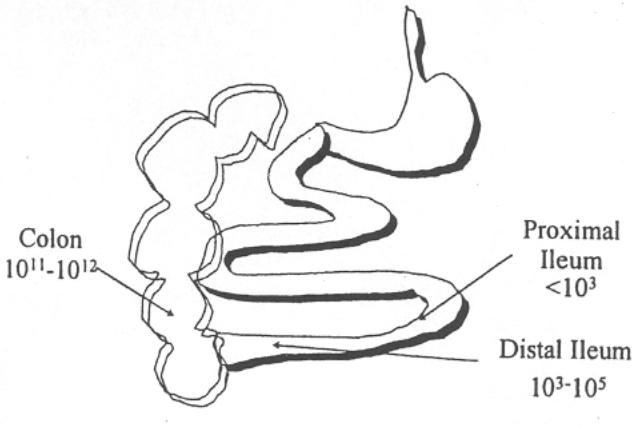
** p<0.01 v control

Gwee et al. Gut 1999;400

Mechanism # 2

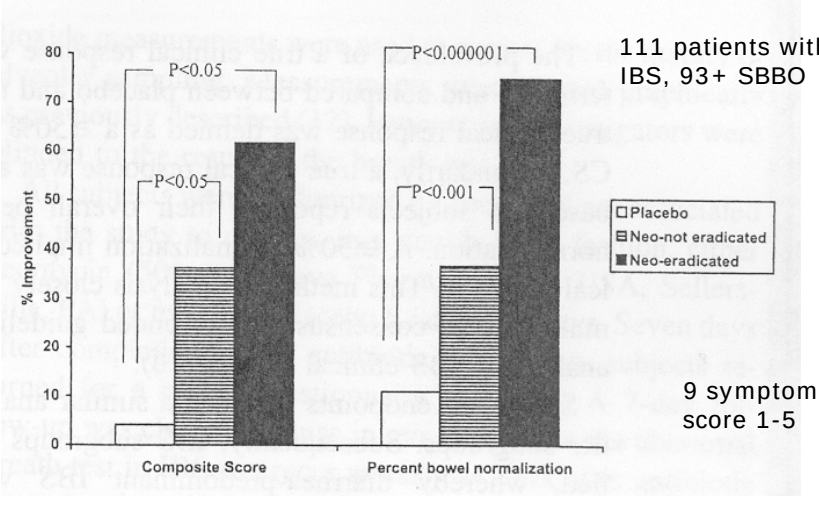
- Acute inflammation leads to Small bowel bacterial overgrowth (SBBO)

Gut Flora



Levitt MD. NEJM 1969:281:122

Eradication of Small Bowel Bacterial Overgrowth Reduces Symptoms of IBS



111 patients with IBS, 93+ SBBO

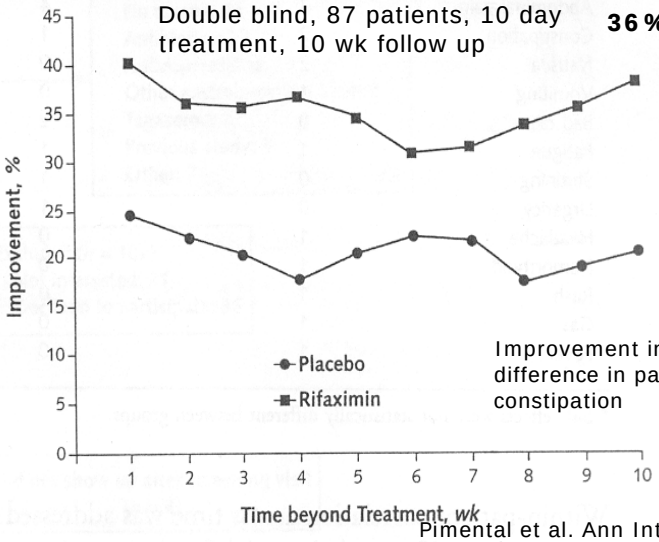
9 symptoms, score 1-5

Pimental et al. Am J Gastroenterol 2003;98(2):200

Global Improvement: IBS with Rifaximin

Double blind, 87 patients, 10 day treatment, 10 wk follow up

36% vs. 21%



Improvement in bloating, no difference in pain, diarrhea, constipation

Pimental et al. Ann Intern Med. 2006,

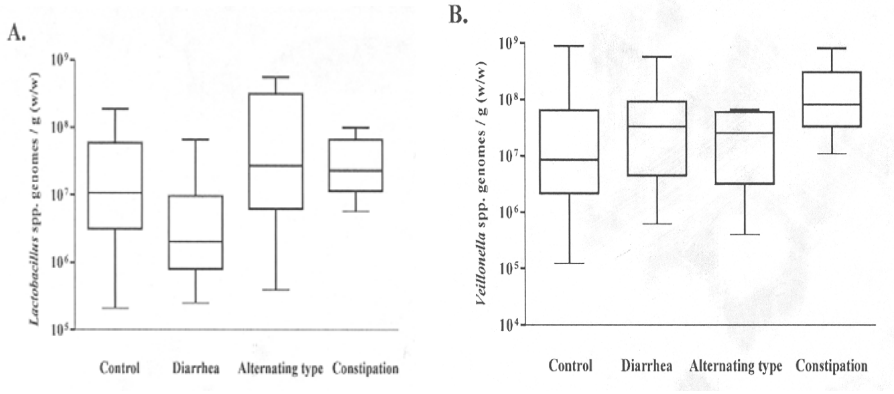
Mechanism#3: Change in Bacterial Flora

- Gut flora in IBS patient is different than normal
- Acute Gastroenteritis lead to change in bacterial flora.

*Bradley et al. J Med Microbiol 1987:23:29

*Balsari et al. Microbiologica 1982:5:185

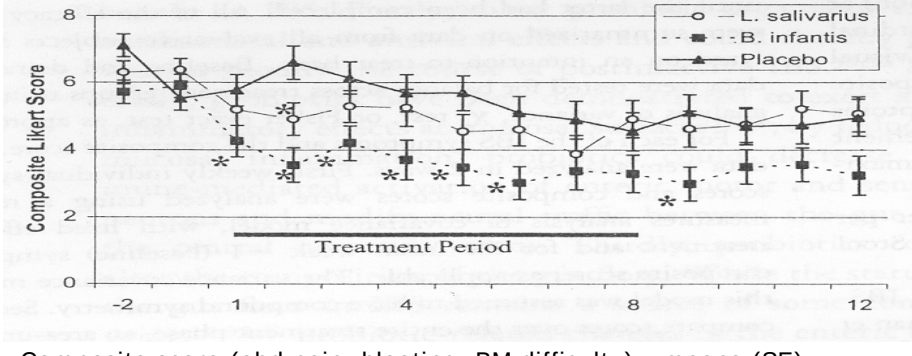
Stool Genomic DNA
A) Lactobacillus B) Veillonella



Malinen et al. Am J Gastro 2005:100:373

Probiotics in IBS

(N=75, IBS-A 45%, 28% IBS-D, 26% IBS-C)



Composite score (abd pain, bloating, BM difficulty) = means (SE)

O'Mahony et al. Gastroenterol 2005;128:54

Mechanism #4

- Psychological factors--Altered brain-gut communication

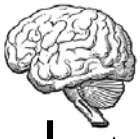
Effect of Stress on GI Motility

	Stressor	Parameter	Effect	
			Healthy	IBS
Esophagus	Mental	Contractile amplitude/ frequency	↓↑	↓↑
Stomach	Anger	Antral Motor activity	↑	↓
Small Intestine	Mental Stressor	MMC	↓	↓
Colon	Cold, mental stressor, anger	Motility index	→	↑

Dig Dis 2001:19:201

Pathophysiology of IBS
Altered Brain-Gut Communication

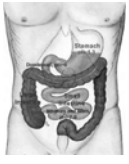
Central Control



Psychological Stress

Autonomic/Endocrine Control

Visceral Hypersensitivity/ Hypervigilance



Enteric Control
neual/immunological/hormonal

Gut Function: Food, infection/inflammation

Conclusion-1 Infection and IBS

Acute Gastroenteritis

~~Steroids~~

~~COX-2~~

Persistent Inflammation/ altered Immunity

?mesalamine

?cromoglycate

Impaired Small bowel motility

Antibiotics — **SBBO**

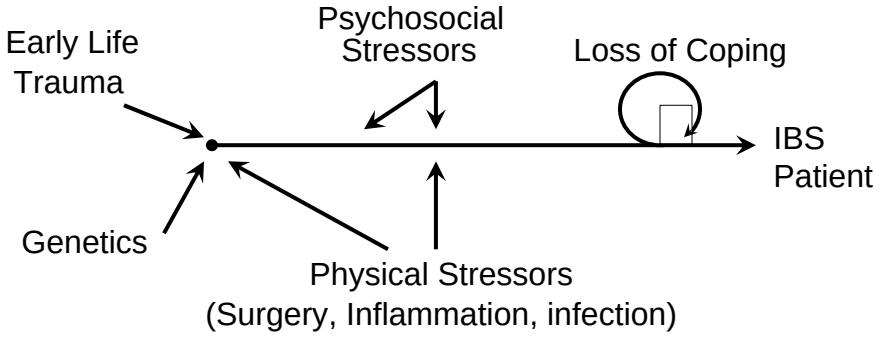
Altered Gut Flora — Probiotics

Genetics

Stress

Conclusion- Disease model of IBS

Perinatal	Early Life	Adult
Vulnerability Factors	Trigger Factors	Perpetuating Factors



Adopted from Mayer et al., 2001

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- Stacy Batt,
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Presentation 4 - Avlin Imaeda & Fred Gorelick

Perspectives on Gastrointestinal Illness in Gulf Veterans

Avlin Imaeda M.D., Ph.D
Fred Gorelick M.D.
4/7/08

Case #1
Operation Desert Storm

- 39 y/o man deployed to the Persian Gulf 1990-1991 and developed diarrhea while there with 5-6 watery stools per day, no blood, some mucous
- No nausea or vomiting
- Some cramping relieved by bowel movement
- Re-deployed to Northern Iraq with no change in symptoms
- Continues with about 4 episodes watery diarrhea per day, temporally related to eating, no weight loss or other symptoms
- Normal colonoscopy and EGD and labs including CRP
- No response to loperamide, but peanut butter helps

Case #2
Operation Iraqi Freedom

- 29 y/o man deployed in Iraq for Operation Iraqi Freedom
- C/O abdominal cramping, daily nausea and diarrhea since 5/2003, episodic flairs with worsening symptoms and vomiting
- Only other symptoms, GERD
- Multiple ER visits
- Unremarkable evaluation including: CT scan, gastric emptying scan (rapid emptying), small bowel series, EGD, colonoscopy, capsule endoscopy, laboratory studies including malabsorption evaluation, stool pathogen evaluation
- Unresponsive to loperamide, promethazine, omeprazole, dicyclomine

Case #3
War in Afghanistan

- 25 y/o man recently returned from deployment in Afghanistan
- He c/o soft to watery stools 30-60 minutes after eating
- Mild cramping that is relieved with a bowel movement
- He doesn't notice blood or mucus and doesn't awaken at night for bowel movements
- He has no other symptoms
- Labs and stool infection work-up are unrevealing
- Loperamide lead to constipation

Subjective Overview WHVA Experience

- Veterans from Saudi Arabia, Kuwait, Iraq, and Afghanistan
- Crampy abdominal pain not generally prominent
- GERD occasionally associated and can be prominent
- Nausea +/- vomiting sometimes associated
- Headaches occasionally associated
- Most poorly responsive to opioid antagonism ie. loperamide
- Constipation not commonly seen/referred to our clinic
- Associated Gulf War syndrome complaints not commonly seen/referred to our clinic
- Prominent lymphoid hyperplasia in the ileum
- Low range but normal **B12** and carotene sometimes seen not universally checked
- No systematic follow-up, many Gulf War Veterans lost to follow-up

Syndrome Related vs Non-Syndrome Diarrhea

- Are there overlapping etiologies?
- Is there a final common pathway?
- Will treatment be the same?

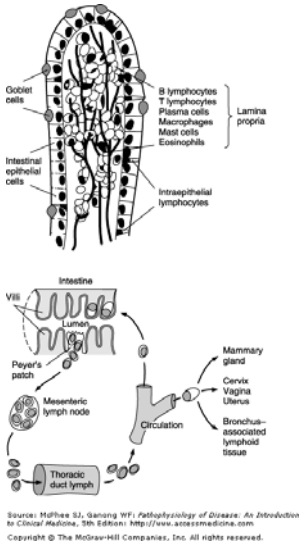
Possible Etiologies

- Post-infectious IBS
- Toxin or exposure related: Gut vs CNS injury
  - Pyridostigmine bromide pills
  - Pesticides
  - Sarin
  - Other exposures
- IPSID- Immunoproliferative small intestinal disease
- Chronic unidentified infection

Immune and Neuroendocrine Systems Control Intestinal Function

- Enteric immune system
- Enteric nervous system
- Parasympathetic and Sympathetic Nervous System
- Enteric Endocrine System

Enteric Immune System



- Innate immune system
  - Mucosal protection system
  - Innate immune cells
- Adaptive immune system

IBS is a Model to Understand Intestinal Function

Chronic Inflammation Occurs in IBS

- Heterogeneous group of patients
- Chronic low grade inflammation in some patients with IBS
- Changes in mucosal bacterial populations
- Mucosal gene expression profiling reveals alterations in expression of several genes involved in the mucosal immune response

Mucosal Bacteria is Altered in IBS

Malinen et al. Amer J Gastroenterol 2005; 100:373

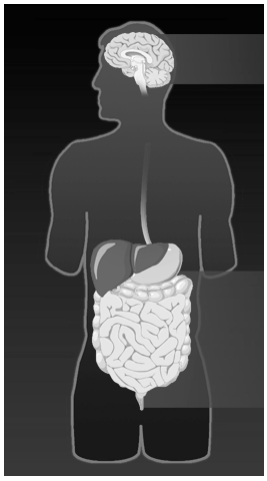
- Real-Time PCR for 16s rRNA from 20 bacterial groups
- 22 controls, 12 IBS-D, 9 IBS-C, 6 IBS-M
- IBS-D decreased *Lactobacillus* spp.
- IBS-C increased *Veillonella* spp.

Immune System Genes are Altered in IBS

Aerssens et.al. Clin Gastroenterol Hepatol 2008; 6:194

- **Human Genome U133 Plus 2.0 Gene Chips with RNA from 36 IBS vs 25 controls**
- 3 genes, lower in IBS- MHC class I antigen processing
- 3 genes, lower in IBS- involved in innate immune responses
- Multiple related genes involved in oxidative burst increased or decreased
- 6 genes involved in various aspects of the immune response significantly increased or decreased in IBS vs controls

The Gut Nervous System is Immense



5%

95%

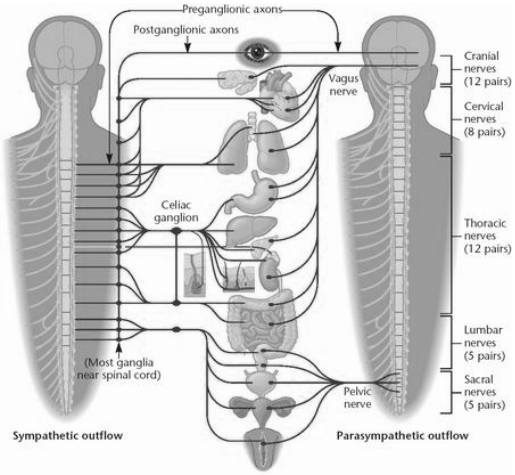
- Central Nervous System
  - Neurons

Autonomic Nervous System
- Neurons

Enteric Nervous System
- Neurons
- Enterochromaffin Cells
- Mast Cells

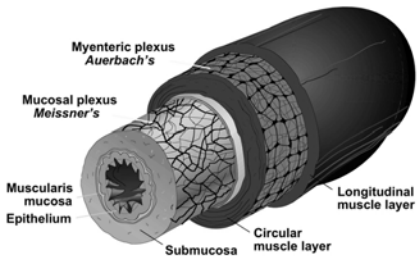
The Autonomic Nervous System Links ENS to CNS

- Parasympathetic: acetylcholine stimulates motility and secretion
- Sympathetic: norepinephrine inhibits motility and secretion

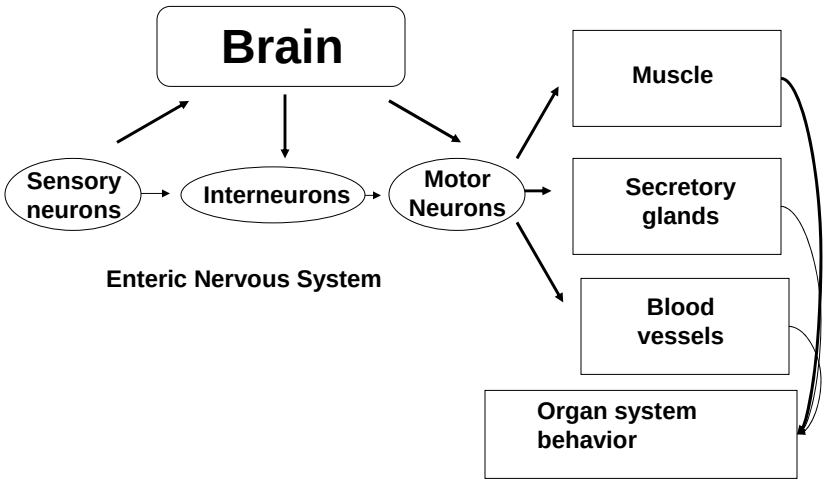


ENS Can act independently of CNS

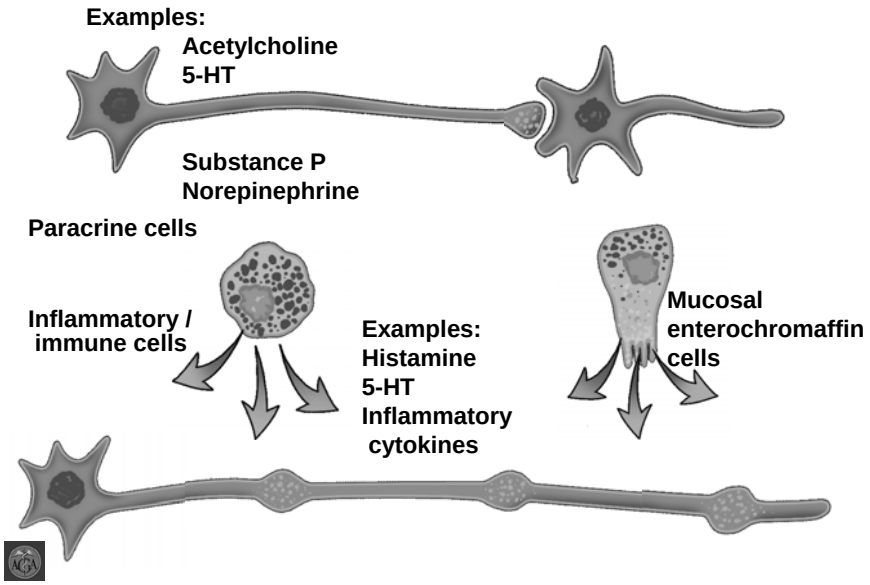
- Sensory nerves: detect mechanical (stretch), thermal, osmotic, chemical stimuli
- Motor nerves: control motility, secretion, ?absorption (Acetylcholine)
- Interneurons: integrate



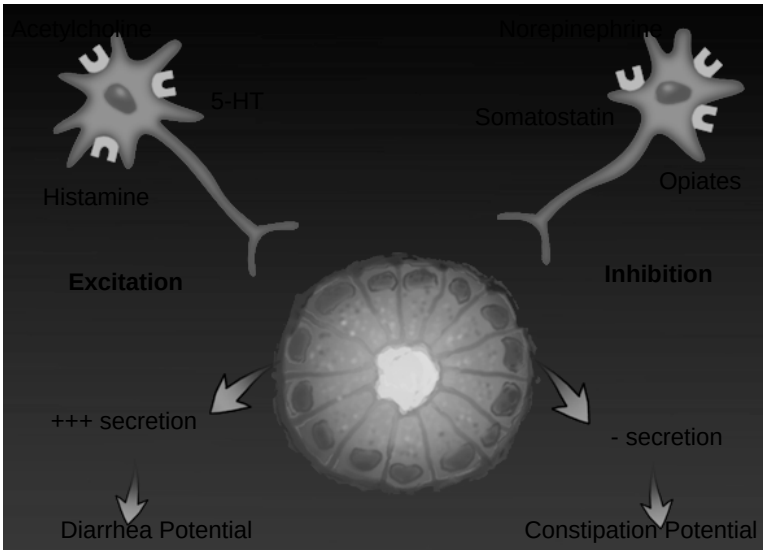
Enteric Nervous System Model



Signaling in the Enteric Nervous System is Complex



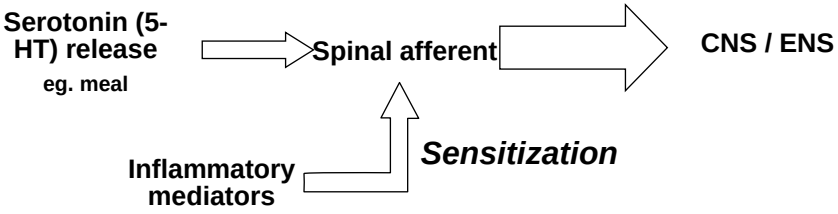
Efferent Signals Direct Function



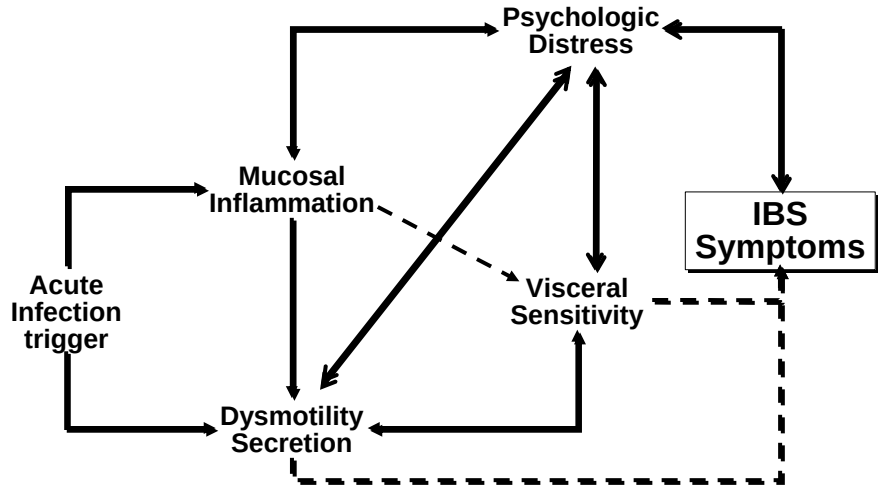
The Autonomic Nervous System Links ENS to CNS

