

Research Advisory Committee on Gulf War Veterans' Illnesses

August 14-15, 2006, Committee Meeting Minutes

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



**DEPARTMENT of VETERANS AFFAIRS**

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

I hereby certify the following minutes as being an accurate record of what transpired at the August 14-15, 2006, 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

## Table of Contents

<b>Attendance Record.....</b>	<b>5</b>
<b>Abbreviations .....</b>	<b>6</b>
<b>Welcome, introductions, and opening remarks.....</b>	<b>10</b>
<b>Gulf War Illnesses, CNS Pro-Inflammatory Processes, and Autonomic Dysregulation .....</b>	<b>11</b>
<b>The Cholinergic Anti-inflammatory Pathway in the Inflammatory Reflex .....</b>	<b>11</b>
<b>Immune abnormalities associated with CFS in the general population.....</b>	<b>13</b>
<b>Gulf War Exposures and Dysregulation of CNS Inflammatory Processes: Research Previously Considered .....</b>	<b>14</b>
<b>Low Level Chemical Toxicity Study of Autonomic Neural Balance .....</b>	<b>14</b>
<b>Role of inflammation in the pathogenesis of neurodegenerative diseases: models, mechanisms, and therapeutic interventions.....</b>	<b>19</b>
<b>Peripheral Benzodiazepine Receptor (PBR) imaging of central nervous system inflammation and injury.....</b>	<b>21</b>
<b>Committee Discussion.....</b>	<b>24</b>
<b>Public Comment – Day 1 .....</b>	<b>29</b>
<b>Day 2.....</b>	<b>30</b>
<b>Gulf War illnesses and chronic fatigue syndrome comparative studies – The Miami experience ....</b>	<b>34</b>
<b>Public Comment – Day 2 .....</b>	<b>36</b>
<b>Gulf War-related Research at Wright State University .....</b>	<b>37</b>
<b>Overview of the Congressionally Directed Medical Research Programs (CDMRP).....</b>	<b>39</b>
<b>Gulf War Update.....</b>	<b>41</b>
<b>Appendix.....</b>	<b>50</b>
<i>Presentation 1 – Lea Steele.....</i>	<i>50</i>
<i>Presentation 2 - Kevin Tracey .....</i>	<i>87</i>
<i>Presentation 3 – Nancy Klimas.....</i>	<i>105</i>
<i>Presentation 4 – Lea Steele.....</i>	<i>125</i>
<i>Presentation 5 – Marina Morris .....</i>	<i>126</i>
<i>Presentation 6 – Mohan Sopori .....</i>	<i>140</i>
<i>Presentation 7 – Jau-Shyong Hong .....</i>	<i>160</i>

*Presentation 8 – Tomás Guilarte*..... 177

*Presentation 9 – Nancy Klimas*..... 194

*Presentation 10 – Mariana Morris* ..... 203

*Presentation 11 – Janet Harris*..... 222

*Presentation 12 – William Goldberg* ..... 233

**Attendance Record**

**Members of the Committee**

James H. Binns, Chairman  
Carrolee Barlow  
Daniel J. Clauw  
Beatrice A. Golomb  
Joel Graves  
Anthony Hardie  
Marguerite L. Knox  
William J. Meggs  
Mary D. Nettleman  
James P. O'Callaghan  
Steve Smithson  
Lea Steele  
Hugh H. Tilson

**Committee Staff**

Laura Palmer  
Barbara LaClair

**Guest Speakers**

Tomás Guilarte  
Robert Haley  
Janet Harris  
Jau-Shyong Hong  
Nancy Klimas  
Marina Morris  
Mohan Soporì  
Kevin Tracey