Normal

Inflammation



The Brain Interprets Signals and Directs Function

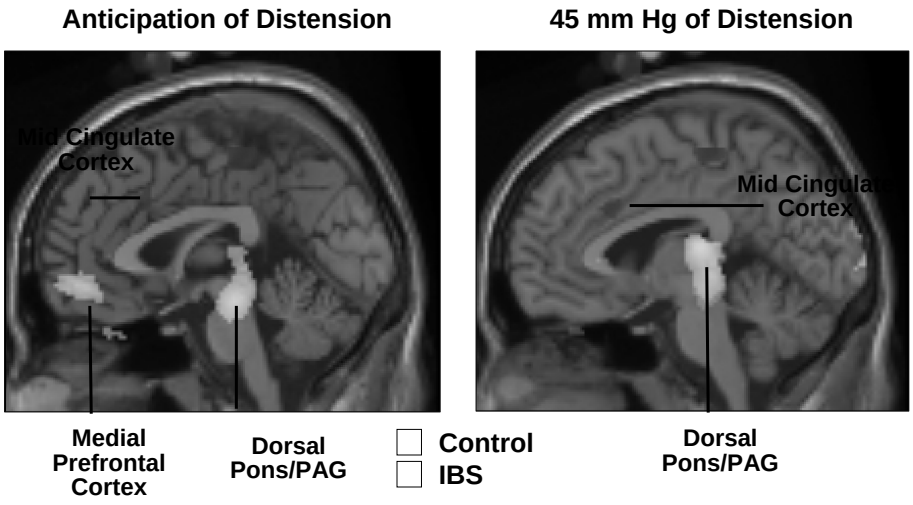


Drossman, Gut 1999; 44:306
Gwee et. al., Gut 1999; 44:400

Neurologic Changes Occur in IBS

- Diverse group of patients
- Altered perception of visceral stimulation: rectal and stomach (+/- altered somatic hypersensitivity)
  - Altered visceral sensitivity
  - Altered central processing and modulation
- PET and fMRI show altered responses to rectal stimuli
- Altered intestinal transit time
- EC cell hyperplasia

Brain Activation (PET) Differs in Normals vs. IBS



Naliboff, et. al., Psychos Med 2001; 63:365

Serotonin and the Enteric Endocrine System

- Complex array of molecules
- Enterochromaffin Cells (EC): serotonin (5-HT)-activates secretory and peristaltic reflexes and vagal afferents (5HT3)
- Receptors 5-HT1, 5-HT3, 5-HT4, 5-HT7 in the gut

Serotonin (5-HT) Controls the Human Gut

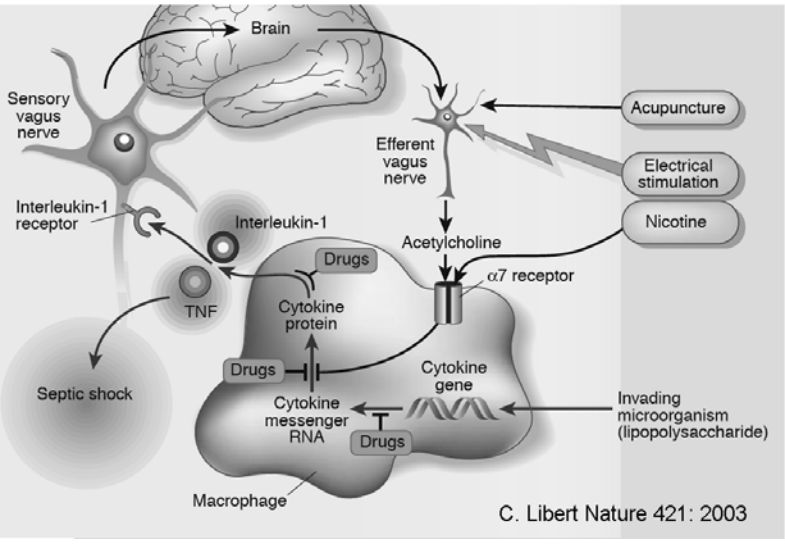
	5-HT_1	5-HT_3	5-HT_4
Gastric accommodation	⇧		⇧
Transit	⇩	⇧	⇧
Colonic tone	⇩	⇧	
Sensation		⇧	?
Secretion		⇧	

Altered serotonin metabolism may have a role in IBS

- Increased bowel serotonin levels in diarrheal IBS
- 5-HT4 (Tegaserod), 5-HT3 (Alosetron) therapies effective in some IBS patients
- SERT regulates serotonin uptake; p11 increases 5HT receptor expression
- p11 levels are changed in depression and affected by anti-depressants
- p11 expression in sigmoid colon increased in some IBS patients; SERT equivocal

Spiller and Bennett. Gastroenterology 132: 2007

Acetylcholine and Macrophages
The Neuroendocrine-Inflammatory Axis

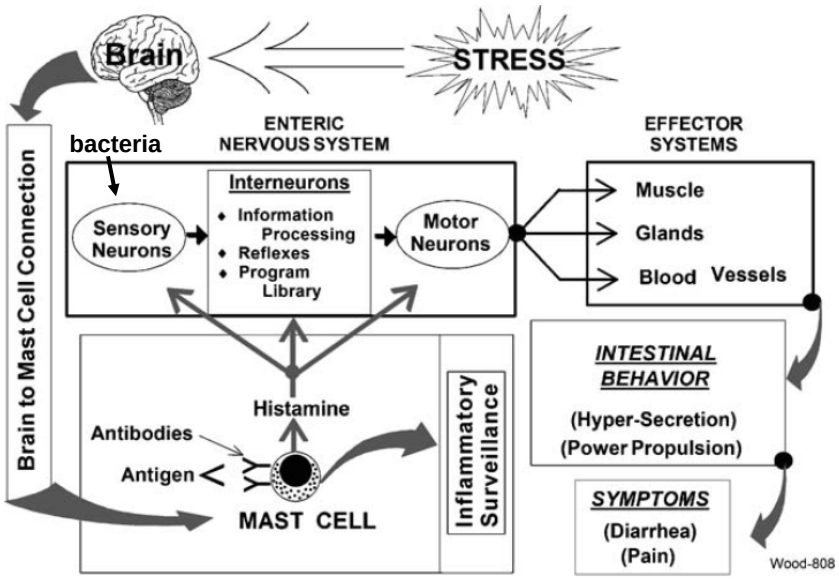


Summary

- Diarrhea is common in Veterans from all recent conflicts. Some also experience UGI symptoms
- Etiology may be multi-factorial
  - Post-infectious IBS
  - Toxin related
- Stimulus to immune system, peripheral nervous system or central nervous system may lead to diarrhea and abdominal pain
- Further evaluation is needed

Mechanistic Overveiw

- Fixed change in function represents a new steady state
  - Sensitization: a change in the degree or type of response to a physiological stimulus (IBD). This could be induced by a fixed change in “receptive” pathways involving mucosa, inflammatory cells or nerves
  - New stimuli:
    - Change in bacterial flora
    - Viral
    - Chemical



Wood J Clin Gastro 2007

GI Symptoms in GW Veterans: Directions

- Characterize phenotypes
- Determine prevalence
- Evaluations
  - Autonomic, gut transit, gastric volumes (GV), satiation, rectal compliance and sensation (thresholds and pain ratings) testing
  - Mucosal genomics
  - Bacterial genomics
  - Psychological testing

Presentation 5 - Richard Valente

Health Study of Rhode Island Veterans

Brigadier General (Ret) Richard J. Valente
Rhode Island Persian Gulf War Information Relief Commission (RIPGWIRC)

April 7, 2008

Rhode Island Persian Gulf War Information Relief Commission

- Established February 1998
- Mission Statement:

The mission of the RIPGWIRC is to identify the medical, administrative and social needs of the Rhode Island Veterans of the Persian Gulf War and to ensure that all information pertinent to these veterans is identified and distributed to them in an expeditious manner; to provide advice to the director of the department of human services on all appropriate matters and to render an annual report to the Governor and the General Assembly.

Rhode Island Persian Gulf War Information Relief Commission

4 Objectives:

- Creation of a RI Persian Gulf War Registry
- Collection of Pertinent Data
- Conducting a Comprehensive Health Status Survey
- Conducting a Communications Campaign

Overview

Four Research Questions:

1. Was there a problem? (Gulf War Illness, Symptoms, Work and Social Interference)
2. If so, what percentage of RI veterans were affected?

Overview (cont.)

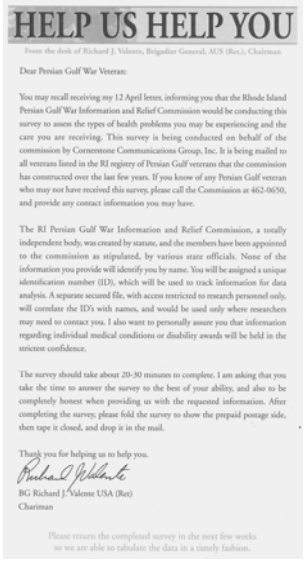
Four Research Questions:

3. Was there a history of exposures that increased the chances of having Gulf War Illness?
4. What was the use and satisfaction of VA services?

Source Population

- 6,500 veterans recognized by the Department of Defense that were active, reserve or in the national guard (August 1990 - August 1991) from RI
- We identified 3,500 veterans from rosters of units deployed, searching DD 214s from the RI veterans archives, public forums (1999-2002) and sent them a health survey
- 2,000 surveys sent to valid addresses
- 241 valid replies (12% response rate)

Health Study of RI Veterans



HELP US HELP YOU

From the desk of Richard J. Valente, Brigadier General, AUS (Ret.), Chairman

Dear Persian Gulf War Veteran:

You may recall receiving my 12 April letter, informing you that the Rhode Island Persian Gulf War Information and Relief Commission would be conducting this survey to assess the types of health problems you may be experiencing and the care you are receiving. This survey is being conducted on behalf of the commission by Cornerstone Communications Group, Inc. It is being mailed to all veterans listed in the RI registry of Persian Gulf veterans that the commission has constructed over the last few years. If you know of any Persian Gulf veteran who may not have received this survey, please call the Commission at 462-0650, and provide any contact information you may have.

The RI Persian Gulf War Information and Relief Commission, a totally independent body, was created by statute, and the members have been appointed to the commission as stipulated, by various state officials. None of the information you provide will identify you by name. You will be assigned a unique identification number (ID), which will be used to track information for data analysis. A separate secured file, with access restricted to research personnel only, will correlate the ID's with names, and would be used only where researchers may need to contact you. I also want to personally assure you that information regarding individual medical conditions or disability awards will be held in the strictest confidence.

The survey should take about 20-30 minutes to complete. I am asking that you take the time to answer the survey to the best of your ability, and also to be completely honest when providing us with the requested information. After completing the survey, please fold the survey to show the prepaid postage side, then tape it closed, and drop it in the mail.

Thank you for helping us to help you.

BG Richard J. Valente USA (Ret)
Chairman

Please return the completed survey in the next few weeks so we are able to tabulate the data in a timely fashion.

PERSIAN GULF WAR
Health Study of Rhode Island Veterans

When answering this survey, please check the most correct response, to the best of your knowledge.

1. Were you on active duty or mobilized any time between August 1990 and December 1991?
   ☐ YES ☐ NO
2. What is your gender? ☐ Male ☐ Female
3. Please indicate your service component as of 1 Aug 90.
   ☐ Active ☐ National Guard ☐ Reserves
4. Indicate your branch of service.
   ☐ Army ☐ Navy ☐ Marines ☐ Air Force ☐ Coast Guard
5. What rank did you hold during this period? _____
6. What was your Military Occupational Specialty? _____
7. Were you deployed to the Persian Gulf area any time?
   ☐ YES Please check the South West Asia countries you were in during the period Aug 90-Dec 91.
   ☐ NO Please list countries or areas you were in, and the period of time i.e. (days, months, year)
   ☐ Saudi Arabia ☐ Kuwait ☐ Iraq ☐ Bahrain ☐ United Arab Emirates
   List any others _____ (mo/yr)
   _____ (mo/yr)
   _____ (mo/yr)
8. What month and year did you arrive in the Persian Gulf? _____ (mo/yr)
9. When did you depart the Persian Gulf? _____ (mo/yr)
10. What units did you serve with between Aug. 90 through Dec. 91? (For Army and Marines, be specific as possible, i.e., Division, Brigade, Battalion, Co, detachment.) For Air Force, list bases. For Navy/Coast Guard, list ship or shore duty station)
    _____
    _____
11. Have you ever received a notification from the Department of Defense that your unit may have been in the downwind fallout area from the demolition of the ammunition dump at Khamisiyah?
    ☐ YES ☐ NO ☐ DON'T KNOW
12. Do you believe you were in the vicinity of the Khamisiyah demolitions, or in the downwind fallout area in Mar. 91? ☐ YES ☐ NO ☐ DON'T KNOW
13. Are you currently in the military?
    ☐ YES ☐ ACTIVE COMPONENT ☐ NATIONAL GUARD ☐ RESERVE ☐ NO
14. Have you changed component or branch of service since returning from the Persian Gulf?
    ☐ YES changed to _____ on _____ (mo/yr) ☐ NO
15. Have you undergone an official Persian Gulf War Physical?
    ☐ YES _____ (mo/yr) ☐ NO ☐ DON'T KNOW
16. If you answered YES to #15, for the Persian Gulf Physical Registries in which you were enrolled?
    ☐ DOD CLINICAL EVALUATION PROGRAM _____ (mo/yr)
    ☐ VETERAN'S ADMINISTRATION REGISTRY _____ (mo/yr)
17. Did you apply for a VA service connected disability rating?
    ☐ YES _____ (mo/yr) ☐ NO
18. Did you receive a VA service connected disability rating?
    ☐ YES _____ (mo/yr) ☐ NO
18a. If YES, was it for MEDICAL or OTHER, such as PTSD _____ % _____
19. Currently, how would you classify your health?
    ☐ EXCELLENT ☐ GOOD ☐ FAIR ☐ POOR ☐ DON'T KNOW
20. Looking back to just before Aug 90, would you say your health was:
    ☐ EXCELLENT ☐ GOOD ☐ FAIR ☐ POOR ☐ DON'T KNOW

Demographics

- Gender: 92% men, 8% women
- Employed: 80% yes, 15% no
- Branch of Service:

  Army - 44%,Navy - 20%, Air Force - 17%, Marines - 13%
- Service Component:

  Active - 51%, National Guard - 31%, Reserves - 15%

Definition of Gulf War Illness

3 out of the 6 criteria from
Lea Steele's AJE 2000 paper

Gulf War Illness

6 Symptoms	N
• Fatigue/Sleep Problems	124
• Pain Symptoms	103
• Neurologic/Cognitive Mood Symptoms	159
• GI Symptoms	84
• Respiratory Symptoms	86
• Skin Problems	80

n=241

Outcomes

Number of Individuals fitting the criteria of Gulf War Illness (3 of 6 symptoms)

122 (51%)

n=241

Definition of Gulf War Illness

2 out of 3 criteria from CDC definition

CDC Gulf Illness

Symptoms	N
• Fatigue	108
• Mood/Cognition	144
• Musculoskeletal	103

n=241

Outcomes

Number of Individuals fitting the criteria of CDC Gulf Illness (2 of 3 symptoms)

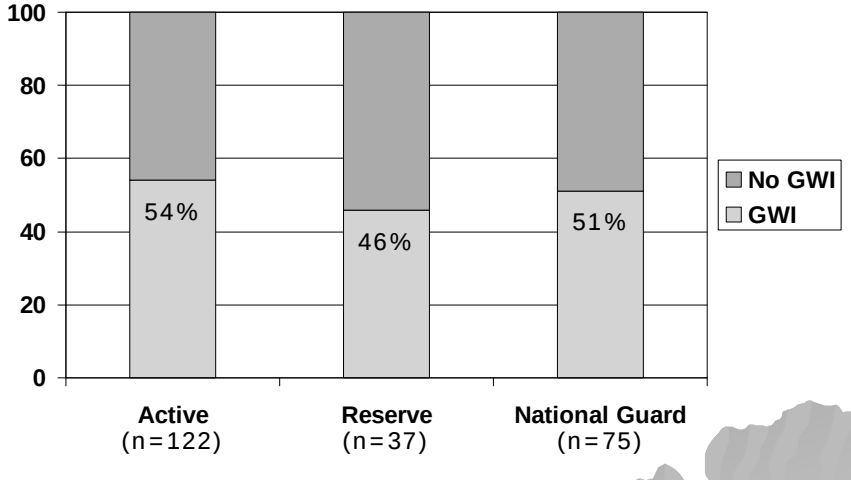
118 (49%)

n=241

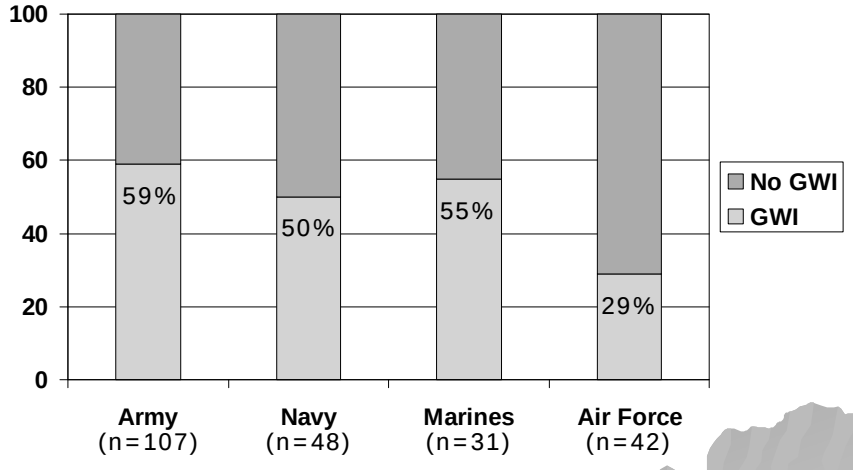
Selected Health Conditions Developed After 1991

Symptoms	N
• Skin Problems*	58
• GI Symptoms*	84
• Cancer	35
• Depression	49
• Post-Traumatic Stress	28
• Chronic Fatigue*	35
• Asthma/Bronchitis*	30

Each Service Component Equally Affected



Army and Marines Most Affected With GWI



Research Questions 1 & 2

1. Was there a problem? (Gulf War Illness, Symptoms, Work and Social Interference) Yes!
2. If so, what percentage of RI veterans were affected?

   Our study: 49% - 51%

Gulf War Illness

Steele article:

- If deployed to Iraq and/or Kuwait 42%
- Persian Gulf War Veterans (PGW) 34%
- Non-PGW Veterans (with vaccine) 12%
- Non-PGW veterans (without vaccine) 4%

n=1548 veterans who served in the PGW, 482 veterans who served elsewhere, Total n=2030

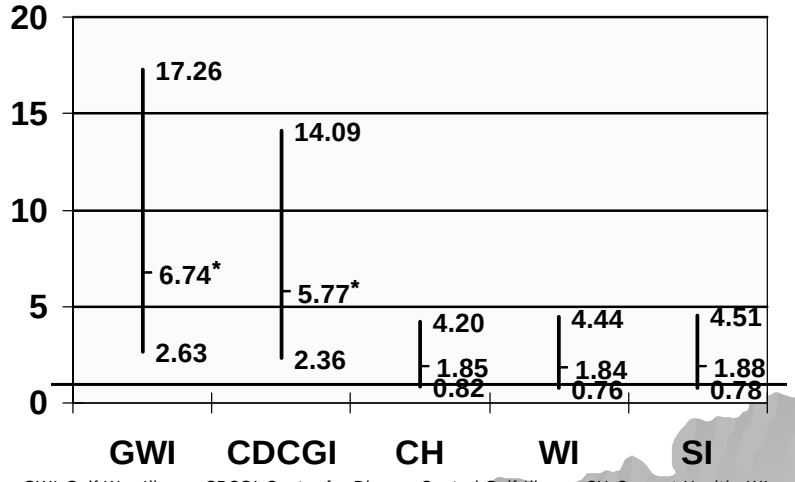
Gulf War Illness

CDC Definition:

- Iowa - 17% (n=4886)
  (JAMA 1997;277:238-245)
- Air Force - 39% (n=3881)
  (JAMA 1998;280:981-988)
- Blanchard - 29% (n=2189)
  (AJE 2006;163;66-75)

What caused the problem?

Deployed to Iraq and/or Kuwait (n=129)



Key: GWI-Gulf War Illness, CDCGI-Center for Disease Control Gulf Illness, CH-Current Health, WI-Work Interference, SI-Social Interference. *Significant odds ratio at p=0.05.

What caused the problem?

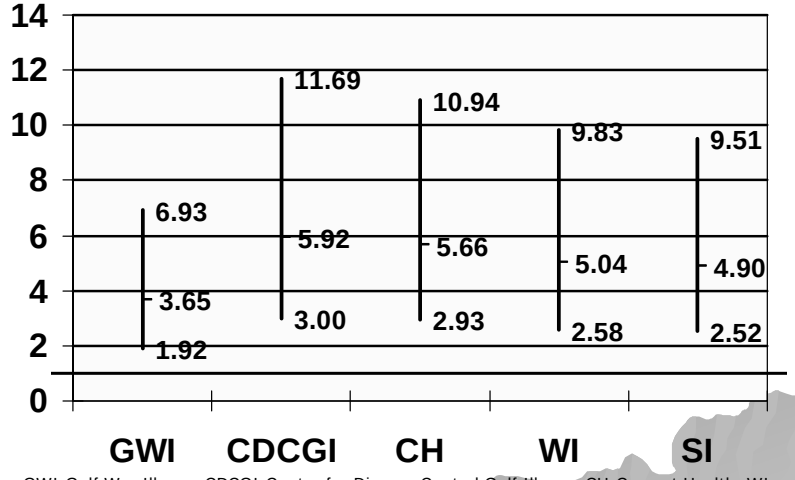
Deployed to Iraq and/or Kuwait (n=129)

Gulf War Illness Symptoms	OR	95% CI	
Fatigue/Sleep Problems	**4.88**	**2.07**	**11.49**
Pain Symptoms	**3.46**	**1.47**	**8.14**
Neurologic/Cognitive/Mood Symptoms	**5.18**	**2.36**	**11.38**
GI Symptoms	**5.94**	**1.99**	**17.76**
Respiratory Symptoms	**2.89**	**1.18**	**7.08**
Skin Problems	**3.38**	**1.32**	**8.67**
CDC Gulf Illness Symptoms			
Fatigue	**3.26**	**1.43**	**7.45**
Mood/Cognition	**6.68**	**2.89**	**15.43**
Musculoskeletal	**3.46**	**1.47**	**8.14**
Selected Health Conditions			
Developed Skin Problems	**2.69**	**0.95**	**7.65**
GI Symptoms	**5.94**	**1.99**	**17.76**
Developed Any Cancer	**9.53**	**1.25**	**72.69**
Depression		not est	
PTSD	**8.80**	**1.13**	**68.24**
Chronic Fatigue	**8.04**	**1.04**	**62.19**
Asthma/Bronchitis	2.68	0.58	12.40

OR = Odds Ratio: odds of having an adverse outcome given exposure. 95% CI = Confidence Interval for associated odds ratio. not est = not able to estimate odds ratio & 95% CI.

What caused the problem?

Exposed to Khamisiyah Dump Demolition (n=63)



Key: GWI-Gulf War Illness, CDCGI-Center for Disease Control Gulf Illness, CH-Current Health, WI-Work Interference, SI-Social Interference. All 5 categories indicate significant odds ratio at p=0.05.

What caused the problem?

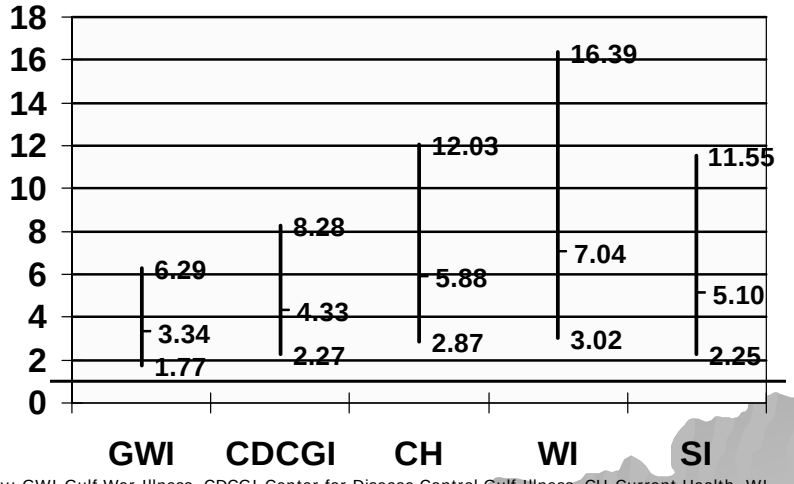
Exposed to Khamisiyah Dump Demolition (n=63)

Gulf War Illness Symptoms	OR	95% CI	
Fatigue/Sleep Problems	**6.07**	**3.01**	**12.24**
Pain Symptoms	**2.81**	**1.52**	**5.21**
Neurologic/Cognitive/Mood Symptoms	**4.72**	**2.16**	**10.34**
GI Symptoms	1.78	0.95	3.32
Respiratory Symptoms	**2.15**	**1.16**	**4.01**
Skin Problems	**2.21**	**1.17**	**4.15**
CDC Gulf Illness Symptoms			
Fatigue	**4.74**	**2.48**	**9.05**
Mood/Cognition	**7.18**	**3.28**	**15.72**
Musculoskeletal	**2.81**	**1.52**	**5.21**
Selected Health Conditions			
Developed Skin Problems	**4.36**	**2.06**	**9.22**
GI Symptoms	1.78	0.95	3.32
Developed Any Cancer	**3.17**	**1.42**	**7.09**
Depression	**4.78**	**2.26**	**10.11**
PTSD	**10.57**	**3.79**	**29.49**
Chronic Fatigue	**7.18**	**3.06**	**16.85**
Asthma/Bronchitis	2.34	0.96	5.72

OR = Odds Ratio: odds of having an adverse outcome given exposure. 95% CI = Confidence Interval for associated odds ratio. not est = not able to estimate odds ratio & 95% CI.

What caused the problem?

Took Pyrodistigmine Bromide (n=93)



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What caused the problem?

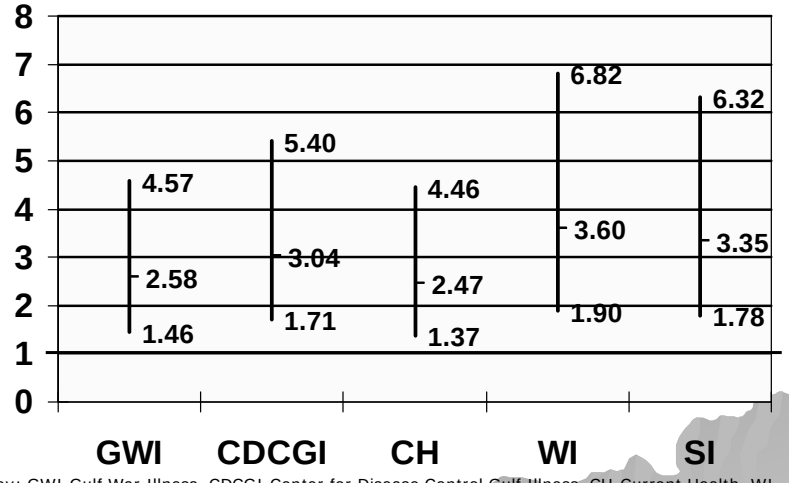
Took Pyrodistigmine Bromide (n=93)

Gulf War Illness Symptoms	OR	95% CI	
Fatigue/Sleep Problems	**4.24**	**2.22**	**8.10**
Pain Symptoms	**2.92**	**1.53**	**5.57**
Neurologic/Cognitive/Mood Symptoms	**3.51**	**1.78**	**6.89**
GI Symptoms	**3.67**	**1.85**	**7.28**
Respiratory Symptoms	**2.58**	**1.33**	**4.99**
Skin Problems	**3.73**	**1.74**	**8.01**
CDC Gulf Illness Symptoms			
Fatigue	**3.96**	**2.07**	**7.61**
Mood/Cognition	**5.60**	**2.85**	**11.00**
Musculoskeletal	**2.92**	**1.53**	**5.57**
Selected Health Conditions			
Developed Skin Problems	**3.80**	**1.57**	**9.23**
GI Symptoms	**3.67**	**1.85**	**7.28**
Developed Any Cancer	**3.83**	**1.47**	**9.99**
Depression	**6.14**	**2.36**	**15.99**
PTSD	**6.76**	**1.89**	**24.21**
Chronic Fatigue	**31.50**	**4.13**	**240.00**
Asthma/Bronchitis	1.80	0.70	4.62

OR = Odds Ratio: odds of having an adverse outcome given exposure. 95% CI = Confidence Interval for associated odds ratio. not est = not able to estimate odds ratio & 95% CI.

What caused the problem?

Area Where Chemical Alarms Sounding (n=92)



Key: GWI-Gulf War Illness, CDCGI-Center for Disease Control Gulf Illness, CH-Current Health, WI-Work Interference, SI-Social Interference. All 5 categories indicate significant odds ratio at p=0.05.

What caused the problem?

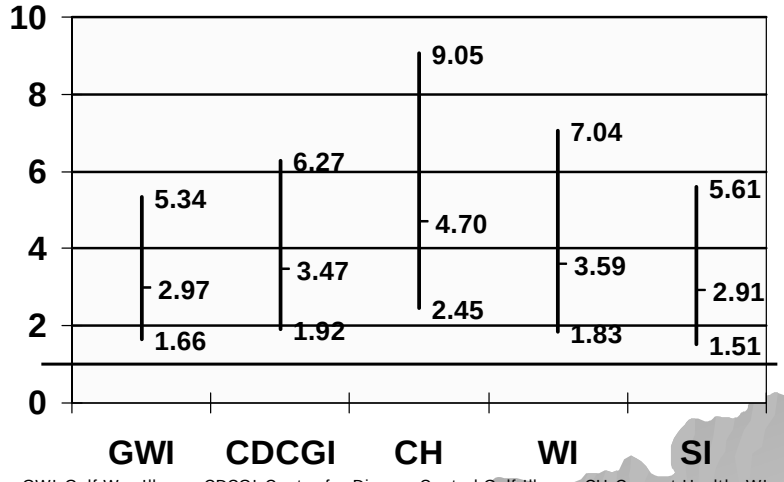
Area Where Chemical Alarms Sounding (n=92)

Gulf War Illness Symptoms	OR	95% CI	
Fatigue/Sleep Problems	**2.88**	**1.61**	**5.12**
Pain Symptoms	**2.67**	**1.51**	**4.74**
Neurologic/Cognitive/Mood Symptoms	**3.00**	**1.61**	**5.59**
GI Symptoms	**2.24**	**1.25**	**4.03**
Respiratory Symptoms	1.50	0.84	2.69
Skin Problems	**4.24**	**2.26**	**7.93**
CDC Gulf Illness Symptoms			
Fatigue	**2.68**	**1.51**	**4.75**
Mood/Cognition	**3.64**	**1.97**	**6.72**
Musculoskeletal	**2.67**	**1.51**	**4.74**
Selected Health Conditions			
Developed Skin Problems	**4.35**	**2.10**	**8.99**
GI Symptoms	**2.24**	**1.25**	**4.03**
Developed Any Cancer	1.94	0.90	4.19
Depression	**3.57**	**1.72**	**7.42**
PTSD	**3.92**	**1.52**	**10.10**
Chronic Fatigue	**2.94**	**1.27**	**6.78**
Asthma/Bronchitis	1.10	0.45	2.68

OR = Odds Ratio: odds of having an adverse outcome given exposure. 95% CI = Confidence Interval for associated odds ratio. not est = not able to estimate odds ratio & 95% CI.

What caused the problem?

Participated in Combat Operations (n=105)



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What caused the problem?

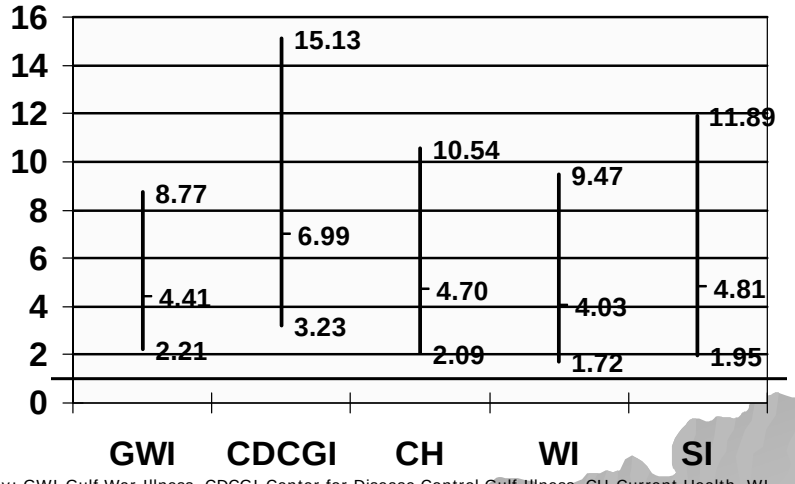
Participated in Combat Operations (n=105)

Gulf War Illness Symptoms	OR	95% CI	
Fatigue/Sleep Problems	**3.12**	**1.73**	**5.61**
Pain Symptoms	1.57	0.89	2.79
Neurologic/Cognitive/Mood Symptoms	**3.53**	**1.88**	**6.61**
GI Symptoms	**2.51**	**1.36**	**4.62**
Respiratory Symptoms	1.63	0.90	2.95
Skin Problems	**2.25**	**1.20**	**4.20**
CDC Gulf Illness Symptoms			
Fatigue	**2.65**	**1.47**	**4.78**
Mood/Cognition	**4.06**	**2.21**	**7.45**
Musculoskeletal	1.57	0.89	2.79
Selected Health Conditions			
Developed Skin Problems	**2.48**	**1.19**	**5.18**
GI Symptoms	**2.51**	**1.36**	**4.62**
Developed Any Cancer	**3.47**	**1.42**	**8.50**
Depression	**3.70**	**1.66**	**8.21**
PTSD	**7.27**	**2.04**	**25.97**
Chronic Fatigue	**4.06**	**1.55**	**10.64**
Asthma/Bronchitis	1.48	0.56	3.86

OR = Odds Ratio: odds of having an adverse outcome given exposure. 95% CI = Confidence Interval for associated odds ratio. not est = not able to estimate odds ratio & 95% CI.

What caused the problem?

Any Exposure (n=187)



Key: GWI-Gulf War Illness, CDCGI-Center for Disease Control Gulf Illness, CH-Current Health, WI-Work Interference, SI-Social Interference. All 5 categories indicate significant odds ratio at p=0.05.

What caused the problem?

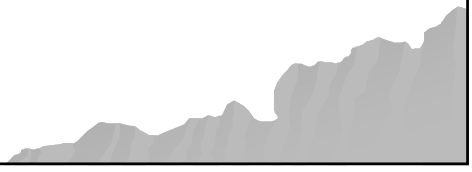
Any Exposure (n=187)

Gulf War Illness Symptoms	OR	95% CI	
Fatigue/Sleep Problems	**4.08**	**2.08**	**8.01**
Pain Symptoms	**4.35**	**2.07**	**9.16**
Neurologic/Cognitive/Mood Symptoms	**4.43**	**2.34**	**8.37**
GI Symptoms	**2.88**	**1.37**	**6.08**
Respiratory Symptoms	**2.29**	**1.13**	**4.64**
Skin Problems	**3.60**	**1.61**	**8.06**
CDC Gulf Illness Symptoms			
Fatigue	**4.21**	**2.05**	**8.67**
Mood/Cognition	**6.52**	**3.29**	**12.91**
Musculoskeletal	**4.35**	**2.07**	**9.16**
Selected Health Conditions			
Developed Skin Problems	**3.18**	**1.33**	**7.59**
GI Symptoms	**2.88**	**1.37**	**6.08**
Developed Any Cancer	**11.78**	**1.57**	**88.16**
Depression	**21.28**	**2.85**	**158.82**
PTSD		not est	
Chronic Fatigue		not est	
Asthma/Bronchitis	2.11	0.69	6.44

OR = Odds Ratio: odds of having an adverse outcome given exposure. 95% CI = Confidence Interval for associated odds ratio. not est = not able to estimate odds ratio & 95% CI.

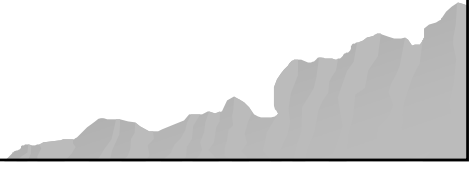
Research Question 3

3. Was there a history of exposures that increased the chances of having Gulf War Illness? Yes, including…
   - Deployment to Iraq and/or Kuwait
   - Participating in a combat operation
   - Exposure to Khamisiyah Dump demolition



Research Question 3 (cont.)

3. Was there a history of exposures that increased the chances of having Gulf War Illness? Yes, including…
   - Taking pyrodistigmine bromide
   - Being in an area where chemical alarms were sounding



VA Use and Satisfaction

- Please review the following list of benefits and services you might have been entitled to as a veteran, or private citizen. Check the appropriate responses regarding your usage since 1991.
- Treatment at a Veterans Administration hospital?
- Applied for veteran's disability benefits?
- (If yes) Did you receive veteran's disability benefits?

VA Use and Satisfaction (cont.)

- If you have used VA services since 1991, how would you rate your experience?
  - ❑ Excellent
  - ❑ Good
  - ❑ Fair
  - ❑ Poor

Research Question 4

- VA Benefits and Services

  Used the VA hospital?: 41% yes, 51% no

  Applied for disability?: 28% yes, 64% no

  (If yes) Did you receive veteran's disability?: 64% yes, 27% no

- Rating VA Services:

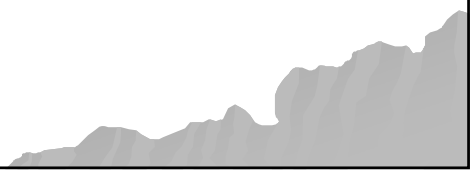
  16% Excellent
  44% Good
  25% Fair
  15% Poor

Summary

- Exposure to Khamisiyah Dump demolition, taking pyrodistigmine bromide, and/or being in an area where chemical alarms were sounding are associated with increased risk of Gulf War Illness (as defined by Lea Steele in American Journal of Epidemiology, 2000) and CDC defined Gulf War Syndrome.

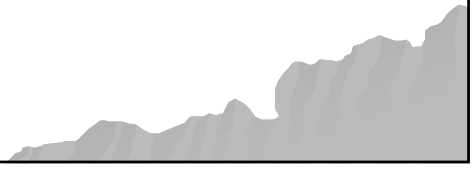
Summary (cont.)

- Of 189 surveys returned, almost half could be categorized as having Gulf War Illness (by either CDC (49%) or Steele definitions (46%)). This rate may be explained by sampling bias but could also be a real event.



Future Research

- Further analysis of exposures adjusting for other factors such as rank and/or branch of service is recommended.



Next Steps

- Plans for Operation Iraqi Freedom and Operation Enduring Freedom RI veteran survey
- Currently finalizing questionnaire
- Database of 1000+ individuals who have demobilized has been proactively developed
- Obtained funding for survey design, implement, analyze, and publish results

Related Articles

1. Prevalence and Patterns of Gulf War Illness in Kansas Veterans: Association of Symptoms with Characteristics of Person, Place, and Time of Military Service. Steele, L. American Journal of Epidemiology, 2000, pp. 992-1002.
2. Combat duty in Iraq and Afghanistan, mental health problems, and barriers to care. Hoge CW, Castro CA, Messer SC, McGurk D, Cotting DI, Koffman RL. New England Journal of Medicine, 2004, pp. 13-22.

Related Articles

3. Chronic widespread pain and psychiatric disorders in veterans of the first Gulf War. Kuzman JM, Black DW. Curr Pain Headache Rep, 2006, pp. 85-89.
4. Why people believe they were exposed to biological or chemical warfare: a survey of Gulf War veterans. Brewer NT, Lillie SE, Hallman WK. Risk Analysis, 2006, pp. 337-345.

Related Articles

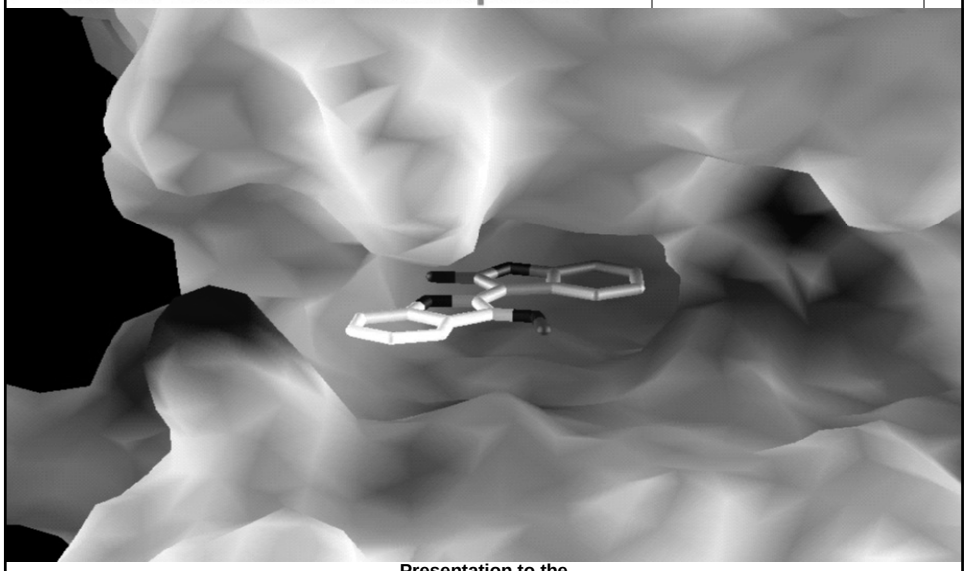
5. Military deployment to the Gulf War as a risk factor for psychiatric illness among US troops. Fiedler N, Ozakinci G, Hallman W, Wartenberg D, Brewer NT, Barrettt DH, Kipen HM. British Journal of Psychiatry, 2006, pp. 453-459.
6. Veterans Administration. Texas earmark allots millions to disputed theory of Gulf War illness. Couzin J. Science, 2006, p. 668.

Related Articles

7. Is there an Iraq war syndrome? Comparison of the health of UK service personnel after the Gulf and Iraq wars. Horn O, Hull L, Jones M, Murphy D, Browne T, Fear NT, Hotopf M, Rona RJ, Wessely S. Lancet, 2006, pp. 1742-1746.
8. Interpreting symptoms in military personnel after combat. Axelrod BN. Lancet, 2006, pp. 1709-1710.