**Guest Participants**

Allen Fienberg  
James Baraniuk  
Bellina Veronesi  
Malu Tansey  
Roberta White

**Abbreviations**

ACTH	Adrenocorticotrophic hormone
ALS	Amyotrophic lateral sclerosis
CDC	U.S. Centers for Disease Control
CDMRP	Congressionally Directed Medical Research Programs
CFS	Chronic fatigue syndrome
CNS	Central nervous system
CRADO	Chief Research and Development Officer (VA)
CRP	C-reactive protein
DM	Dextromethorphan
DoD	U.S. Department of Defense
EPA	Environmental Protection Agency
FY	Fiscal year
GDNF	Glial cell derived neurotrophic factor
GFAP	Glial fibrillary acidic protein
GLAST	Glutamate aspartate transporter
GWI	Gulf War illness
GWVIRP	Gulf War Veterans' Illnesses Research Program (DoD)
HHS	U.S. Department of Health and Human Services
HPA	Hypothalamic-pituitary-adrenal axis
ICU	Intensive care unit
IL	Interleukin
LPS	Lipopolysaccharide
MEG1	Maternally-expressed gene 1
MHC	Major histocompatibility complex
MoD	UK Ministry of Defence
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
NADPH	Nicotinamide adenine dinucleotide phosphate
NIEHS	National Institute of Environmental Health Science (US)
NIH	National Institutes of Health (US)
NK	Natural killer
NMDA	N-methyl-d-aspartate
NMR	Nuclear magnetic resonance
NMRIS	Nuclear magnetic resonance imaging spectroscopy
OIF	Operation Iraqi Freedom
ORD	Office of Research and Development (VA)
PB	Pyridostigmine bromide
PBR	Peripheral benzodiazepine receptor

PET	Positron emission tomography
PHOX	Phagocytic oxidase
PMSF	Phenylmethylsulfamyl fluoride
POMS	Profiles of Mood State
PTSD	Post traumatic stress disorder
RAC-GWVI	Research Advisory Committee on Gulf War Veterans' Illnesses
SPECT	Single photon emission computed tomography
TH	Tyrosine hydroxylase
TLR	Toll-like receptor
TNF	Tumor necrosis factor
UK	United Kingdom
VA	U.S. Department of Veterans Affairs

**Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses**

**August 14-15, 2006**

**VA Headquarters, 810 Vermont Ave., N.W., Room 230**

**Washington, D.C.**

***Agenda***

**Monday, August 14**

<b>8:00 – 8:30</b>	<b>Informal gathering, coffee</b>	
<b>8:30 – 8:45</b>	<b>Welcome, introductory remarks</b>	<b>Mr. Jim Binns, Chairman Res Adv Cmte Gulf War Illnesses</b>
<b>8:45 – 9:45</b>	<b>Overview: Gulf War illnesses in relation to central proinflammatory processes and autonomic dysregulation</b>	<b>Dr. Lea Steele Res Adv Cmte Gulf War Illnesses</b>
<b>9:45 – 10:30</b>	<b>The cholinergic anti-inflammatory pathway in the inflammatory reflex</b>	<b>Dr. Kevin Tracey Feinstein Institute for Medical Research</b>
<b>10:30 – 10:45</b>	<b>Break</b>	
<b>10:45 – 11:15</b>	<b>Immune abnormalities associated with chronic fatigue syndrome</b>	<b>Dr. Nancy Klimas Univ of Miami School of Medicine, Miami VAMC</b>
<b>11:15 – 11:30</b>	<b>Overview: Research on autonomic and neuroimmune dysregulation associated with Gulf War exposures</b>	<b>Dr. Lea Steele</b>
<b>11:30 – 12:00</b>	<b>Autonomic dysfunction following low-level sarin exposure</b>	<b>Dr. Mariana Morris Wright State Univ Boonshoft School of Medicine</b>
<b>12:00 – 1:00</b>	<b>Lunch</b>	
<b>1:00 – 1:30</b>	<b>Summary: Alterations in cholinergic receptors, cytokines, glucocorticoids, and immunity following low-level exposure to cholinergic agents</b>	<b>Dr. Mohan Sopori Lovelace Respiratory Research Institute</b>
<b>1:30 – 2:00</b>	<b>Summary: Persistence of regional indicators of CNS inflammation following Gulf War-related exposures</b>	<b>Dr. Mohamed Abou-Donia Duke University Medical School</b>
<b>2:00 – 2:45</b>	<b>Microglial activation following neurotoxic exposure: the self-propelling cycle of neuroinflammation in neurodegenerative disease</b>	<b>Dr. John Hong Nat'l Inst Environmental Health Sciences</b>
<b>2:45 – 3:00</b>	<b>Break</b>	
<b>3:00 – 3:30</b>	<b>Noninvasive imaging of inflammation in the central nervous system</b>	<b>Dr. Tomas Guilarte Johns Hopkins School of Public Health</b>
<b>3:30 – 5:00</b>	<b>Discussion: Priority study questions and methods in investigating the potential role of neuroinflammatory processes in Gulf War illnesses</b>	
<b>5:00 – 5:15</b>	<b>Public questions/comments on scientific presentations</b>	

**Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses  
August 14-15, 2006  
VA Headquarters, 810 Vermont Ave., N.W., Room 230  
Washington, D.C.**