Presentation 6 - Allen Fienberg

Intra-Cellular Therapies



Presentation to the
Committee for Gulf War Veteran's Illnesses
Allen A. Fienberg, Ph.D.
April 7th, 2008

Intra-Cellular Therapies

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Intra-Cellular Therapies

ITI Program to Characterize the Biochemical Effects of Nerve Agent Exposure in the Brain and the Development of Therapeutic Drugs

3

Intra-Cellular Therapies

Background

- Long-standing effort at ITI to characterize the intracellular signaling pathways associated with nicotinic and muscarinic receptor signaling
- Nerve agents such as sarin lead to supraphysiological levels of acetylcholine, overstimulation of nicotinic and muscarinic receptors and the resultant downstream signaling pathways and in the release of multiple neurotransmitters

4

Intra-Cellular Therapies

Background (cont)

- High level activation of cholinergic receptors leads to a number of acute and chronic pathological brain conditions that are characteristic of nerve agent exposure i.e. seizures, convulsions, fatigue, memory loss, and other cognitive impairments
- The precise biochemical pathways that link nerve agent exposure to these neurological impairments are unknown

5

Intra-Cellular Therapies

Background (cont)

- Gulf War Illness is a multi-symptom based syndrome of unknown etiology. However, it is suspected that its development may be related to acute or sub-chronic exposure to multiple agents experienced in the war theater, including sarin
- Effects of low level exposure of nerve agents on intracellular signaling pathways in the brain have never been characterized and may lead to a better understanding of Gulf War and related illnesses

6

Intra-Cellular Therapies

Goals of ITI Research on Gulf War Illness

- Characterize effects of nerve agent exposure on changes in phosphorylation state of key intracellular signaling targets
- Develop agents as antidotes/treatments based upon inhibition of CDK5
  - target implicated in the biochemical effects of nerve agent exposure

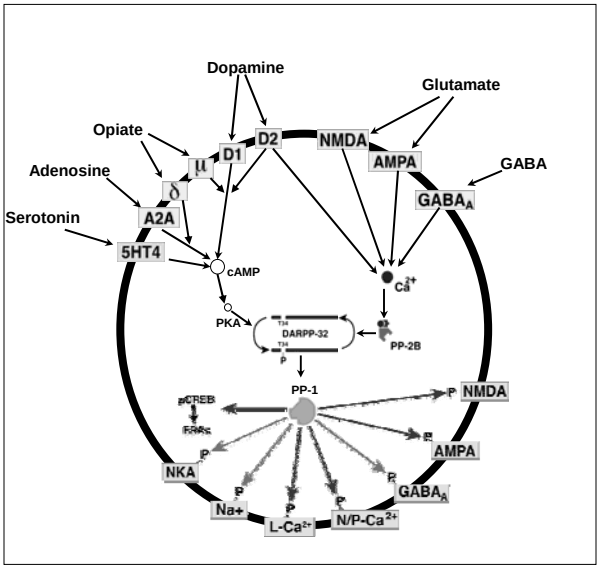
7

Intra-Cellular Therapies

The Inside Track for CNS Drug Discovery

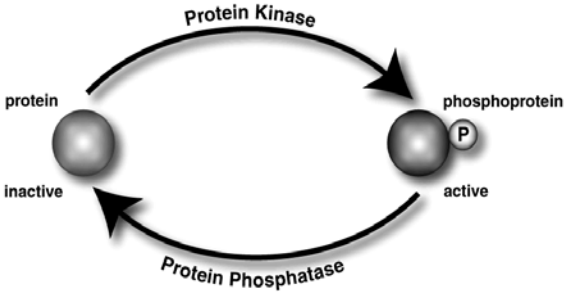
Dopamine, Glutamate, GABA and many other neurotransmitters have intracellular actions that converge on signaling proteins in the striatum such as DARPP-32 that are regulated by protein phosphorylation

We are characterizing DARPP-32 and other key phosphoproteins as potential downstream targets for the actions of nerve agents



Intra-Cellular Therapies

Strategy for Measuring Protein Phosphorylation in the CNS



- Protein kinases and protein phosphatases determine the state of phosphorylation of proteins
- *In vivo* methods are designed to minimize artifactual post-mortem changes in kinase and phosphatase activity
- Thus, phosphorylation state is preserved following drug treatment to provide a 'snapshot' of the state of phosphorylation of multiple phosphoproteins

Intra-Cellular Therapies

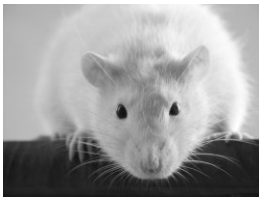
Phosphorylation sites studied using CNSProfile

Target Site	*Target Protein*	*Type of Protein*
S831	GluR1	Glutamate receptor
S845	GluR1	Glutamate receptor
S897	NR1	Glutamate Receptor
T34	DARPP-32	Phosphatase or Kinase inhibitor
T75	DARPP-32	Inhibitor
S102	DARPP-32	Inhibitor
S137	DARPP-32	Inhibitor
S94	Spinophilin	Cytoskeletal Anchor
T183	ERK1/2	Protein kinase
S133	CREB	Transcription Factor
S40	Tyrosine Hydroxylase	Enzyme
S549	Synapsin I/II	Synaptic Vesicle Protein
S603	Synapsin I/II	Synaptic Vesicle Protein
S9	GSK3β	Protein kinase
T308	Akt	Protein kinase
S473	Akt	Protein kinase

Selection of intra-cellular and receptor phosphorylation sites relevant to the action of nerve agents

Intra-Cellular Therapies

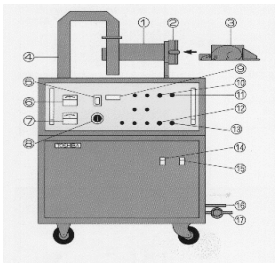
Measuring Neuronal Phosphoproteins Using *In Vivo* Techniques



In Vivo Phosphoprotein Measurements

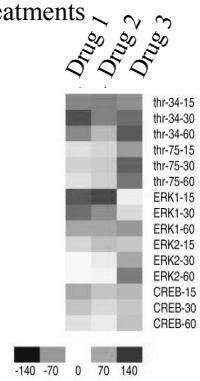
Systemic administration of nerve agents to rodents in the absence or presence of palliative treatments

Focused microwave irradiation
Used to preserve phosphoproteins



PhosphoProfile of
Nerve Agent Effects

- Monitor effects of nerve agents at multiple phosphorylation sites
- Time-dependent responses
- Dose responses
- Multiple brain regions

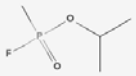


Intra-Cellular Therapies

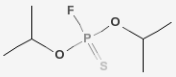
Characterization of Signaling Pathways Activated by Nerve Agents

Intra-Cellular Therapies

Outline



Sarin



DFP

- Sarin exposure at convulsant doses
- Effects of DFP in various brain regions
- Exposure to low-level Sarin aerosol

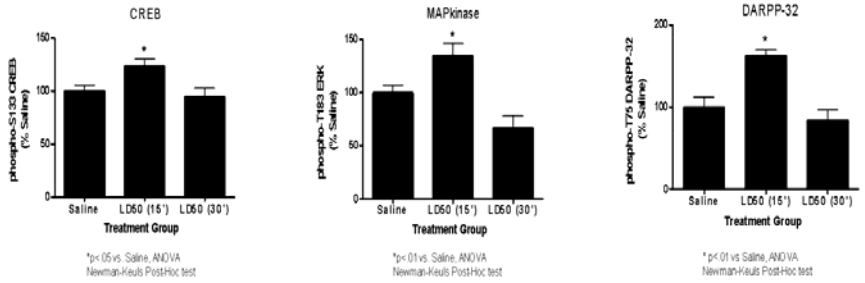
Intra-Cellular Therapies

- Sarin exposure at subconvulsant and convulsant doses
  - Acute IP exposure to sarin at 0.5 and 1 LD50
  - rats
  - Performed in collaboration with the Institute for Chemical Defense, Fort Detrick, MD

Intra-Cellular Therapies

Sarin (LD50) activates pathways involved in gene expression and neuronal differentiation

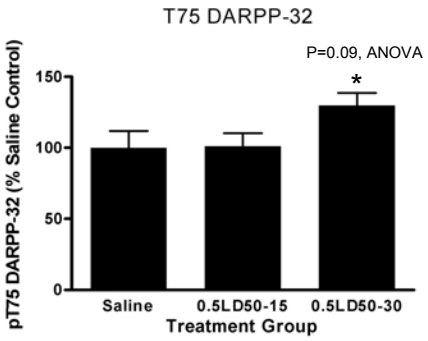
- *Increased phosphorylation and activation of gene expression factors*
  - *CREB*
  - *MAP kinase*
  - *DARPP-32 at T75, a substrate for CDK5*



Intra-Cellular Therapies

DARPP-32 T75 phosphorylation is elevated after sub-convulsant Sarin exposure

- *Increase in phosphorylation of DARPP-32 at T75 occurs after exposure to a sub-convulsant dose of Sarin*
  - *Effect of Sarin on T75 DARPP-32: a marker for Sarin exposure*



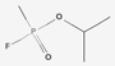
Intra-Cellular Therapies

Conclusions

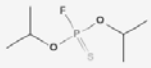
- Convulsant doses of Sarin (i.e., LD50) elicit significant increases in the CDK5-phosphorylated (T75) form of DARPP-32, an intracellular signaling proteins that subserves the actions of the neurotransmitter dopamine.
- Increases in T75 DARPP-32 may serve as an important early marker for exposure to sub-convulsive doses of Sarin

Intra-Cellular Therapies

Outline



Sarin

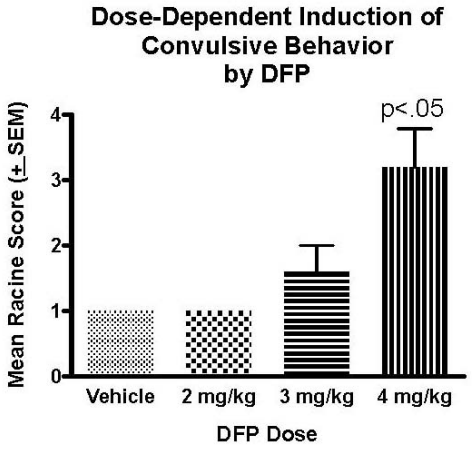


DFP

- Effects of DFP in various brain regions
  - In collaboration with James O'Callaghan PhD, Center for Disease Control
  - Practical considerations led us to use DFP, a close relative to Sarin, to perform detailed studies of OP action
  - DFP used as an appropriate and convenient model of rodent nerve agent exposure
  - Relationship between convulsive behavior and the appearance of phosphorylation changes

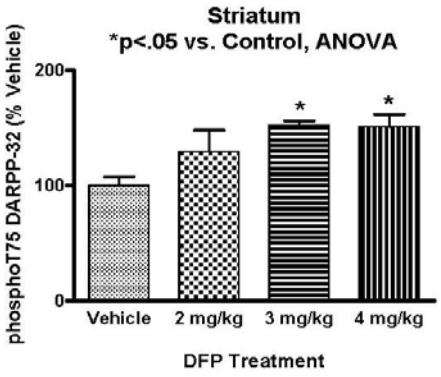
Intra-Cellular Therapies

DFP induces a dose-dependent increase in convulsive behavior in mice



Intra-Cellular Therapies

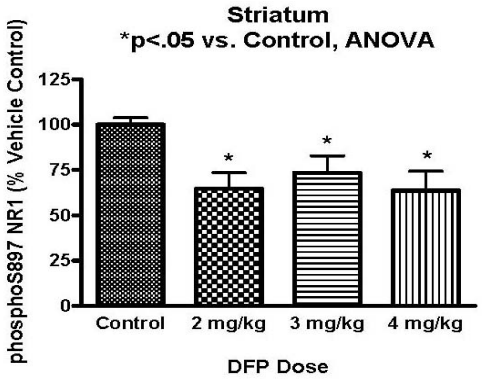
DFP exposure results in an <u>increase</u> in phosphorylation of DARPP-32 in mouse striatum



Increase in T75 DARPP-32 phosphorylation

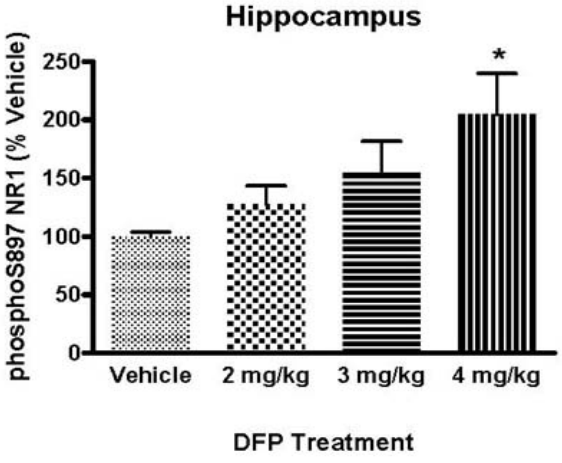
Intra-Cellular Therapies

DFP induces a dose-dependent decrease in NMDA receptor phosphorylation in mouse striatum



Intra-Cellular Therapies

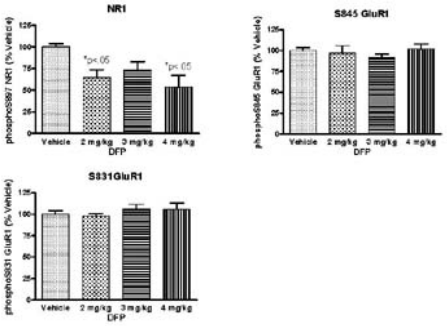
DFP induces a dose-dependent increase in NMDA receptor phosphorylation in mouse hippocampus



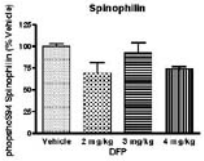
Additional Changes in Phosphorylation: Striatum

Intra-Cellular Therapies

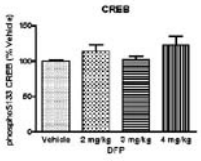
A. Glutamate Receptors



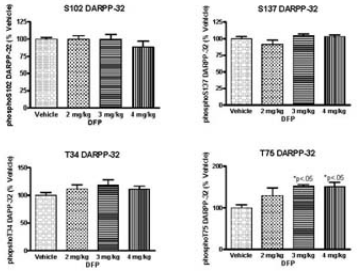
B. Phosphatase Targeting Protein



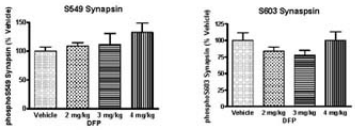
C. Transcription Factor



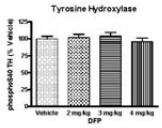
A. DARPP-32



B. Presynaptic Protein



C. Postsynaptic Protein



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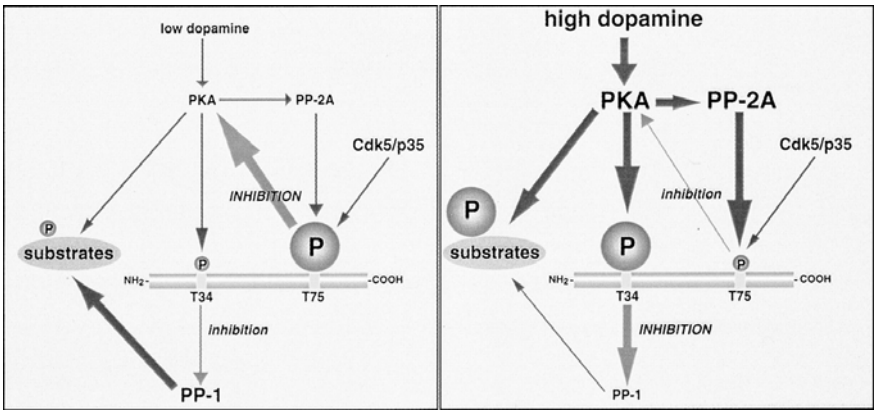
Intra-Cellular Therapies



Nishi, Akinori et al. (2000) Proc. Natl. Acad. Sci. USA 97, 12840-12845

Intra-Cellular Therapies

Summary of DFP experiments

- DFP, an organophosphorus (OP) compound, induces convulsive behavior in female mice at high doses.
- Exposure to DFP increases phosphorylation of DARPP-32
- Significant decreases in phosphorylation NMDA receptor, NR1, at Ser897, a residue that enhances functional activity of the receptor.
- In contrast to its effects in striatum, DFP exposure results in a significant increase in NR1 receptor phosphorylation at Ser897 in hippocampus

Intra-Cellular Therapies

Low-level Sarin Exposure

- We sought to determine if low-level, non-convulsive doses of Sarin impacted T75 DARPP-32 phosphorylation in rat striatum
- We investigated this using a whole-body inhalation technique for exposure.
- We studied the regulation of T75 DARPP-32 as a function of the temporal pattern of Sarin exposure

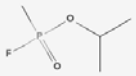
Intra-Cellular Therapies

Conditions for inhalation exposure to low-level sarin

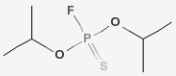
- ACUTE: 8 min to 200 ug/m3 GB
- CHRONIC: 10 x 8 min to 20 ug/m3 GB
- SUB-CHRONIC (SC): 300 min to 5.3 ug/m3 GB
- SUB-CHRONIC-DELAY (SC-Delay): 300 min to 5.3 ug/m3 GB, 8 weeks later euthanized

Intra-Cellular Therapies

Outline



Sarin

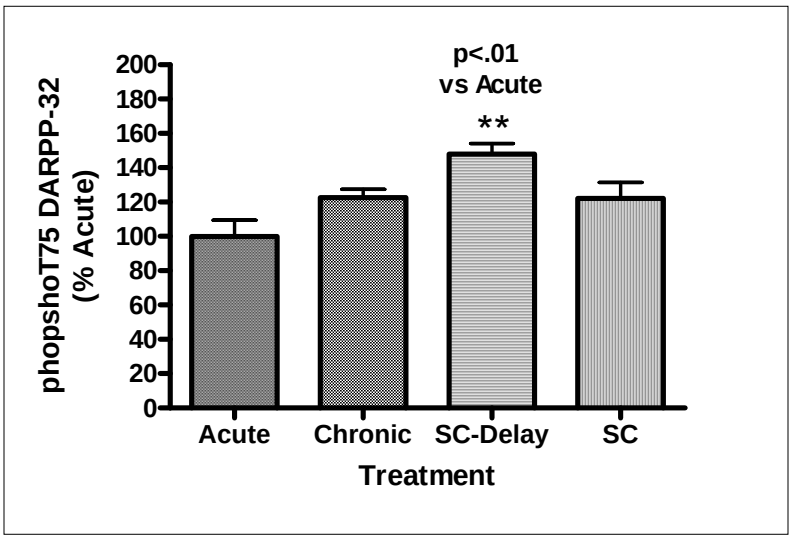


DFP

- Exposure to low-level Sarin aerosol
  - Performed with TNO – The Netherlands
  - Lower level and longer duration exposure in rats to Sarin

Intra-Cellular Therapies

T75 DARPP-32 is elevated in rats exposed to low-level aerosol sarin after a sub-chronic delay paradigm relative to acute low-level exposure



Intra-Cellular Therapies

Summary

- Exposure to Sarin and Sarin-like agents results in persistent changes in key brain signaling proteins
  - CDK5 and NR1 phosphorylation
- These signaling pathways are involved in processes related to synaptic plasticity
  - learning and memory, cognition

Intra-Cellular Therapies

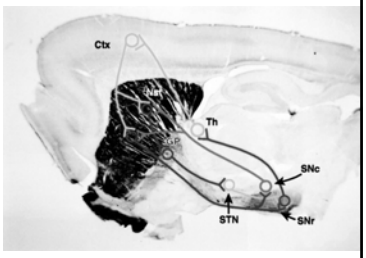
CDK-5 – p35 Program

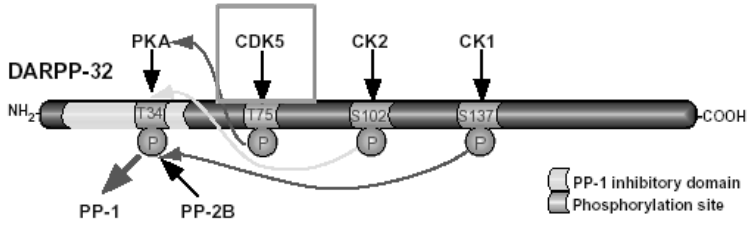
- CDK-5 is a protein kinase implicated in sarin toxicity and in neurodegenerative diseases
  - High localization in the brain
- Two approaches to find inhibitors:
  - Inhibition of p35 (p25) interaction with CDK-5
  - Inhibitors of CDK-5:p25 Holo-enzyme

Intra-Cellular Therapies

DARPP-32

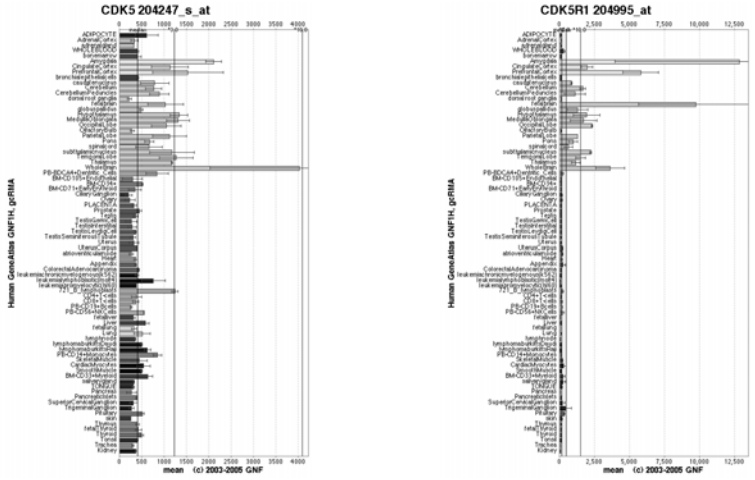
Dopamine and cAMP Regulated Phosphoprotein, 32 kDa, a dual-function protein kinase/protein phosphatase inhibitor.





Intra-Cellular Therapies

CDK-5 : p35
Inhibition for neuro-protection

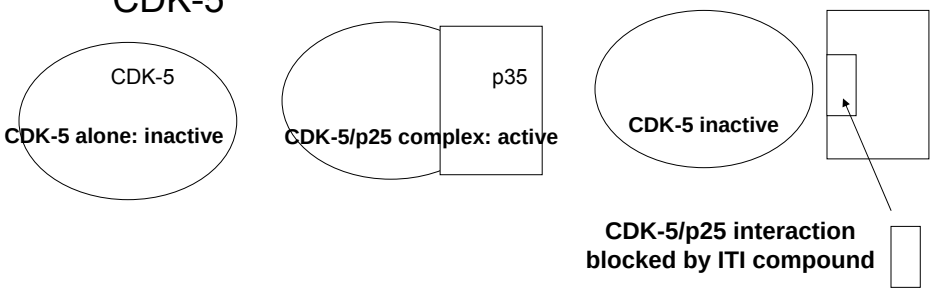


CDK-5 and p35 are highly localized to neuronal tissue, offering the potential for discovery of highly-specific inhibitors

Intra-Cellular Therapies

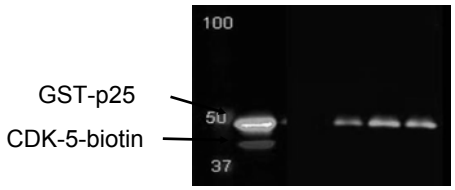
Approach #1: Interaction assay for CDK-5/p35 Kinase

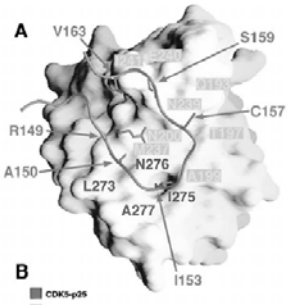
- p35 (and truncated forms such as p25) activate CDK-5
- Inhibition of this protein–protein interaction will specifically prevent activation of neuronal CDK-5

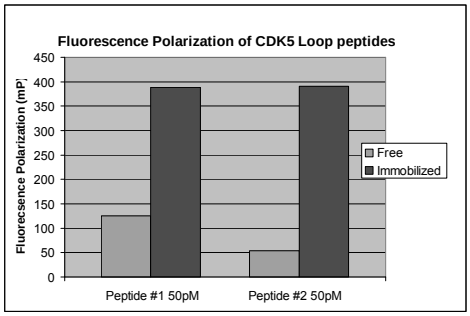


Intra-Cellular Therapies

Interaction assay

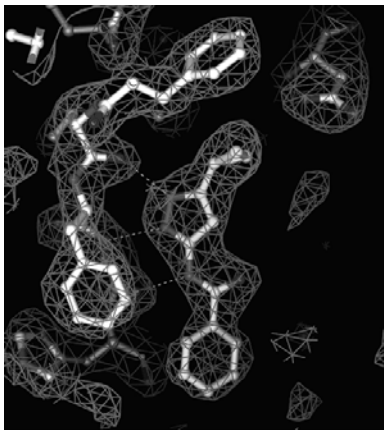


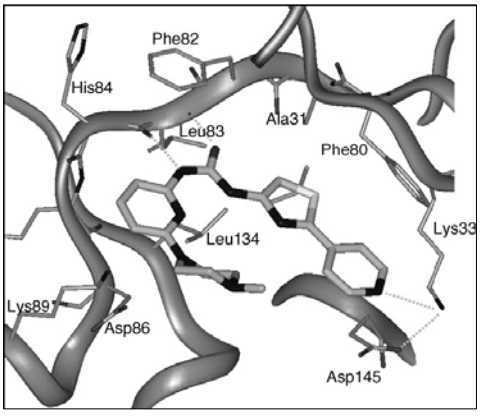




Intra-Cellular Therapies

Design of novel CDK5 Inhibitors is driven by sophisticated modeling and other methods





Inhibitors in models derived from crystal structures of CDK5

Intra-Cellular Therapies

Design of Kinase-Targeted Library

ITI kinase targeted library

Combined ligand-based and structure-based drug design

Medicinal chemistry filter: Reactive and toxic groups;

No. of halogen atoms <=4

Drug-like property filters

- Lipinski rule of 5
  100 < MW≤ 500,
  -2 ≤ ClogP ≤ 5,
  HDO ≤ 5 ,
  HAC ≤ 10,
- $LogS_w \geq -6$
- tPSA ≤ 140 Å^2,
- RTB ≤ 10
- RNG ≤ 5 and RNG ≤ 2 for fused aromatic rings

Database diversity and clustering analysis

Fingerprint, tanimoffo coeff = 0.85;

<=3 compounds in the same cluster

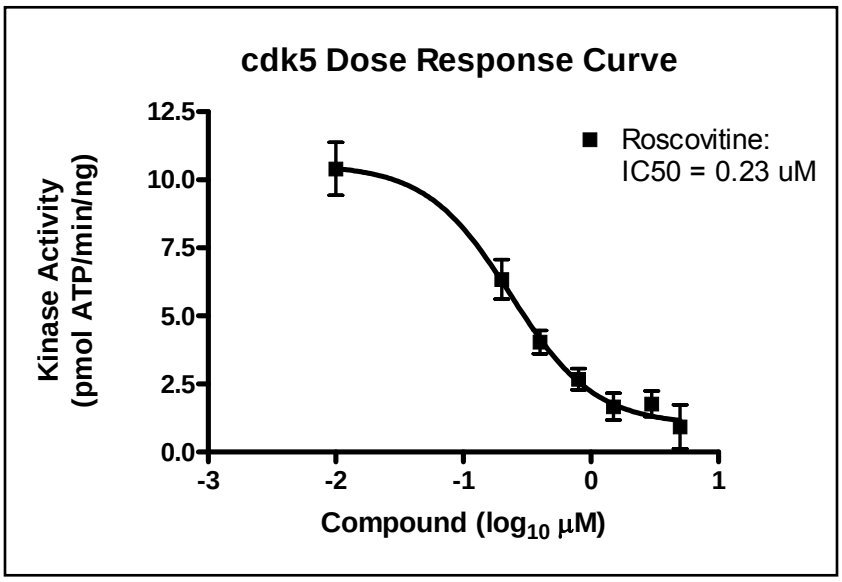
Intra-Cellular Therapies

Approach #1: p25/CDK-5 interaction - progress

- Recombinant GST-p25/CDK-5-biotin expressed in HEK293 cells
- Complex purified via affinity chromatography on glutathione-agarose
- However, the protein-protein interaction (reconstitution) assay developed to date is not a robust and high-volume screening assay
  - Primary high-capacity screening activity has used a holo-enzyme screen

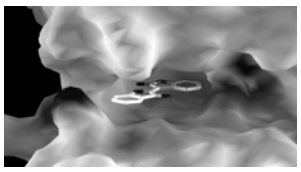
Intra-Cellular Therapies

Roscovitine IC50 curve



Intra-Cellular Therapies

High Capacity Screen for CDK-5 Inhibitors



- Current focus is to screen large chemical diversity libraries for CDK-5 inhibitors
  - High Capacity enzyme assay in operation
  - Standard agents show potent activity
- In silico efforts to identify potent and selective inhibitors
  - Several high diversity chemical libraries have been purchased and screening is ongoing

Intra-Cellular Therapies

ITI Chemical Screening Approach

- ITI chemical library developed though purchase of compounds with high diversity and maximum value as CDK-5 inhibitors
- Currently: ~35,000 compounds
  - Continual expansion to address specific targets
  - Focused CDK-5 library has been defined

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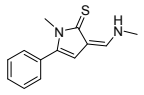
Intra-Cellular Therapies

Chemi-informatics used to filter libraries for "drug likeness"

Physical-chemical properties (Lipinski Rule of Five):

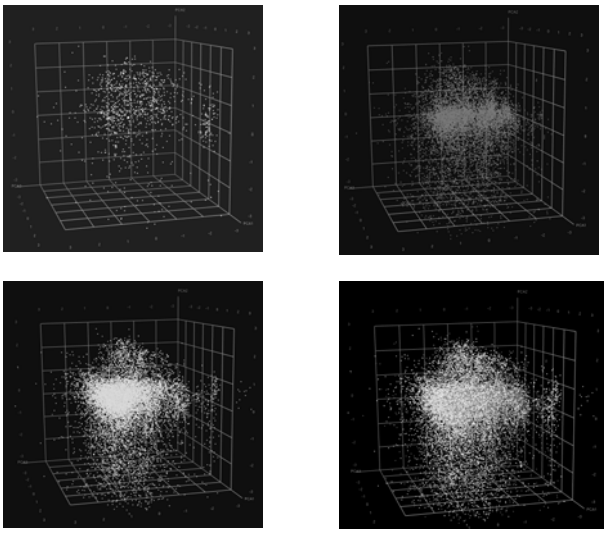
- MW ≤ 500,
- ClogP ≤ 5.0, HBA ≤ 10, HBD ≤ 5
- Number of Rotatable Bonds (RB) ≤ 10
- Polar Surface Area (PSA) ≤ 140 Å^2



MW:230
ClogP:1.89
HBA:3
HBD:1
RB:2
PSA:16 Å^2

42

Intra-Cellular Therapies



3D plots of principal component analysis of ITI diverse libraries. PCA1-3 are combinations of a series of molecular descriptors, e.g., charges, logP, rotatable bonds, etc.

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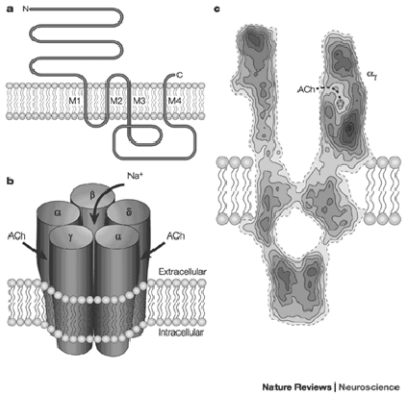
Intra-Cellular Therapies

Other Programs Relevant to Neural Protection

- GSK-3 Beta
  - Potent and specific inhibitors identified and are being optimized in cell culture models of neurodegeneration
- CK-1 Kinase Inhibitor
  - Potent and specific inhibitors identified and are being optimized in cell culture models of neurodegeneration
- Cholinergic Receptor Agents
  - Antidotes to Nerve Agent Exposure
  - Neuroprotection for Alzheimer's Disease and Parkinson's Disease, Cognitive Function, Anti-convulsant
  - Plan to test for protection in the DFP animal model

Intra-Cellular Therapies

Nicotinic Acetylcholine Receptor (nAChR) agents as neuroprotectants



Intra-Cellular Therapies

Potent and specific cholinergic ligands synthesized for animal testing

- Nicotinic Cholinergic Receptor – potent, specific agents synthesized
  - Alpha4Beta2 Agonist
  - Partial Alpha4Beta2 Agonist
  - Alpha7 Agonist
- Muscarinic Cholinergic Receptor – potent agents synthesized
  - PCH – Phencynonate HCL- IC200335

Intra-Cellular Therapies

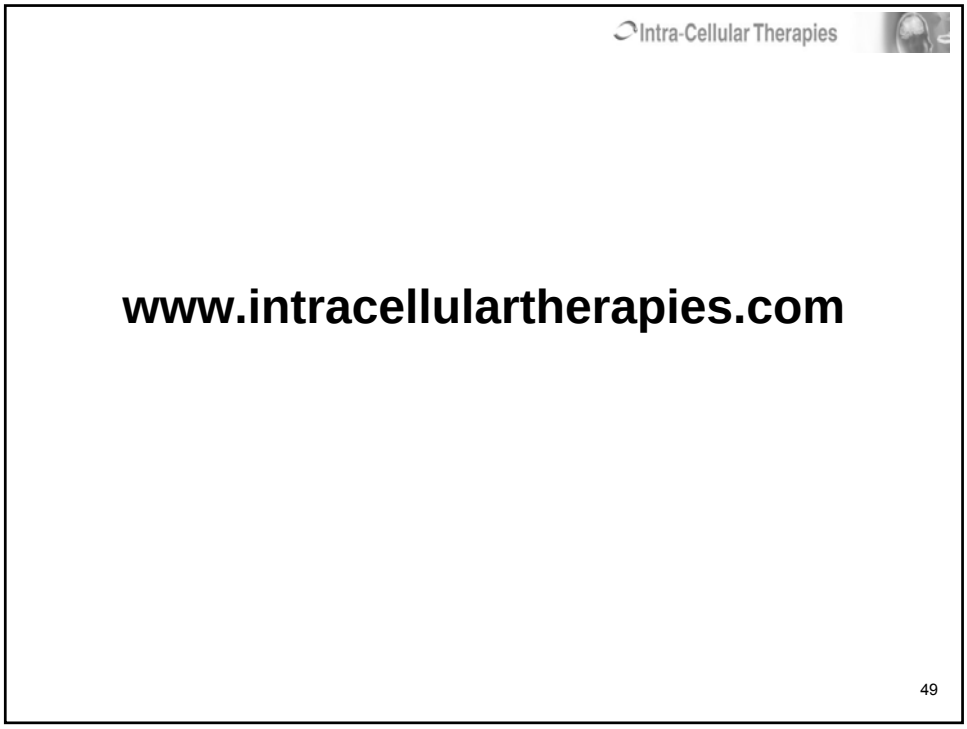
Summary – CDK5 and other targets

- Significant progress to discover CDK-5 inhibitors as antidotes to Nerve Agents and with potential dual use
- Identification of four other target areas (GSK3, CK1, cholinergic receptor agents and dual reuptake inhibitors) where small molecule drug candidates have been identified and are currently being optimized
- Planned animal testing of promising ligands

Intra-Cellular Therapies

Additional ITI programs relevant to Gulf war Illness

- ITI-722
  - Clinical evaluation ongoing for sleep maintenance insomnia
- ITI-002
  - Pre-clinical development for cognitive dysfunction due to dopaminergic hypofunction in the pre-frontal cortex
- Dual inhibitors
  - Potent bio-available dual reuptake inhibitors for norepinephrine and serotonin transport systems


Intra-Cellular Therapies
www.intracellulartherapies.com
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Presentation 7 - Beatrice Golomb

Update on Research in Persian Gulf War Veterans Illnesses

April 2008

Beatrice Alexandra Golomb, MD, PhD

Golomb (PNAS)

Finding: Convergent evidence increasingly suggests a causal role for ACHEi in illness in GWV

Design: Follow-up on epi results and results of research approaches I outlined in my RAND report.

1. **Epi**
2. **Dose response**
3. **Genetics and activity levels of detoxifying substances**
4. **Relation repeat low level exposures in animals to chronic effects on Ach system and other physiol**
5. **Other exposed groups**

Golomb BA PNAS 2008105: 4295

Epi Data

Author	PB	Pesticide	NAgent
Australian	+	+	+
Bell	o	+	o
Cherry 01	+	+	
Gray 02	+	+	+
Haley 97	+	+	+
Kang 00	+	+	+
Kang 02			+
Kroenke 98	o	o	
McCauley 01			+
McCauley 02			~/-

+ = signif pos; - = signif neg ; o=nonsignificant; blank = not examined

Golomb BA PNAS 2008105: 4295

Epi Data

Author	PB	Pesticide	NAgent
Nisenbaum00	+	+	+
Proctor 98	o	+	+
Proctor	+	+	+
Schumm 01	+		
Schumm 02	+		
Spencer 01	o	o	o
Steele 00			+
Sullivan 03	+		
Unwin 99	+	+	+
White 01		+	+
Wolfe 02	+	+	+

p < 0.0001: Sign test, 2-sided, spanning AChE, not double counting

Golomb BA PNAS 2008105: 4295

Dose Response: PB pills

PB category	Wolfe OR severe illness	SchummFem % excellent health	SchummM %poor/fair health	Australian % fxl imprmt
1	1	51 %	16 %	15 %
2	2.3	15 %	23 %	14 %
3	3.7	6 %	32 %	24 %
4			41 %	31 %
5			41 %	
6			75 %	
p	<0.0001	<0.0001	<0.0001	<0.0001

Golomb BA PNAS 2008105: 4295

Genetic and Biological data

If there is a causal relationship, those with genetic variants poor at detoxifying AChEi should have higher rates of illness (assuming similar exposure status; or confined to those exposed)

Also, those with lower concentrations/ activity levels of enzymes should be more likely to be ill. (BUT: in principle, this could be affected by exposure.)

Golomb BA PNAS 2008105: 4295

BChE low metabolizer variants

Described Health	N	# w BChE variant	# alleles affected
Well	74	22	25
Maybe ill	61	29	30
Ill	91	43	48

P = 0.03 Kendall's Tau rank correlation coefficient (p = 0.012 combining maybe ill and ill)

Lockridge 1999 (p values calculated from data provided)

Low PON activity and concentrations

Haley: 25 GWV, 20 age/sex/education controls

1. PON ACTIVITY: Lowest quartile activity vs top 3
Linked to cases: OR 4.5, p = 0.02
Linked to "syndrome 2": OR 9.0, p < 0.001
2. PON Genotype: R allele vs not
Linked to cases OR 3.4 (p = 0.05)

Mackness: 152 ill GWV vs 152 age-sex matched ctrl

1. PON activity by hydrolysis (nmol/min/ml)
100 (ill) vs 215 (ctrl), p < 0.001
2. PON1 concentration (micrograms/ml)
75.5 (ill) vs 88.2 (ctrl), p < 0.00025

PON variants

Another Mackness study:
Did not segregate by GWV criteria.
Looked at disabled GWV, nondisabled (but ill, high sx level) GWV, disabled nondeployed and Bosnia groups.
Sx counts as high in nondisabled GWV group as in disabled other group: 'disability' doesn't mean the same thing (as we will see!)

Both ill GWV groups had lower PON activity.
-Trend still lower in more ill GWV.

The more symptomatic GW group had
-- less 55LM than less symptomatic p = 0.002 calc'd from the data;
-- less 192 QR than the disabled from other groups p < 0.005.

The more unhealthy GWV group were signif. less likely to be heterozygotes

Triangulating evidence from nonGW

If there is a causal relationship, it might be expected that persons with AChEi exposures from other settings may show similar *symptoms*.

Don't look at studies failing to show relation to outcomes that aren't increased in GWV;
Looked for articles examining low level exposures and symptoms.

Chem Terrorist Exposed vs Sx

Long term problems with fatigue, cognition, and muscle, multiple studies

Agriculturally Exposed vs Sx

Design: Mail survey UK agricultural area, N = 175

Exposure: OP exposure over prior 10 years

Outcome: # of 10 health symptoms yes/no, including muscle, fatigue, cognitive

Mean # sx: 2.7 vs 0.24

At least 1 sx: 59% vs 13%, p < 0.001

Among those with sx, mean # sx: 4.5 vs 1.8

(Also: Female Polish greenhouse workers with OP exposure vs similar gardening worker without: significant problems with fatigue, muscle, cognitive.)

Davies 1999 J Nutr Environ Med 9:123-34

(Bazylewicz-Walczak 1999: Neurotoxicology 20: 819-26)

Triangulating Genetics

Sheep dippers: known diazinon exposure

With Chronic symptoms: 175

Without chronic symptoms (sim age): 234

PON Genetics:

192QR: Any R: 2.3 (1.5-3.4) p = 0.001

55 LM: LL vs any M: 1.9 (1.3-2.9), p = 0.002

PON Activity:

Diazoxonase activity < median: 1.8 (1.1-2.8)

Cherry 2002 Lancet 359: 763-4

Look at ACh and nt regulation

Ach is involved in regulation of:

- **Memory**
- **Muscle**
- **Sleep**
- **GI**
- **Skin**
- **Immune**

And in presynaptic regulation of other nts (DA, glu etc): so altered regulation could provide a mech

Look at ACh and nt regulation

A nonphysiological increase in ACh signaling may lead to later downregulation (and dysregulation) of Ach signaling at many levels.

Look at

- **Long term effects after stopping,**
- **Throughout ACh regulation pathway (receptor #, affinity, activity, Ach production, release, AChEi etc)**
- **Including repeated exposure,**
- **Separately for different brain/body areas.**

Also look at neurotransmitters regulated by Ach

(And look at other physiol effects, e.g. mt fxn)

Chronic & delayed Chgs Regulation of ACh are Being Found

Change density of cholinergic receptor subtypes

- **Delayed decrease M1 rec subtypes selected brain regions after repeated low level exposures**
- **Persistent increase M3 rec if coexposure with heat**
- **Persistent change select nicotinic receptors after repeat low level OP, coupled with memory impairment that reverses with nicotine**

Altered splicing of mRNA for AChE, depressing ACh fxn

Induce multigene transcriptional fdback resp that depresses cholinergic action

ALS

AChEi relation fits with reports of excess ALS in GWV*:

Emerging literature linking PON genotype to sporadic ALS and possibly gene-pesticide interactions**

***E.G studies by Haley; Coffman; Horner**

****E.g. studies by Saeed; Slowik; Morahan;**

Hills Presumptive Criteria for Causality

Association has:

- **Strength**
- **Consistency**
- **Temporality: Healthier or as healthy before exposures**
- **Biological Gradient: E.g. PB dose response**
- **Biological Plausibility: Low level exposure changes Ach, glu regulation in animal studies**
- **Coherence with other literature (sarin, agricultural exp)**
- **Specificity: not finding abnormalities with these exposures that are absent in GWV**

Other Studies

Disabled GWV Differ vs Disabled Comparator Veterans

Design: "2-phase cohort": from pop based x-sexl postal survey of >10K current and ex-UK milit persons

Subjects: Persons with disability by SF36 PCS

GWV n = 111; nonGWV n = 133 Bosnia and Nondeployed

Analysis: adjusted OR.

Adjusted for: age, sex, rank, marital status, alcohol disorder

Results:

Vs disabled persons nondeployed and Bosnia, Gulf war show:

Increase CFS:	**OR 7.8, CI 2.5-25**	**N = 20 (18%) vs N = 4 (3%)**
No increase chronic fatigue not CFS:		**N = 4 vs 3**
No increase fibromyalgia		**N = 3 vs 0**

If deployed and have anxiety or depression OR increases to 10 (suggesting anxiety and depression when seen in GWV are more often there as part of this more complex picture)

Limitations: small numbers. (Persistent probs in infer direction of causation fm assn)

Ismail 2007 Psychological Medicine

Animal (mechanism): glutamate rec effec

Finding: repeat sublethal OP (soman) affects glutamate subunit expression in Hippocampus w delayed changes

Design: experimental animal study

Ss: 20/trial guinea pigs (10wk male diet ctl, sedentary)

Exposure: soman 0.4xLD50 vs saline control sq neck x 10 days (mon-fri): highest dose with no clin signs @1dose

Assessments: Morris Water Maze; Neurocytoskeletal

4 glu receptor subtypes immunoreactivity

Result: No change water maze, (Nfilament lite/med, synaptophysin)

3 mo incr GluR2, NMDA-R2, NMDA-R26 (gone6mo)

6 mo: Decr NMDA-R1

Johnson EA 2008 glutamate receptor pathology is present in the hippocampus following repeated sub-lethal soman exposures… NeuroToxicology 29: 73-80

Individual Differences: More on Metab.

Individual Differences in OP metabolism. Focus e.g. on chlorpyrifos.

Desulfuration: Activates: to more toxic oxon form. CYP 2B6

Dearylation: Detoxifies. Eg. CYP2C19. ?CYP3A4

CYP3A4 said important for "metabolism" – didn't specify which direction.

Note: Main enzymes CYP 2B6; CYP3A4: in metab of 50% prescription drugs

Polymorphic variants in CYP3A4 vary much in ability to 'metabolize" chlorpyrifos

In human liver microsomes, there is 10-fold variation in ratio of

Activation: Detoxification (Desulfuration: Dearylation)

Also:

Hydrolases: to detoxify oxon forms

Dehydrogenases

Hodgson 2007 J Biochem Mol Toxicol 21: 182-6 Human Metabolic Interactions of Environmental Chemicals

Exposure Interactions

Interactions among Agents:

CPS oxon: inhibits permethrin metabolism

complete inhibition, at very low concentrations, noncompetitive, irreversible.

(Permethrin metabolism involves a couple steps; this inhibition is of the initial hydrolysis step):

Carbaryl also inhibits permethrin metabolism, less complete, even at high concentration

It is thought that there are a spectrum of esterases involved in permethrin, and that all are inhibited by CPS, and only some by carbaryl

Hodgson 2007

Animals: Objective Changes

Effects on Human Markers

Steroid hormone metabolism is affected

- **Testosterone**
- **Estrogen**

Hodgson 2007

Animals: Mitochondrial Changes

Subjects: Rats (male, albino, wistar, 120-160g)

Exposure: OP (dichlorvos) in corn oil, or corn oil sq

Duration: 6mg/kg/d x 12 weeks: then sacrificed

Rat brain mitochondria isolated

Kaur 2007 Neurotoxicology 28:1209-19, Impaired mitochondrial energy metabolism and neuronal apoptotic cell death after chronic dichlorvos (OP) exposure in rat brain

Animals: Mt Changes

Oxidative stress induction: Mt calcium uptake

(Ca++ uptake is 1 of most common inducers of mt derived ROS & impaired mt E metabolism)

Energetics:

Oxygen uptake (mt oxygen respiration) (polarographically measured)

Mt complex 1 activity (NADH dehydrogenase):

Mt complex 2 activity (succinate dehydrogenase)

Mt complex 4 activity (cytochrome oxidase)

Kaur 2007 Neurotoxicology 28:1209-19

Animals: Mt Changes

Antioxidant state

- **Mt superoxide dismutase activity**

Apoptotic signals

- **Western blot analysis of cytochrome c and caspase 3**

Apoptosis

- **DNA fragmentation by gel electrophoresis**
- **Electron microscopy**

Kaur 2007 Neurotoxicology 28:1209-19

Animals: Mt Changes

Comparing Control to OP-exposed:

Mt calcium uptake: dramatic increase ~700nmol/10min vs ~20, p < 0.001

- **Ca uptake is 1 of most common inducers of mt derived ROS & impaired mt E metabolism**

Oxygen uptake (mt oxygen respiration) (polarographically measured)

- **Respiratory rate (atoms of O2 consumed/min/mg protein)**
- **Basal (state 4): ~30 to ~15, p < 0.001**
- **Active (state 3): > 100 to < 50, p < 0.001**

Mt complex 1 activity (NADH dehydrogenase):

- **NADH oxidized/ min/ mg protein**
- **reduced from >60 to <30, p < 0.001**

Mt complex 2 activity (succinate dehydrogenase)

- **Succinate oxidized nmol/min/mg protein**
- **Reduced to >30 to < 15, p < 0.001**

Mt complex 4 activity (cytochrome oxidase)

- **Cytochrome C oxidized / sec/ mg protein**
- **Reduced from ~145 to to ~85, p < 0.001**

Kaur 2007 Neurotoxicology 28:1209-19

Animals: Mt Changes

↓Mitochondrial antioxidants	ctrl	OP	p
Glutathione content (nmol/mg pr)	38.7 (11.0)	21.6 (11.2)	< 0.005
Mn SOD (U/mg pr)	23.8 (3.8)	5.58 (1.18)	< 0.001
Oxidative damage: Mt lipid peroxidation, pr oxidation, mtDNA oxidation in rat brain			
Lipid peroxidn (nM TBARs/mg pr)	142 (29.0)	42.5 (16.0)	< 0.001
Protein Carbonyl (nM/mg pr)	1.11 (.58)	4.80 (1.6)	< 0.005
MtDNA oxidation (8-OHdG ng/ml)	2.0 (0.06)	8.0 (0.30)	p < 0.001

∴ oxidatively modify mt proteins, lipids, and mtDNA as I had outlined in prior talk, assoc with oxidation/antiox imbal.