***Agenda*  
Tuesday, August 15**

<b>8:00 – 8:30</b>	<b>Informal gathering, coffee</b>	
<b>8:30 – 9:00</b>	<b>Public comment period</b>	
<b>9:00 – 9:30</b>	<b>Remarks, presentation of certificates to new Committee members</b>	<b>Dr. Michael Kussman, VA Principal Deputy Undersecretary for Health</b>
<b>9:30 – 10:15</b>	<b>Immune, genomic studies of Gulf War veterans at Miami VAMC</b>	<b>Dr. Nancy Klimas</b>
<b>10:15 – 10:30</b>	<b>Break</b>	
<b>10:30 – 11:15</b>	<b>Gulf War-related research at Wright State University</b>	<b>Dr. Mariana Morris</b>
<b>11:15 – 11:45</b>	<b>2006 Gulf War illness research initiative at the Department of Defense</b>	<b>COL Janet Harris, Ph.D. Director, U.S. Army Congressionally-Mandated Medical Research Program</b>
<b>11:45 – 12:00</b>	<b>Update: Gulf War research program at University of Texas Southwestern</b>	<b>Dr. Robert Haley Univ of Texas Southwestern Medical School</b>
<b>12:00 – 1:00</b>	<b>Lunch</b>	
<b>1:00 – 1:45</b>	<b>Overview and Update: VA Gulf War Illness Research</b>	<b>Dr. Joseph Francis Dr. William Goldberg VA Office of Res and Development</b>
<b>1:45 - 2:30</b>	<b>RAC Business</b>	
<b>2:30</b>	<b>Adjourn</b>	

Dr. Floyd Bloom and Mr. Adrian Atizado, Committee members, and Dr. Jack Melling, Committee consultant, were not able to be present at this meeting. The meeting was held both days in Room 230 of the U.S. Department of Veterans Affairs (VA) Headquarters, 810 Vermont, NW, Washington, DC.

### **Welcome, introductions, and opening remarks**

James H. Binns, Jr., Chairman

Chairman James Binns called the meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses (hereinafter referred to as "the Committee") to order at 8:31 a.m. He welcomed the Committee members, invited scientists, managers of government research programs, Congressional staff, veterans and other members of the public. He extended a special welcome to Lord Morris of Manchester, an advocate for ill Gulf War veterans in the United Kingdom. Lord Morris was accompanied by Ms. Sue Freeth of the British Royal Legion. Chairman Binns noted that the Committee would be meeting later with Dr. Michael Kussman, the newly appointed VA Undersecretary for Health. Dr. Kussman would be presenting certificates of appointment to the members appointed within the last year.

At the Committee's May 2006 meeting, Chairman Binns noted that the Committee had entered a new phase of activity, having completed the review of research relating to the extent and nature of Gulf War illnesses, and would now focus on identifying the mechanisms potentially underlying Gulf War illnesses, which hopefully would lead to a basis for diagnosis and treatment. Chairman Binns stated that this was the first meeting devoted to this new focus and represented a departure from previous meetings in many respects. It was focused on a single scientific topic, which was relatively narrow, and was designed to explore this topic in depth and offer insights into the mechanisms underlying Gulf War veterans' illnesses. Chairman Binns noted that Dr. Lea Steele, the Committee's Scientific Director, had identified an impressive array of speakers to address this topic from the different aspects and perspectives of their work. In addition, several scientists had been invited to join the Committee in its discussions. Some of these scientists had spoken before the Committee at previous meetings. Chairman Binns noted that all of these scientists were seated in the area opposite the Committee table, and had been provided microphones to facilitate their participation in the discussion. To ensure that the discussion was productive, Chairman Binns stated that discussion would be structured and lead by Dr. Steele and Dr. Jim O'Callaghan. Chairman Binns noted that there was a full 1 hour and 45 minutes allocated at the end of the day for discussion. He stated that the Committee would not be entertaining comments and questions from the members of the public during this period of time in order to ensure ample time for discussion with the invited scientists. He asked that members of the public address their questions and comments during the afternoon public comments session to the scientific topic of the day.

Chairman Binns commented that this was an exciting day for him as chairman of the Committee. He believed that the presentations and discussions had significant potential to advance the state of Gulf War illness research. Chairman Binns noted that there were a number of individuals on the Committee who were not scientists. He stressed that he did not wish to constrain any of the scientists from having a good, in-depth discussion, but asked that they include lay references, when possible, so the non-scientist members and attendees could follow the more technical discussions.

The Committee and the guest speakers and participants then introduced themselves and gave a brief description of their background.