↑ Apoptotic neuronal cell death

1. cytochrome c release fm mt to cytosol = major event in triggering apoptosis: immunoblotting
   Cytochrome C (immunoreactive band) brain cytosol fraction: OP treated, NOT control
2. Caspase 3 activation is the signal for apoptosis: immunoblotting to assess
   Caspase 3 activation (rat brain cytosol): in OP treated only, not control
3. DNA fragmentation (gel electrophoresis): OP exposed rat brain only, not in control

Explains apoptotic neuronal cell death in chronic OP exposure

Kaur 2007 Neurotoxicology 28:1209-19

Animals: Mt Changes

Transmission EM for morphology. In OP exposed group only, see:

- Mt matrix swelling
- Loss of mitochondria cristae
- Change in nuclear shape
- Chromatin condensation
- Vacuolization in brain

∴ **Thus, EM supports mt dysfxn and apoptosis as a cause of OP induced neuron death**

Kaur 2007 Neurotoxicology 28:1209-19

Mt Effects – in vitro

In vitro: incubate rat brain mitochondria with diff doses dichlorvos on mt cplx1, cplx 2, cplx 4 activity:

***dose dependent reductions in each**

Kaur 2007

Mito Changes

In summary: these findings support prooxidant effects leading to loss of mt energetics, more ROS induction, and triggering of apoptosis with OP exposure – at least, in rat brains, at high dose, with no delay (hypothesis: lower dose will take more delay to see).

These processes are known to be involved in neurodegenerative conditions, like ALS. These mechanisms may explain the increased ALS (and I propose may, in attenuated form, also explain clinical pathology) in ill GWV.

Kaur 2007

The End

Presentation 8 - Daniel Clauw

Recent Studies in Fibromyalgia

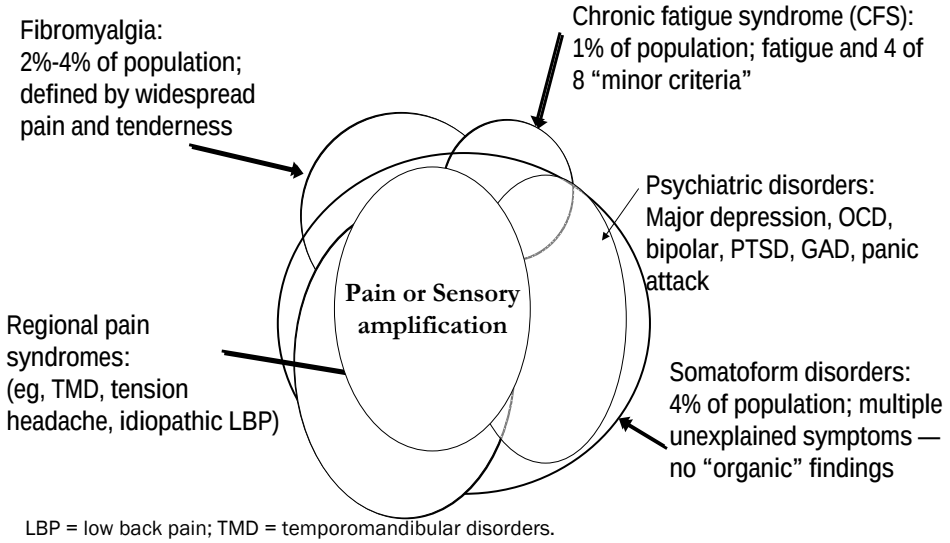
Daniel J. Clauw, MD

Professor of Medicine (Rheumatology) and Psychiatry
Associate Dean for Clinical and Translational Research
Director, Chronic Pain and Fatigue Research Center
University of Michigan Medical Center
Ann Arbor, Michigan

Recent Studies in Fibromyalgia

- **Latest theories re: pathogenesis**
  - **Increased "gain" in pain and sensory processing**
  - **Multiple ways to get to this final common pathway**
    - **Strong genetic predisposition**
    - **Multiple environmental "stressors" can trigger**
  - **Subsets of individuals with different underlying causes will respond to different treatments**
- **Evidence for globally increased sensory processing**
- **Evidence for decreased descending analgesic activity**
- **Is chronic pain a neurodegenerative disease?**

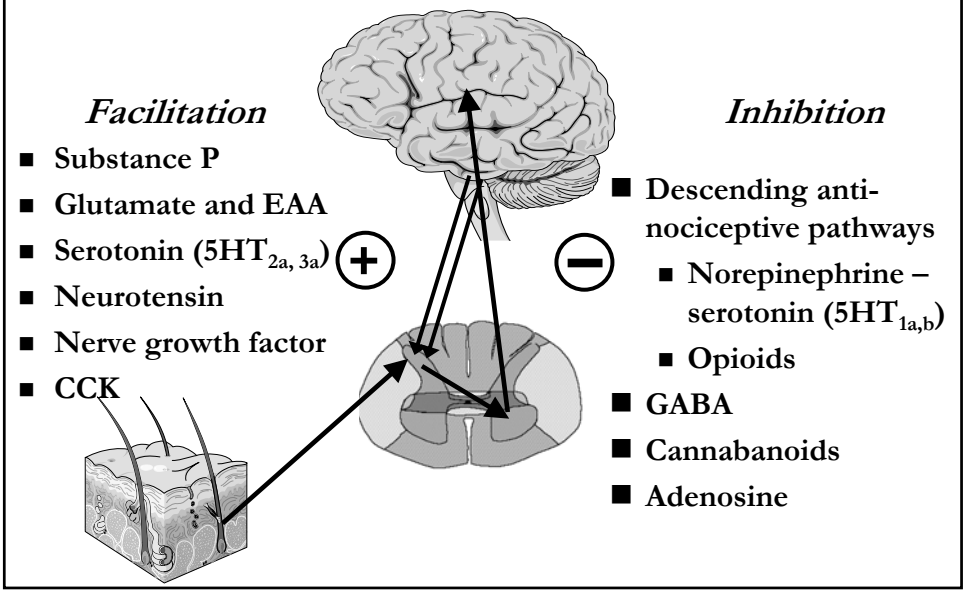
Overlap Between Fibromyalgia and Related Syndromes



LBP = low back pain; TMD = temporomandibular disorders.

Clauw DJ, et al. *Neuroimmunomodulation*. 1997;4:134-153.

Supraspinal Influences on Pain and Sensory Processing



fMRI of Evoked Pressure Pain in Fibromyalgia and Related Conditions

- Is there objective evidence of augmented pain processing in fibromyalgia?[1]
- Role of depression in pain processing in FM[2]
- Role of cognitive factors in pain processing in FM
  - Locus of control
  - Catastrophizing[3]
- fMRI changes of augmented central processing of pain also seen in idiopathic low back pain

1. Gracely et al. *Arthritis Rheum*. 2002;46:1333-1343; 2. Giesecke et al. *Arthritis Rheum*. 2003;48:2916-2922; 3. Gracely et al. *Brain*. 2004;127:835-843; 4. Giesecke et al. *Arthritis Rheum.* 2004;50(2):613-623.

Specific Underlying Mechanisms in Fibromyalgia

- Global problem with sensory processing (i.e. interoception)
  - FM patients equally sensitive to loudness of auditory tones[1]
  - Insular hyper-reactivity consistently seen[2-4]
  - H-MRS studies of glutamate levels in posterior insula[5]

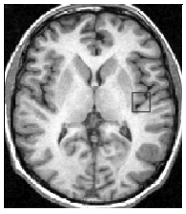
1. Geisser et. al. *J. Pain* (2008); 2. Gracely et. al. *Arthritis Rheum.* **46**, 1333-1343 (2002); 3. Giesecke et. al. *Arthritis Rheum.* **50**, 613-623 (2004); 4. Cook *J Rheumatol.* **31**, 364-378 (2004); 5. Harris et. al. *Arthritis Rheum.* **58**, 903-907 (2008).

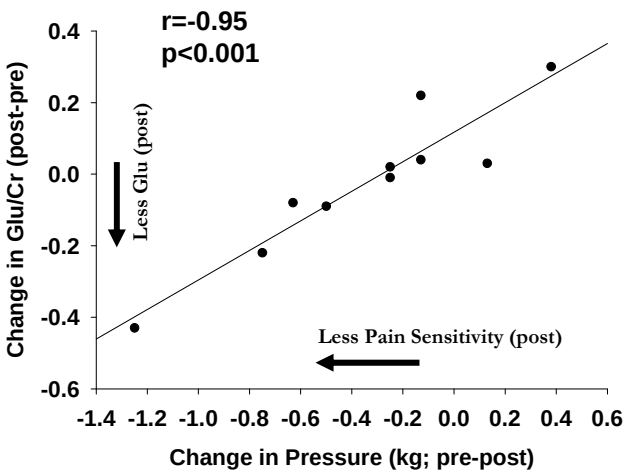
Longitudinal Study of Changes in Glutamate within Insula of FM

- 10 female FM patients randomized to receive acupuncture or sham acupuncture.
- H-MRS of right insula (anterior and posterior) performed at baseline and post treatment (9 sessions over 1 month).
- Clinical and experimental pressure pain evaluated before each H-MRS session.
- Correlated changes in Glu with changes in pain.

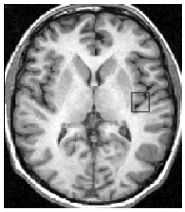
Harris et al. *Arthritis Rheum* 2008

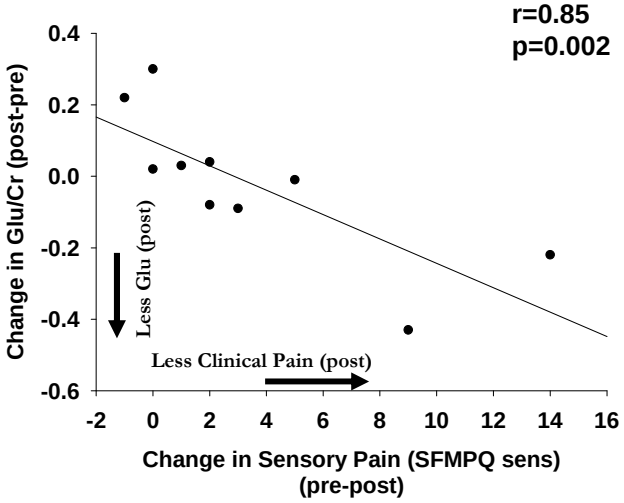
Reduction in Glu is Associated with Reduced Experimental Pressure Pain in FM





Reduction in Glu is Associated with Reduced Clinical Pain in FM





Specific Underlying Mechanisms in Fibromyalgia

- Decreased descending analgesic activity
  - Absent or attenuated descending analgesic activity in FM and IBS[1-3]
  - Brainstem activations with conditioning stimulus seen in controls but not in FM patients[4]
  - DLPFC variability in Cho/Cr greater in FM patients than controls[5]

1. Kosek et. al. *Pain* **70**, 41-51 (1997); 2. Julien et. al. *Pain* **114**, 295-302 (2005); 3. Wilder-Smith et. al. *World J. Gastroenterol.* **13**, 3699-3704 (2007); 4. Gracely et. al. Arthritis Rheum 2006 (abstract); 5. Petrou et. al., *Am J Neuroradiology,*

There is a Deficiency of Descending Analgesic Activity in FM [1,2] Which one?

Opioids

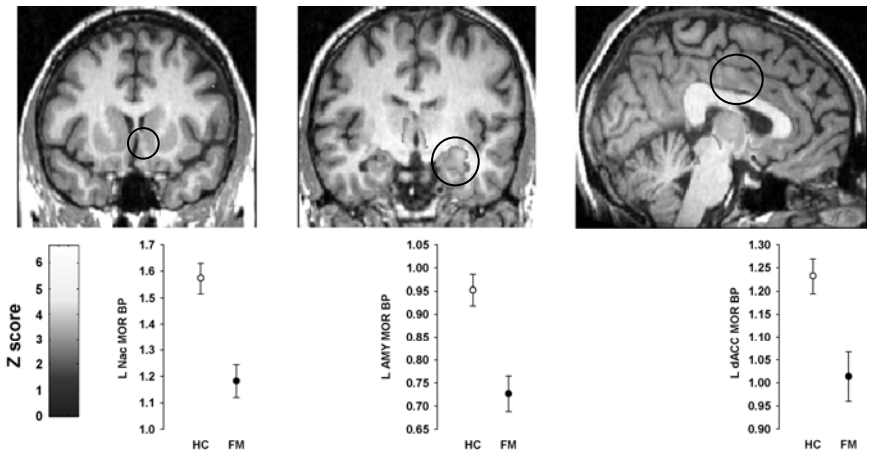
- Normal or high levels of CSF enkephalins[3]
- Never been administered in RCT but most feel that opioids are ineffective or marginally effective
- Harris recently used PET to show decreased mu opioid receptor binding in FM[4]

Noradrenergic/Serotonergic

- Low levels of biogenic monoamines in CSF in FM[5]
- Nearly any class of drug that raises *both* serotonin and norepinephrine has demonstrated efficacy in FM

1. Kosek et. al. *Pain* 1997
2. Julien et. al. Pain 2005.
3. Baraniuk et al. *BMC Musculoskelet Disord*. 2004.
4. Harris, *J Neuroscience, 2007*
5. Russell et al. *Arthritis Rheum*. 1992.

FM Patients Have Reduced MOR Availability



	L NAcc	lAMY	L dCC
Z	4.12	4.21	3.39
p-value*	<0.05	< 0.05	<0.05
%Δ BP	33.1(7.1)	31.1(7.0)	21.5(6.4)

*corrected

Harris et al. JNeurosci 2007

Is Chronic Pain a Neurodegenerative Disease?

- Apkarian[1] was first to show that chronic pain may be a neurodegenerative disease, showing
  - Decreased gray matter density in DLPFC and thalamus
  - Related to length of pain
- More recently seen in other pain states including headache (insula and ACC)[2], IBS (insula and ACC)[3], and fibromyalgia[4] (multiple regions), as well as PTSD[5] (insula)

1. Apkarian, J Neurosci. 2004; 24(46):10410-5, 2. Schmidt-Wilcke T et. al., 2007, *Pain*; 3. Davis KD et. al., Neurology 2008:70:153-154. 4. Kuchinad et. al. J Neuroscience, 2007:27(15):4004-7. 5. Chen et. al. Psychiatry Res 2006:146:65-72.

Implications for GWI Research

- Need to be extremely careful about interpreting mechanistic studies that find differences in levels of neurotransmitters in CNS, and or damage to brain regions, as indicative of toxin exposure
- Patients in GWI studies should be extensively "phenotyped" for conditions such as FM, IBS, PTSD, and if mechanistic studies are used for causal inference these groups should be included in studies (i.e. comparing ill GWV to healthy GWV or healthy controls cannot control for these confounds

Presentation 9 - Louis Fiore & Neil Kowall

The VA Gulf War Brain Bank

Louis Fiore MD, MPH
Neil Kowall MD
Anil Prasad MD

Gulf War Brain Bank

- Provision of high quality tissue and histology services and associated clinical data to Gulf War researchers
- Provide clinical information on tissue samples such that basic research findings can be correlated with clinical outcomes for discovery and validation efforts

- VA Boston: Administrative Core and Blood Bank
- Southern Arizona VA: Tissue Bank
- New York Brain Bank: Tissue Processing
- VA Durham: ALS Registry
- Gulf War Advocates
- Gulf War Researchers
- Gulf War Veterans

Gulf War Brain Bank

SACTL Start Date: May 8, 2006

Project Phases:

✓ Planning:	Completed
✓ Personnel:	3 positions filled
✓ Equipment:	Purchased
✓ IT:	Purchased, *in progress*
✓ Installation and set up:	Completed
✓ Equipment testing phase:	*in progress*
✓ Procedures:	SOPs, *in progress*
✓ IRB:	Completed

SACTL Workflow

Receiving Tissue

Data Entry

Gross examination

Sample=Aliquot preparation:
- snap-freezing
- formalin fixation
- DNA/RNA

Gross Aliquot Data Entry

Storage

Quality Control: Sections (frozen or paraffin embedded) of each aliquot are assessed for proper diagnosis by:
- H & E Staining
- Immunohistochemical marker staining
- Special Stainings

- Digital Imaging and Documentation
- Data base entry

DNA/RNA Quantity & Quality Control:
- Agilent 2100 Bioanalyzer

Storage-Freezer Facility

SAVAHCS's Bldg 56:
- High Security
- Video camera surveillance
- Restricted access: ID card reader
- Alarm systems for unauthorized entry

Freezers equipped with:
- Emergency power
- Temperature controlled environment
- Analog phone line
- Forma 1535 Temperature Monitoring System
- Sensaphone 2000 Remote Monitoring System
  - Calls pagers and cell phones
  - Computer-based data tracking and storage

Gulf War Brain Bank

3 x 28 ft^3 freezers

Following NBI Blueprint Good Practice Guidelines:

- 2 Freezers used for storage
- 1 Freezer kept empty as back up

Current Storage capacity:

1 Freezer: 600 boxes

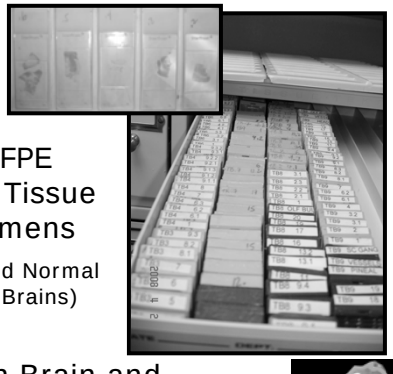
- 1 Brain yields ~ 175 brain tissue samples
  - Specific areas: 60 samples
  - Spinal cord: 35 samples
  - Vials for Molecular Biology: 80 samples

4 boxes filled by one brain with spinal cord

- 150 brains including spinal cords per freezer
- 2 Freezers capacity: 300 brains incl. spinal cords

Total storage capacity:
52,500 samples / 300 brains

VABT-SACTL Current Inventory

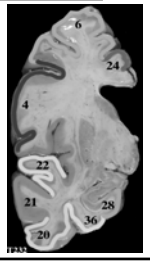


100 FFPE Brain Tissue Specimens

(ALS and Normal Control Brains)

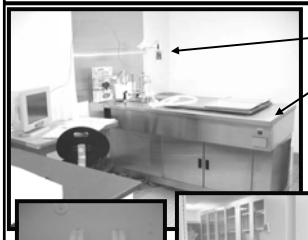
900 Frozen Brain and Spinal Cord Tissue Specimens

(ALS and Normal Control Brains)



289 Formalin-fixed Coronal Brain Slices

SACTL: Grossing and Histology

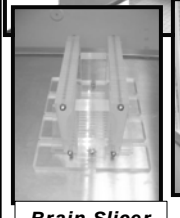


Macropath Digital Imaging System

Thermo Electron Gross Station

Tissue Processor

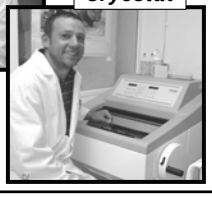
Embedding Station



Brain Slicer

pH Meter and Balance

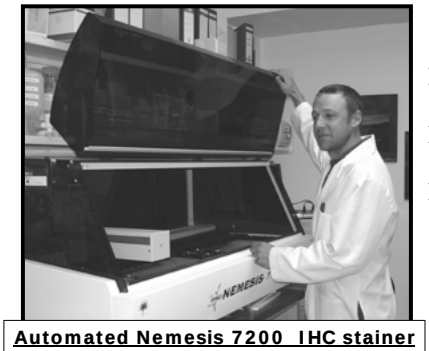
Cryostat



Microtome

Automated Tissue Microarrayer

Immunohistochemistry Core



Automated Nemesis 7200 IHC stainer

- Open system
- Customized & standardized protocols
- Reagents

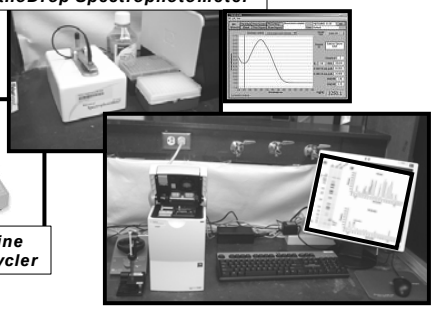
- Single stainings
- Double stainings
- Triple stainings
- Quadruple stainings

– Biomarker correlations
- Multiple marker analysis in one tissue section

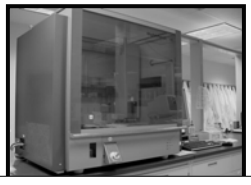
Molecular Laboratory

Covaris Ultra-sonic tissue disruptor

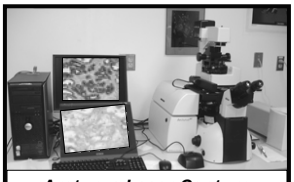
NanoDrop Spectrophotometer



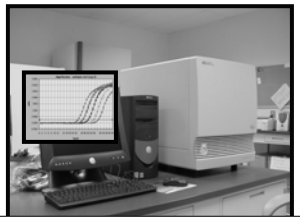
DNA Engine Thermal Cycler



The MagNA Pure LC instrument

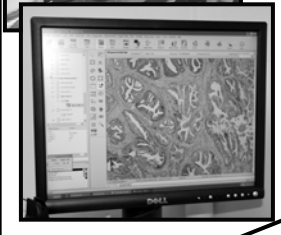


Arcturus Laser Capture Microdissection System



Applied Biosystems 7900HT Fast Real-time PCR System

Digital Imaging Systems



Ariol SL 50, Applied Imaging Corp.

Automated Scanner:
50 slide capacity

Additonal:
- Dual headed Zeiss Axiophot with digital camera
- Dual headed Nikon microscope

Automated Digital Image Analysis for IHC Stained Tissue:

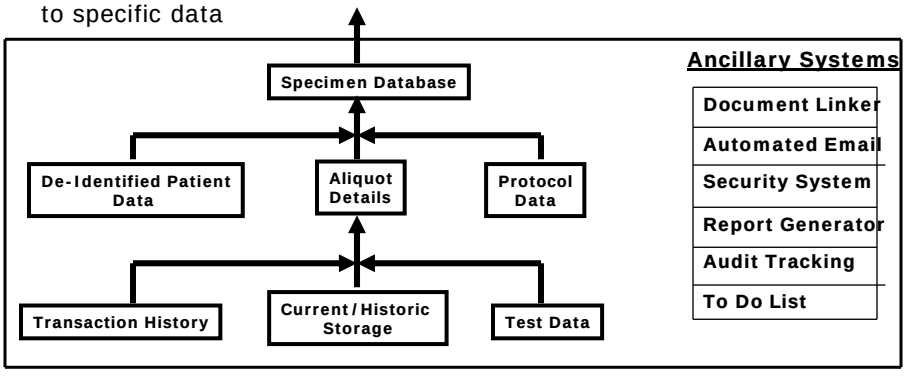
- Customization of the Scoring Criteria to fit
- Individual Protocols
- Morphometric Analysis
- Karyometry
- Tissue Microarray
- High Throughput

d·metrix
MICROSCOPY MULTIPLIED

- Whole Slide Imaging
- Automation
- Digital Pathology
- Images stored in TissueMetrix database and accompany tissue requests

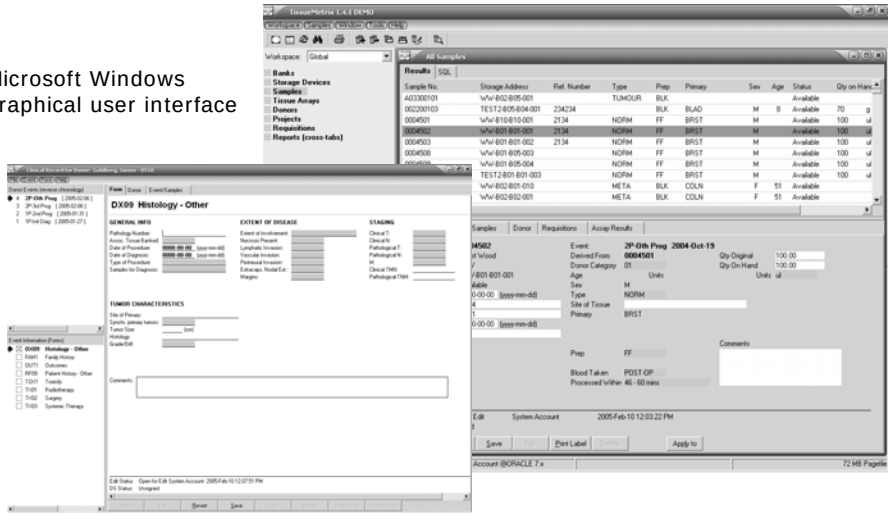
TissueMetrix-SACTL Database

- Configurable tissue banks and storage devices
- Efficient sample registration system
- Ability to restrict users' functional privileges
- Ability to restrict users' access to specific data
- Configurable interface with user-defined data fields
- Digital signatures for data authenticity verification
- Active contention control for data editing

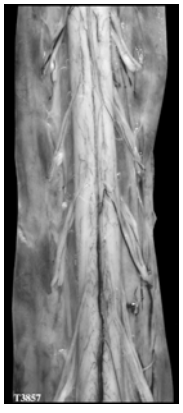


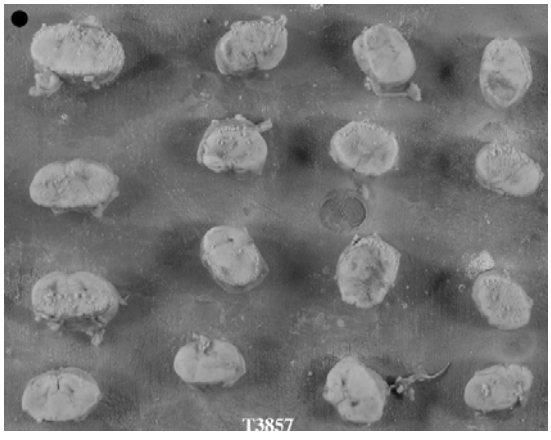
SACTL Database

Microsoft Windows graphical user interface



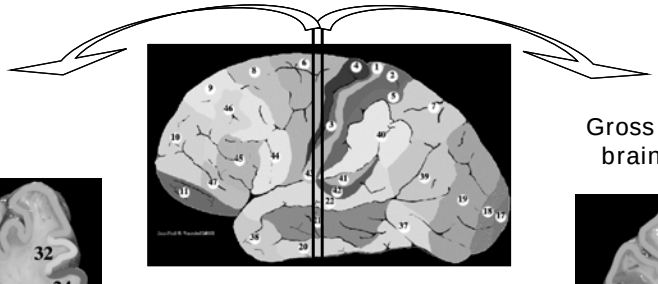
ALS Protocol: Spinal Cord Gross Examination & Dissection



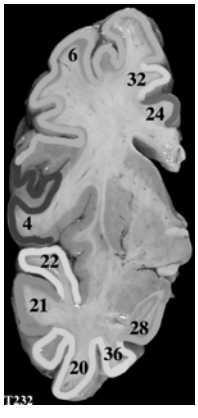


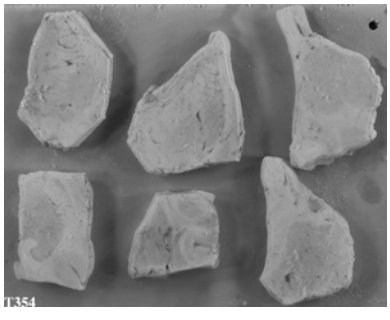
Alternate segments : Frozen -> Research / Fixation -> Diagnosis

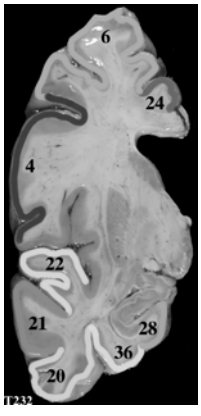
ALS Protocol: Brain Dissection



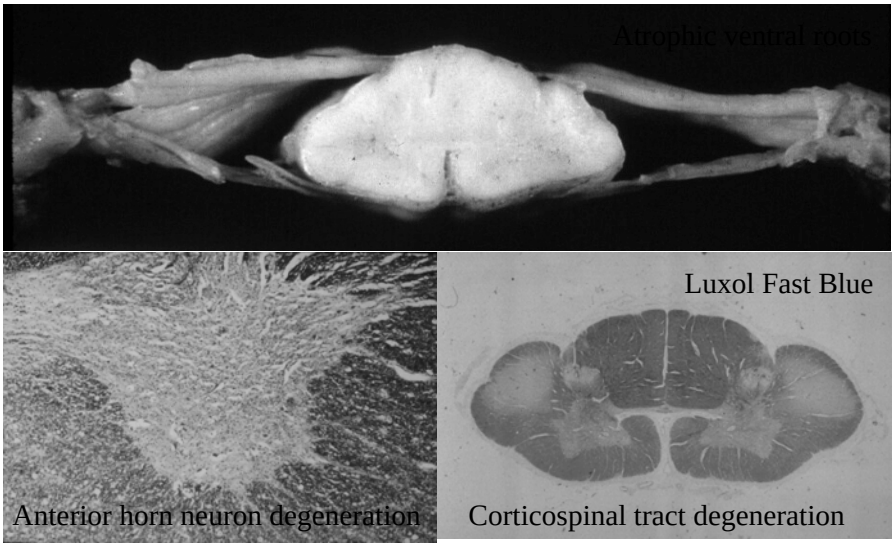
Gross coronal brain slices







Gross and Microscopic Examination: ALS



Quality Control / Quality Assurance

Review of clinical data

- SOPs for case review
- Standardization and coding
- Correlation of clinical data with histopathology

Quality control of preserved tissue samples

- Confirmation of histopathology
- Confirmation of DNA/RNA quality
- Confirmation of sample usability

Senior investigators responding positively to solicitation

- ROBERT BOWSER PhD
- FLINT BEAL MD
- SERGE PRZEDBORSKI PhD
- JEFF ROTHSTEIN MD PhD
- MATT FROSCH MD
- JEREMY SHEFNER MD
- MERIT CUDKOWICZ MD
- ROBERT FERRANTE PhD
- HOON RYU PhD
- JUNGHEE LEE PhD
- BEN WOLOZIN PhD

Gulf War ADVISORY COMMITTEE BRAIN BANK RESOURCE UTILIZATION UPDATE

APRIL 7, 2008

Status Report

- Now have sufficient postmortem case material to begin distributing tissue
- Poised to move into the next phase and support meaningful research

SENIOR INVESTIGATORS RESPONDING POSITIVELY TO BRAIN TISSUE AVAILABILITY

- ROBERT BOWSER PhD (Director, Center for ALS Research, U Pittsburgh)
- FLINT BEAL MD (Chair Neurology, Cornell Med)
- SERGE PRZEDBORSKI MD PhD (Professor, Columbia U)
- JEFF ROTHSTEIN MD PhD (Director, ALS research center, Johns Hopkins)
- MATT FROSCH MD PhD (Director, Neuropathology MGH)
- JEREMY SHEFNER MD PhD (Chair Neurology, Upstate Med)
- MERIT CUDKOWICZ MD (Co-Director ALS clinic and trials unit, MGH)
- ROBERT FERRANTE PhD (Professor, Boston University)
- BEN WOLOZIN PhD (Professor, Boston University)

Examples of proposed projects

BEN WOLOZIN PhD (Professor, Boston University):

- Tar DNA Binding Protein-43 (TDP-43) is the major protein that accumulates as inclusions in neurons in the spinal cords of subjects with ALS
- Mutations recently shown to be a rare cause of ALS
- Identified a number of proteins that co-localize with TDP43 aggregates in cell culture
- Determine if co-localize with TDP43 aggregates in ALS spinal cord/brain.
- Will provide insights into the biological processes leading to TDP43 aggregation and potential strategies for inhibiting TDP43 aggregation as well as preventing neurodegeneration in ALS.

Proteomic profiling of postmortem brain in Amyotrophic Lateral Sclerosis (ALS): Jingua Yang PhD
Boston VA

- Objectives:
  - Identify Amyotrophic Lateral Sclerosis (ALS)-related brain proteins in postmortem veterans in order to understand the causes of ALS in gulf war veterans.
  - Establish proteomic databases of different pathological brain structures in postmortem gulf war veterans with/without ALS, in order to get biomarker information for diagnostic and therapeutic targets.

Available instrumentation

- **Nano-LC QSTAR® Elite Hybrid LC/MS/MS System (Applied Biosystems).** It is consisted of a QSTAR® Elite and the Tempo nanoHPLC as a fully integrated LC-MS/MS (Liquid chromatography-mass spectrometry, peptide fingerprinting) solution for robust protein profiling and biomarker discovery.
- **Voyager-DE™ STR Biospectrometry Workstation (Applied Biosystems).** This is a complete MALDI-TOF (Matrix-assisted laser desorption/ionization for large organic molecules) mass spectrometer with both linear and reflection modes for rapid and robust protein identification. It provides excellent resolution, sensitivity, and mass accuracy.
- **2-D gel system coupled with Typhoon Imager (GE Amersham).** This system is capable of resolving tissue/plasma samples by two-dimensional PAGE. It can digitalize 2D protein image and quantitatively analyze the protein spots.
- **Agilent 1200 two-Dimensional Fluorescent HPLC.** This HPLC is consisted of both capillary and analytical pumps and followed with two fluorescence detector for working with cy3 and cy5.

Project: Yang et al

- **Specific aims, methodologies and outcomes**
  - Brain tissue and plasma from postmortem veterans with/without ALS will be analyzed in the following three aims:
    - **differential proteomic analysis of ALS and control postmortem brain by iTRAQ and LC-MS/MS**
    - **proteomic profiling of different pathological structures of ALS brains by DiGE and MALDI-TOF**
    - **systematic analysis of ALS brain proteome by autoimmune imaging.**

Aim 1: Differential proteomic analysis of ALS and control postmortem brain by iTRAQ and LC-MS/MS.

- Methods: soluble proteins from ALS and control postmortem brain will be labeled with iTRAQ (an alternative non-gel based approach using isotope coded affinity tags for the quantitative study of gene expression at the proteome level), analyzed by LC-MS/MS analysis using nano-HPLC Q-STAR Elite (see instrumentation). Matched tissues from different brain structures of ALS and control postmortem veterans will be processed to isolate soluble proteins. Protein samples will be digested with trypsin, labeled with the iTRAQ reagents, profiled on nano-HPLC, and analyzed by MS/MS. Differentially expressed peptides will be identified using the iTRAQ software.
- Outcome: iTRAQ and LC-MS/MS database of ALS-related proteins will be generated, which will be used to identify biomarkers and understand the causes of ALS in gulf war veterans.

Aim2: Proteomic profiling of different pathological structures of ALS brains by DiGE and MALDI-TOF

- Methods: the matched protein samples will be separated by fluorescent-assistant two-dimensional gel, and followed by MALDI-TOF protein identification. Specifically, after soluble proteins are prepared, the undigested full length proteins will be labeled with cy5 and cy3 fluorescent dyes and resolved on 2D gel. This technology is also termed Differential Gel Electrophoresis. Differentially expressed proteins will be identified by software and cut off the gel for MALDI-TOF protein identification.
- Outcome: 2D gel image and proteomic database of ALS-related proteins will be generated, which will be used to identify biomarkers and understand the causes of ALS in gulf war veterans.

Aim3: Systematic analysis of ALS brain proteome by autoimmune imaging.

- Methods: brain proteins will be resolved by 2D gel and differentially immunoblotted with IgG from ALS and control patients; positive proteins will be analyzed by MALDI-TOF. Specifically, total IgG will be isolated from the blood samples of ALS and control postmortem veterans and labeled with cy5 and cy3 fluorescent dyes, respectively. Brain proteins from ALS and control postmortem veterans will be resolved on 2D gel and immunoblotted with Cy5 and Cy3 IgGs. Differential autoimmune image of the 2D gel will be analyzed: positive immunogenic proteins specific to ALS will be identified and cut off the gel for MALDI-TOF protein identification.
- Outcomes: brain 2D autoimmune image of the ALS and control postmortem veterans, and database for the immunogenic brain proteins specific to ALS.

Proteomic profiling of postmortem brain in Amyotrophic Lateral Sclerosis (ALS)

- ROBERT BOWSER PhD (Director, Center for ALS Research, U Pittsburgh)
  - 2D DIGE experiments using lumbar spinal cord tissue from pooled tissue homogenates (same age range, gender ratio and postmortem times) from ALS and control subjects
  - Sequencing spots that appear differentially expressed in ALS will be compared to proteins previously published that exhibit altered levels in ALS tissue and bio-fluids
  - Examine motor cortex tissue to see if similar proteins are differentially expressed in upper versus lower motor neurons
  - Laser capture microscopy to examine protein expression in individual motor neurons.

Other Potential Projects

- Validation of observations in transgenic mice
  - Endothelial damage
- Studies on pathogenesis
  - Validate GWAS hits
  - Dyslipidemia
- Biomarker and therapeutic studies
  - Reverse transcriptase
  - Efficacy of clinical trials: Lithium

What's Next?

- Increase ALS tissue procurement
  - Focus on Gulf Theatre
- Distribution of ALS tissue
  - Genomics (Gulf theater vs era)
- Inclusion of other CNS disorders
  - Gulf War related illness

Targeting Gulf War Veterans

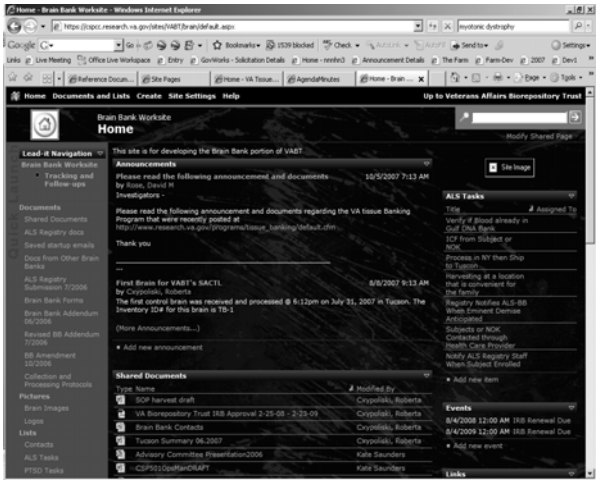
- VA Information Systems – VISTA
  - Gulf Era vets in VA System 99,353
  - Died in VA in 2007 1,267
  - Died in VA with GW Sx ?
- Regional Data Bases
  - Informatics platform
  - I2b2
  - Gulf War Registry

VA GWV Web Based Management System

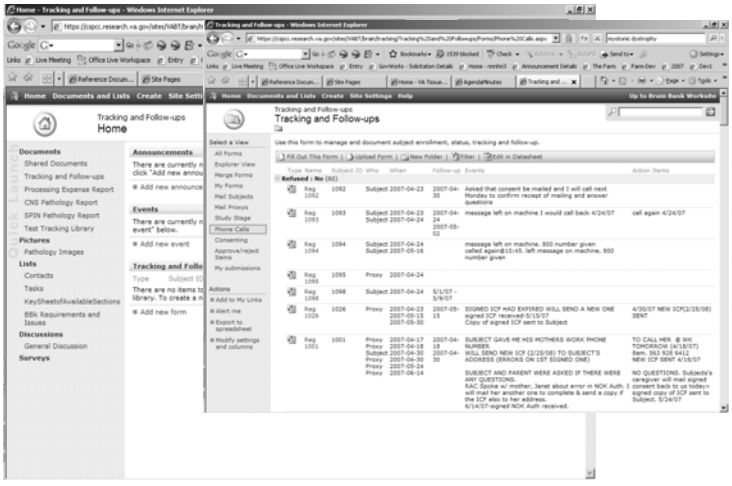
Management Processes in Place

- In-house document and collaboration portal
- Tracking and patient management portal
- Request for tissue sample management portal
- Public GW portal concept preview

Coordinating Center Administration

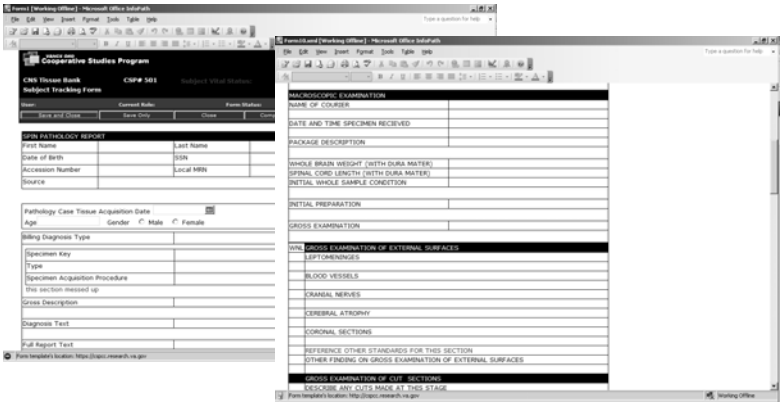


Donor Tracking Pages

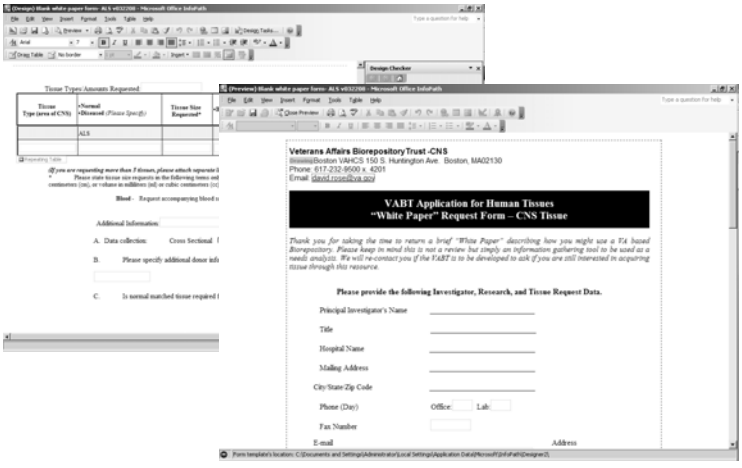


Sample Management

- Standardized Reports
- Standardized Terminologies

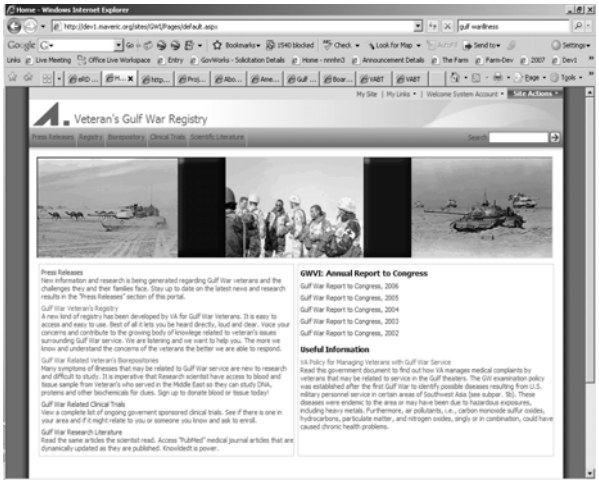


Request Management

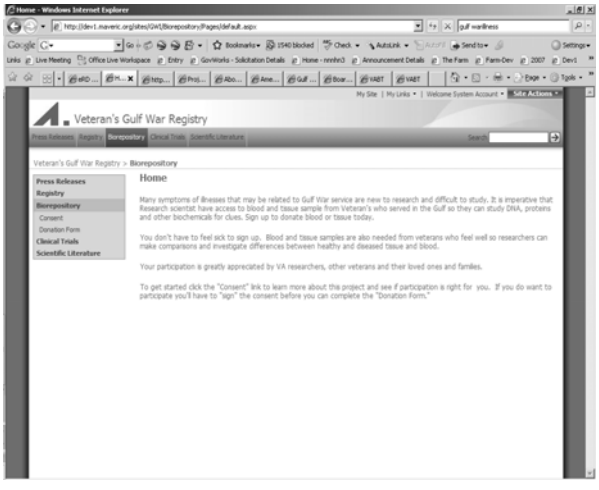


Public GWV Portal Preview

Home Page

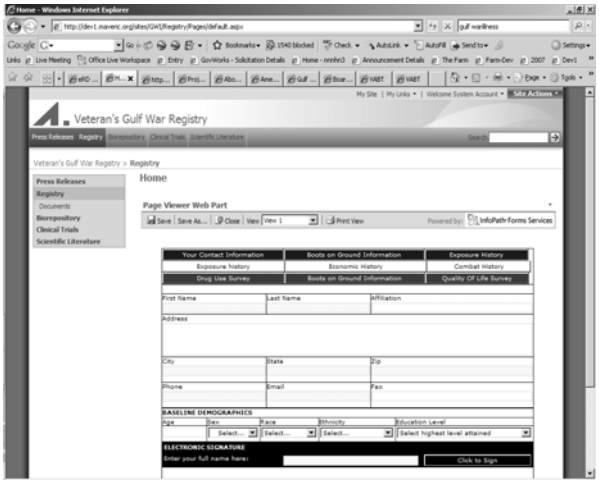


Donate Tissue Request

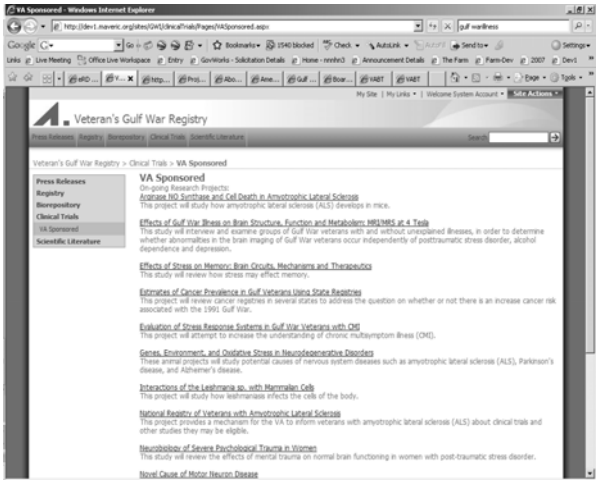


Press Releases and Bank News

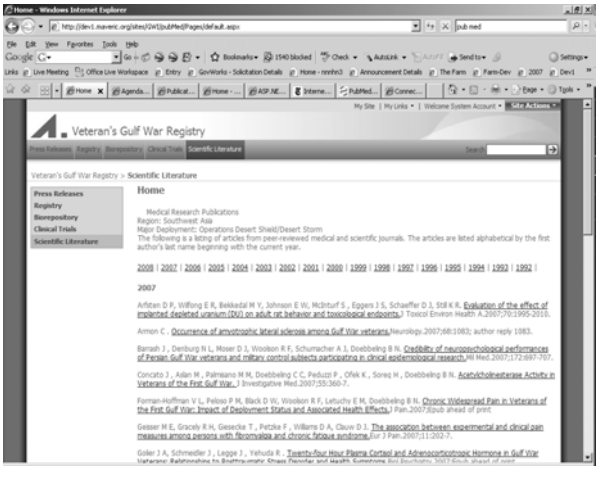
Registry and Surveys



Clinical Trials



Scientific Literature



Presentation 10 - UTSW Discussion

UTSW Gulf War Research Program

- UTSW's Gulf War program to receive $75 million over five years, the largest single allocation for GW illnesses research.
- RAC-GWVI briefed of UTSW research plan in 2006 and on additional details in Dallas July, 2007.
- A subcommittee of the RAC-GWVI met with Dr. Haley and key UTSW investigators to gain further information regarding UTSW research plan in January 2008.