**Gulf War Illnesses, CNS Pro-Inflammatory Processes, and Autonomic Dysregulation**

Lea Steele, PhD

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

Dr. Steele gave a summary of the focus, scope and findings of previous Gulf War illnesses research and an overview of remaining questions regarding the pathophysiology of Gulf War illnesses. ([See Appendix – Presentation 1.](#)) She indicated that the present meeting would focus on the idea that stimulation and dysregulation of proinflammatory processes in the central nervous system (CNS) underlie the chronic symptom complex affecting Gulf War veterans. She provided overview information about innate immunity, cytokines and neuroinflammation and related how this information would shape the day's presentations and discussion.

The meeting recessed at 9:47 a.m. for a break.

The meeting reconvened at 10:00 a.m.

**The Cholinergic Anti-inflammatory Pathway in the Inflammatory Reflex.**

Kevin J. Tracey, MD

CEO, Feinstein Institute for Medical Research

Dr. Tracey spoke about the interactions between the brain and the innate immune system, with a focus on the role of the autonomic nervous system in regulating the inflammatory response. ([See Appendix – Presentation 2.](#))

Dr. Bill Meggs, a Committee member, commented that the research presented by Dr. Tracey could have implications related to the fight-or-flight response. He asked whether this research suggested that if someone in crisis (e.g., an automobile accident) was given something to suppress emotional responses and anxiety (e.g., benzodiazepine), that individual might return to a parasympathetic dominance, preventing the terrible cascade described by Dr. Tracey. Dr. Tracey replied that he did not know, but this was a testable hypothesis and quite plausible based on observations in animals. He stated that this research is causing researchers to go back and look at agents used in intensive care units (ICUs). Several of these agents are cholinergic and anticholinergic and little is known about their immune effects. He stated that one had to be careful, though, with respect to the fight-or-flight response, because both parasympathetic and sympathetic nervous systems are firing "in over drive." However, only the clinical manifestations of the sympathetic nervous system are seen in excess. The benefit of having both systems activated is that they are probably both blocking cytokine production. Dr. Tracey stated that the same thing happened at the cardiovascular level. If one isolated and stimulated the sympathetic nervous system, cardiac output is increased. If the parasympathetic system is isolated and stimulated, cardiac output is decreased. If both systems are stimulated together, there is a synergistic increase in cardiac output to the benefit of the animal, because the heart is slowed enough to increase filling time, resulting in a better contraction. These complex systems interactions must be (1) broken into separate, individual pieces; (2) have their activities examined in combination; and (3) have a determination made as to how best to modulate them clinically.

Dr. Carolee Barlow, a Committee member, asked what afferent signals were involved in this process. Dr. Tracey stated that this was a "black box" and that there were only a handful of papers addressing this issue. Endotoxin can activate vagus nerve signals afferently. Interleukin-1 (IL-1) and other cytokines can activate these sensory or afferent signals. The question has been asked: How does the vagus nerve detect it? This is a very important question. The vagus nerve is going to the entire viscera. It may be that the

vagus nerve is sensing things at the cellular or molecular level, and detects inflammation or problems at very low levels. Dr. Tracey noted that one study, which used a radio-labeled IL-1 receptor antagonist construct to bind to IL-1 receptors, found what looked like cellular or globular structures along the path of the vagus nerve. Dr. Tracey stated that he hadn't seen additional work addressing this question. He noted that this physiological controlling system, which can control cytokine response, was likely occurring at the receptor level. It may not be at the individual cell level, but there are probably regions that are being sampled to detect the endotoxin or IL-1. He commented that there could be a whole list of things identified in twenty years that have differential signals to tell the brain what is going on.

Dr. Mohan Sopori, an invited presenter from the Lovelace Respiratory Research Institute, noted that there was literature which showed that, in terms of the vagus nerve, a behavioral response was only elicited when the stimulus was injected peritoneally and not when given intravenously or subcutaneously. He asked for Dr. Tracey's response to these findings. Dr. Tracey asked if this was in terms of the fever response. Dr. Sopori clarified this related to both the fever response and behavioral response. Dr. Tracey stated that if one gave a low dose in the abdomen, a fever response dependent on the vagus nerve would be generated. If the vagus nerve is cut, the fever response is lost. If a higher dosage is used, it goes to the brain via the bloodstream. If the vagus nerve is then cut, a fever response is still elicited. Several groups have confirmed this finding. Dr. Tracey stated that when his team conducted research using escalating dosages of tumor necrosis factor (TNF) for 7–10 days, it opened a whole universe of complexity of sensitization and desensitization. All other nervous system regulatory effects, even heart rate, are time-dependent. Acetylcholine to the heart is depleted in a few minutes, and then the heart doesn't respond to repeated nervous stimulation. The question is: What is happening with this controlling system? When the vagus nerve fires, does it cause sensitization or desensitization? And what is the biological half-life, i.e., how long does a cell that is turned-off by acetylcholine "remember" to stay turned-off? These are very important questions that are just beginning to be studied. Surprisingly, his team's work suggests that the cell that receives acetylcholine stays turned off for 24-48 hours. He didn't believe this himself at first, but repeated cell-culture trials showed this is the case. If this is occurring in the animal, it may be that a very transient firing of the vagus nerve resets the immune system *in vivo* for a period of time. Dr. Tracey stated the clinical situation may be difficult to tease out, but if one stays focused on the pathway, the "wiring" in animals may be figured out.