UTSW Gulf War Research Program Review and Recommendations

- Congress directed the UTSW research program to implement the recommendations of the Research Advisory Committee on Gulf War Veterans Illnesses, particularly the recommendation in its 2004 Report to create a comprehensive research plan.

RAC-GWVI 2004 Report Summary

1. Suggested a strategic research program to identify and address key research questions regarding the nature, causes, and treatments for Gulf War veterans' illnesses.
2. Suggested this research program work with leading inside and outside scientists to develop comprehensive research protocols most capable of addressing priority Gulf War illnesses (GWI) research questions.
3. Establish an effective management strategy to ensure that studies capable of addressing priority research questions are satisfactorily developed and completed.

RAC-GWVI Subcommittee General Recommendations

- The UTSW GWI Research Program is focused on issues long-identified by the Committee as having high priority.
- It represents a multifaceted effort designed to identify objective markers of GWI and better understand effects of toxic exposures encountered during the Gulf War.
- UTSW has developed an overall research plan with specific research objectives and over forty individual research studies.
- An umbrella research contract and individual task orders for many of these research projects has been signed, and some are underway. The plan is based largely on previous UTSW research concepts.

General Recommendations

1. Establish an "expert panel" of outside experts (including some Committee members) who are knowledgeable in GWI research.

   This panel should identify key research issues and provide guidance on the types and characteristics of studies to be included in the plan, as envisioned in the 2004 Committee Report.

General Recommendations

2. The Committee recommends that UTSW also utilize the expert panel as liaison to the UTSW merit review group, to provide guidance on the degree to which proposed studies address key issues relevant to the health of Gulf War veterans.

General Recommendations

3. The Committee welcomes UTSW's practice to seek the input of knowledgeable outside scientists regarding individual studies.

   The Committee recommends that UTSW continue to contract out studies or functions that outside research teams are best able to provide.

General Recommendations

4. The Committee notes with approval that UTSW envisions adding other research components to the plan in the future, including clinical translation of its research results and the conduct of a genome study.

   The Committee recommends that UTSW add a clinical translation component now. The lead PI should ensure that potential applications of UTSW research to treatments and diagnostic tests are considered at every stage of the program.

General Recommendations

5. The Committee recommends that UTSW add a genetics/genomics component to the plan now.

   The plan should address other physiologic and pathophysiological processes potentially associated with Gulf War illness, including associations between Gulf War illness and central neuroinflammatory processes, immunological measures, and overlaps between systems (e.g., neuroimmune/autonomic processes potentially affected by toxic exposures).

General Recommendations

6. The Committee recommends that UTSW incorporate the overall research plan, and the structures and studies developed to execute the plan, into a program document comparable to the format utilized for a major NIH research center.

   This program document would include information on the objectives and hypotheses of the program and of each study, the design of each study, and how individual studies and components relate to one another. It would also describe the program's management structure, including provisions to ensure the program is managed as a coherent whole.

General Recommendations

7. The Committee applauds UTSW's commitment to managing the program on an industrial model, reviewing all components at the end of pilot and successive phases in light of new developments, and eliminating, modifying, or adding studies as indicated.

   The Committee also notes with approval that UTSW has charged all lead PIs on the program management team with the ongoing responsibility to review existing and emerging external research relevant to their areas of interest, particularly research that may contribute to identifying diagnostic tests and treatments.

   The Committee recommends that formal procedures to carry out these commitments be detailed in the program document.

General Recommendations

8. The Committee notes that UTSW and VA discussed a clinical/research collaboration, and that UTSW developed plans for a Gulf War Illness Treatment Clinic at the Dallas VAMC and committed the first $3 million of its research funding to an Advanced Neuroimaging Center there.

   The Committee recommends that VA and UTSW undertake a clinical/research collaboration to develop a model for evaluating and treating ill Gulf War veterans within VA facilities, which might logically include clinical research utilizing the 3T MRI system provided to the Dallas VAMC with UTSW research program funding.

General Recommendations

9. The Committee notes that VA has no mechanism for providing funding to non-VA organizations as grants and that UTSW and VA have had to develop contract formats for this complex program from scratch.

   The Committee appreciates both parties' efforts to arrive at constructive solutions and recommends that VA Central Office provide guidance to the Dallas VA Contracting Office and its VISN leadership to support the UTSW program's ability to operate with the flexibility and speed necessary to make the program successful.

General Recommendations

10. The Committee recognizes that the hopes of 175,000 Gulf War veterans who suffer from chronic multisymptom illness rest on this program.

    It is not an ordinary research project where an investigator is funded to pursue a particular thesis, while other investigators are also funded to pursue alternative theses.

    The Committee recognizes the considerable effort that has been invested to date by UTSW and VA, and that some of these recommendations envision reconsideration of decisions already made. The Committee encourages UTSW and VA to optimize this vital program for success.

GW Illness Case Definitions Recommendations

The Committee recommends that:

- UTSW plans for developing a "new" Gulf War illness case definition utilizing symptom data collected in the national survey be modified as follows. The revised approach should use a rational method, other than factor analysis, to identify a case definition based on the pattern of symptoms that best characterizes the chronic ill health of Gulf War veterans since the Gulf War.
- At minimum, this approach should identify the pattern of symptom expression that most clearly distinguishes the chronic symptoms affecting Gulf War veterans from ambient symptoms affecting individuals who did not serve in the Gulf War. The case definition should also be "portable", that is, straightforward enough so as to be usable in other research and clinical settings. Additional efforts to identify clinically meaningful illness subgroups would also be highly valuable.

GW Illness Case Definitions Recommendations

The Committee also Recommends:

- That UTSW administer symptom questionnaires to clinical study participants to clearly identify those who, at the time of intake, meet defining criteria for the newly-identified GWI case definition, the CDC CMI case definition, and the Kansas GWI case definition.
- This will allow all clinical measures to be evaluated in relation to differently defined illness outcomes, allow comparisons between those case definitions and the Haley syndromes, and allow findings from the UTSW program to be compared to those from previous clinical studies.

Sampling Strategy Recommendations

1. That UTSW collect serum and DNA samples from all consenting survey participants (approximately 10,000 veterans), to permit a broader range of comparisons between sick and healthy veterans, as well as different exposure subgroups.

2. That UTSW substantially expand the number of subjects to be evaluated clinically. Calculated estimates of the precise sample size needed to address study questions of interest requires data from the survey, the genetics/genomics study, and the pilot phase of the clinical study. Back-of-the envelope estimates, based on effect sizes expected for neuroimaging and neurocognitive studies, suggest that a clinical sample of 400 subjects would be required.

Serum DNA Bank Recommendations

The serum/DNA collection represents an extremely valuable resource, recommendations include:

1. That UTSW add a large scale, unbiased genetic/genomics study as a major program arm now, utilizing the expanded number of serum samples previously recommended.

2. That identified experts, with appropriate expertise in the design and execution of genomic and genetic studies, be contracted to design the plan for experimental design, data collection, statistical data analysis, and the details required to structure the experiments and sampling to achieve the appropriate deliverables.

Serum DNA Bank Recommendations

3. That RNA be collected from a substantial fraction of survey participants using appropriate specialized techniques, as determined by the plan previously described.

4. That the study results be utilized to inform the selection of the optimal neuroimaging evaluations to be included in the full clinical study and the selection of veteran subgroups to be included in the clinical sample, even if that results in a hiatus between the completion of the neuroimaging pilot studies and the full clinical study.

5. That costs of this study be paid for through savings achieved through elimination of other studies in the program.

Neuroimaging and Neuropsychological Projects

The UTSW program includes a broad range of innovative and sophisticated neuroimaging projects.

Since these projects will consume a substantial amount of program funding, the Committee is pleased to note that the UTSW plan incorporates a series of pilot studies to determine which studies are most likely to yield results that can contribute to understanding, diagnosing, and treating Gulf War illness.

Neuroimaging and Neuropsychological Projects

To further refine this arm, the Committee recommends:

1. That UTSW prioritize the neuroimaging studies from the onset, and consider eliminating those with lowest priority.

2. That UTSW conduct its planned pilot protocols on the remaining studies in a subgroup of Seabees evaluated in previous UTSW research, and proceed with an appropriately sized twin sample.

3. That, in addition to the data collected in the survey and pilot phase of the neuroimaging program, UTSW also utilize data collected in the genetics/genomics study to determine the optimal group of neuroimaging and neuropsychological studies to be used in the larger clinical sample.

Other Clinical Evaluations recommendations

The Committee recommends:

1. That UTSW evaluate all previously-identified indicators of objective differences between Gulf War illness cases and controls.

   This evaluation should include consultation with investigators who identified these differences and other experts to determine optimal testing procedures to ensure that previously-identified findings are adequately retested and/or further characterized.

   In particular, tests should be added to assess the association of GWI with mycoplasma and leishmania infections, squalene antibodies, immune parameters, and coagulopathies.

Other Clinical Evaluations recommendations

2. That UTSW consult with researchers with expertise in other multisymptom conditions to identify clinical tests most likely to be informative in Gulf War illness.

3. That UTSW contract out specific clinical tests to laboratories most experienced in testing for the abnormalities targeted for evaluation.

Preclinical Studies Recommendations

- The UTSW program includes diverse animal studies, conducted to shed light on pathophysiological processes resulting from Gulf War exposures.
- The program has adopted a uniform dosing protocol for chorpyrifos pesticide, sarin nerve agent, and pyridostigmine, which will allow comparison effects among studies.
- In order to maximize the utility of these studies in understanding Gulf war illness, it is important that outcomes assessed reflect problems seen in Gulf War veterans that can not be directly evaluated in humans and exposure protocols optimally reflect Gulf War exposures.

Preclinical Studies Recommendations

The Committee Recommends:

1. That UTSW add a comprehensive neuropathological evaluation of exposed animals to the preclinical arm. This evaluation should include sensitive, state-of-the-art measures to evaluate neural degeneration, astrogliosis, and microglial activation to determine if the exposure models are associated with underlying neural damage that is not obvious by traditional histopathology. These studies should be conducted by an experienced outside contractor.
2. That UTSW eliminate the mouse neuroimaging studies. These studies could later be reconsidered if results from the human neuroimaging studies or animal neuropathology studies suggest priority research questions that are best addressed by mouse imaging studies, despite spatial resolution and other limitations associated with these methods.

Preclinical Studies Recommendations

3. That UTSW establish criteria for assessing a "positive" outcome from the findings obtained in the first year's animal studies that is unified across all projects.
4. That the neurotoxin dosing protocol be expanded to include individual and combined effects of DEET and permethrin, and that delayed and persistent effects of exposures be assessed six months and 12 months after exposures.

Preclinical Studies Recommendations

5. That studies in the preclinical arm of the UTSW program be limited to those that specifically explore effects of Gulf War exposures in relation to abnormalities associated with Gulf War illness, reflecting previous research in the field.

   This would require that some currently proposed studies, while of excellent scientific design, should be eliminated, including studies focused on aging, developmental neurotoxicity, fear conditioning and PTSD, vaccine effects previously shown not to be a concern, ALS, and cancer.

Handout 1 - William Goldberg

Goldberg, William J, Ph.D.

From: Baer, Bridgett
Sent: Friday, April 04, 2008 3:35 PM
To: VHA CO 12 ACOS; VHA CO 12 AO; VHA CO 12 All Staff; CSP Directors
Subject: New Clinical Trial Program Announcement
Attachments: CCTA Handbook DRAFT 4.4.08.doc; LOI_Instructions 4.4.08.doc; Guidance CCTA 4.4.08.doc

New Program Announcement: Cooperative Clinical Trial Award (April 4, 2008)

It is critically important that our scientific work continues to lead in advancing new treatments for diseases affecting veterans. As Director of VA's Clinical Science Research and Development Service, I am pleased to announce a new, expanded program for clinical trials, the Cooperative Clinical Trial Award, intended to support further clinical treatment advances. The goal of this program is to provide an opportunity for PIs to collaborate with the VA Cooperative Studies Program (CSP) on smaller clinical trials, with CSP providing statistical, design and implementation expertise.

The attached documents [draft Handbook and guidance for Letter of Intent (LOI) and application] describe the major steps involved, including LOI approval and assignment to our Cooperative Trials Coordinating Center (CTCC). The PI will work closely with the CTCC to plan, implement and complete the trial. The CTCC will convene a national Data Monitoring Committee as an additional resource for certain trials. Funding for the CTCC resources will be supported centrally and will be separate from the budget for the trial.

This CCTA Program provides an opportunity for PIs to work collaboratively with VA clinical trials and biostatistical experts to develop and complete rigorous interventional research. These clinical trials will determine treatment effectiveness and identify clinical advances that may be implemented in the VA healthcare system. We are especially interested in supporting trials that will address new treatment approaches for diseases that are especially relevant to veterans and their unique healthcare needs. Studies that address interventions for the mental and physical health consequences from military/combat experience are encouraged.

Please distribute this new program announcement to VA scientists. We look forward to working together to advance treatment research.

Timothy J. O'Leary, M.D., Ph.D.
Director, Clinical Science R&D Service
Office of Research & Development
Department of Veterans Affairs

Public Comment 1 - Edward Bryan

SSG Edward J. Bryan (Ret.)
Malden, MA 02148

Kimberly Sullivan, Ph.D.
Scientific Coordinator
Research Advisory Committee on Gulf War Veterans' Illnesses

April 7-8, 2008

Dear Dr. Sullivan,

Gulf War veterans from all over this country are expecting this Committee to release treatments for ill veterans. I know this is research, but we need to know what the VA is going to do with all this research. Is the VA-RAC going to recommend treatments to the VA Clinical Side of the house? Medical treatments have been a long-standing issue with the Department of Defense and the Veterans Administration. The clinical doctors, including DOD, VA, and civilian hospitals, still today do not know about Gulf War illnesses. Just read the VA book against veterans "Caring for the War Wounded" published in 2003; **this book is not for the veterans**. There is enough information researched for many years now, and many trials should start soon. Today Gulf War veterans still report being not as healthy as their military counterparts who were not deployed in the Persian Gulf. We are still having medical issues that VA doctors still ignore. Neurology is in and psychiatry is out. The death toll from the Gulf War in 1991 is now closing to over 75,000 veterans, so something biological/environmental happened. This Middle East war started in 1980.

The neurological issues that should be highly driven are Multiple Sclerosis (MS), Amyotrophic Lateral Sclerosis (ALS), Alzheimer's (ALZ) disease and any other neurodegenerative disease. "Therefore, for MS, the use of stem cells may provide a logical solution, since these cells can migrate locally into the areas of white-matter lesions (plaques) and have the potential to support local neurogenesis and rebuilding of the affected myelin...Stem cells were also shown to possess immunomodulating properties, inducing systemic and local suppression of the myelin-targeting autoimmune lymphocytes" (D. Karussis, The potential use of stem cells in multiple sclerosis: An overview of the preclinical experience. *Clin Neurol Neurosurg*. 2008 Mar 27 [Epub ahead of print]). "Several types of stem cells (embryonic and adult) have been described and extensively studied in animal models of CNS diseases and the various models of MS (experimental autoimmune encephalomyelitis [EAE])" (D. Karussis, Use of stem cells for the treatment of multiple sclerosis. *Expert Rev Neurother*. Sept. 2007; 7(9):1189-1201). Along with that, "Lorenzo's oil is a mixture of oleic and erucic acids, that helps to normalize the levels of very long chain of fatty acids in boys with adrenoleukodystrophy. Having low levels of very long chain fatty acids in the body will help to slow and minimize the effects of the disease by preventing them from accumulating in the body. Unfortunately, use of the oil causes gross lowering of platelets in patients" (http://www.ikm.jmu.edu/Buttsjl/ISAT493/Adrenoleukodystrophy/aldtreatment.html).

Another vitamin is lecithin with certain diets, there should be a balance of vitamins. Go to the website: http://tsangenterprise.com/news77.htm. As you can see there is a lot of work to do, but not one treatment released yet from the VA! Dr. Kristin Heaton of Natick labs was another doctor in research who must by now have good solid information on neuropsychological and neuroanatomical findings with respect to sarin and cyclosarin gases.

How many veterans are entitled to the Purple Heart awards? The numbers are in the 1,000,000 or more. When will this information be released? As we are speaking today the Gulf War in 1991 was never really investigated fully for environmental health issues; there were only partial investigations and very limited studies going on. One study is the bombing campaign along with the oil well fires and carbon monoxide poisoning never really came to a true research response because the filtering equipment failed and the DOD had to try and make the numbers work. For carbon monoxide exposure, you have to have a blood test and an MRI for exposures; this is not being done at the VA. As everyone knows, no one can figure those numbers out. We still do not have any true answers today from environmental health issues in 1991. We should have industrial/environmental doctors as primary care doctors for our declining health problems. We need doctors from this Committee to give town hall meetings to doctors so they will know first hand on what is going on with Gulf War veterans' health. The VA/CME credits for doctors didn't work for Gulf War veterans' health problems, how can the veterans get their answer from their doctor? Is this negligence on the VA?

On the brain issues with white matter disease, the spec scan and MRIs are still hard to receive from the VA doctors. The doctors are still in the dark and believe that this is all in our heads. Gulf War veterans should have a neurologist not a psychiatrist for their care. Please see the VA 2006 Gulf War research annual report to Congress, pages 8-12.

On gastroenteritis and chemical exposures from the 1991 war: the water supply, like today's water supply in Iraq, was tainted with manganese and iron and other chemicals above the threshold limit value. The Washington D.C. area gets their drinking water supply from Watertown, Massachusetts (ionic's) so they can drink clean drinking water. "A covert release of a chemical agent might not be identified easily for at least five reasons. First, symptoms of exposure to some chemical agents (e.g., ricin) might be similar to those of common diseases (e.g., gastroenteritis). Second, immediate symptoms of certain chemical exposures might be nonexistent or mild despite the risk for long-term effects (e.g., neurocognitive impairment from dimethyl mercury, teratogenicity from isotretinoin, or cancer from aflatoxin). Third, exposure to contaminated food, water, or consumer products might result in reports of illness to healthcare providers over a long period and in various locations. Fourth, persons exposed to two or more agents might have symptoms not suggestive of any one chemical agent (i.e., a mixed clinical presentation). Finally, health-care providers might be less familiar with clinical presentations suggesting exposure to chemical agents than they are with illnesses that are treated frequently." (from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5239a3.htm).

The sensitizers like pesticides or other lethal chemicals are irreversible inhibitors for cholinesterase or acetylcholinesterase levels and should be fully worked up with toxicology lab results. See: http://www.usaid.gov/our_work/environment/compliance/ane/jordan/completed%20slides/jaghbir_medical_aspects_of_pesticides_antidote__.pdf

SSG Edward J. Bryan (Ret.)

Life Member, D.A.V.
Life Member, V.F.W.
Retired, U.S. Army 1974-2000
Retired Firefighter, Medford, MA 1986-2000
Researcher, Gulf War Illnesses 1992-Present
Health Care Liaison (VA/BU) 1995-2001
Member, VISN-I Mini-Mac 1998-Present
Walter Reed Veteran Health Advisory Council (VHAC) Deployment Health, 2000-2002

Public Comment 2 - Denise Nichols

Dear Research Advisory Committee Members,

This serves as a reminder to keep in mind other organ systems, i.e. cardiac, and potential connecting disorders in Gulf War veterans. Just a plea to keep your mind open past the neurological to other important organs and blood (tissue factor, hypercoagulation), etc. One of the unusual symptoms of fatigue is that components of cardiac function are affected such as blood flow, electrical activity, structure of the heart, and dynamics in the blood.

We forget one of the earliest symptoms: NOSE BLEEDS and bleeding gums! We also had to aero-medically evacuate females out of theater due to uncontrollable large bleeding and prolonged menstrual cycles. Many females in early post-war had hysterectomies to control the continuing problem. We also had many females who had spontaneous abortions because they could not carry to term; these symptoms did not get recorded as possible connections!

It would be interesting if Gulf War veterans had arterial and venous blood tested together for oxygen values. I had this done and oxygen was great arterially but also very high in venous sample. So, it showed a problem with the oxygen being transported out of the blood. A simple and somewhat inexpensive test can show a valuable clinical finding.

Also, half of all animals that had brain lesions had areas of myocardial degeneration and necrosis. Depending upon the point in time at which cardiac tissues were examined, findings varied from areas of acute myolysis and necrosis to areas undergoing resolution of damage.

Sincerely,

Denise Nichols