Dr. Robert Haley, a representative of the University of Texas Southwestern Medical School, commented that a long-standing hypothesis with regards to Gulf War illness was that there is damage to some of these mechanisms in the brain. He stated that the most inviting idea was that there was damage to the muscarinic system. This would then reduce vagal influences that are restraining the cytokine system, resulting in a hyperresponsive cytokine production. He asked if Dr. Tracey was thinking along these lines. Dr. Tracey stated that this was a reasonable hypothesis, and that Dr. Steele and he had been discussing it over the past year. He related a story about a cognitive inflammatory reflex conference that he hosted in Stockholm, Sweden, in September 2005. The first speaker at the conference stated that "We all know that the brain and behavior can control the immune system." And an attendee yelled from the back of the room that he disagreed and that the immune system came first, and cytokines drive behavior. Although some researchers have assumed that our brain is in charge it may be the other way around. Depressed patients clearly have dysregulated peripheral cytokine responses. The question, in context of this meeting, is whether there is a peripheral problem first, which is either sensed by the nerve or "leaks across" the blood-brain barrier, that resets the set point, leading to a decrease in autonomic regulation. Ultimately, this is a "chicken-egg" question.

Chairman Binns thanked Dr. Tracey.

**Immune abnormalities associated with CFS in the general population**

Nancy Klimas, MD

Professor, University of Miami Miller School of Medicine

Director, Chronic Fatigue Syndrome and Gulf War Illnesses Center, Miami VA Medical Center

Dr. Klimas gave an overview of her research findings concerning immune abnormalities associated with chronic fatigue syndrome (CFS), and explained how these findings could inform researchers and clinicians about processes potentially affecting ill Gulf War veterans. ([See Appendix – Presentation 3.](#))

Referencing Dr. Klimas' Hurricane Andrew studies, Dr. Steele asked if Dr. Klimas concluded that there was something in the periphery, such as cytokine levels or natural killer (NK) cell function, that could track symptom severity over time. Dr. Klimas stated that both of these together would do this. She noted that NK cells were the psychologists' favorite cell because it was highly reactive to stress conditions. However, it wasn't the best marker for an individual moment in time because NK cells levels can quickly change based upon individual circumstances at the time. Dr. Klimas noted Dr. Jim Baraniuk's findings, and expressed her excitement over his approach of looking at cerebrospinal fluid to measure chronic inflammation. Dr. Steele commented that Dr. James Baraniuk was present and was part of the Committee's invited scientific discussion panel.

Dr. Malu Tansey, an invited scientist who is with the University of Texas Southwestern School of Medicine, noted Dr. Carla Shatz' research with major histocompatibility complex (MHC) class molecules and findings that these molecules appear to be very involved with synaptic plasticity. Dr. Tansey asked if MHC class upregulation had been examined in chronic fatigue syndrome, e.g., whether it happens in the brains of afflicted individuals. Dr. Klimas stated that it was difficult to do this type of research in humans because of limited access to brain sample specimens. She stated that there were only 5-6 human brain bank specimens available for this type of study.

Dr. Sopori asked if any of the *in vitro* studies had shown a decrease in activity when TNF-alpha was put into NK cells. Dr. Klimas stated there were, and if one just did this one experiment, it would decrease NK cell activity. However, it is difficult to get just one cytokine in real life. This is why researchers use whole blood assays. All of the components may not be known, but the conditions are what matter. Dr. Klimas stated that they have done studies where patients' sera were placed in healthy cells with negative results, indicating that there was something circulating in the serum that reduced NK cell activity. Dr. Sopori noted Dr. Klimas' earlier comments that there was a correlation between the high TNF-alpha level and the decrease in NK cell activity. Dr. Klimas' stated that this could be direct or indirect. She noted that this was a complex system, making it challenging to do *in vivo* research.

Dr. Daniel Clauw, a Committee member, commented that Dr. Klimas had done a wonderful job in presenting a proinflammatory view of these illnesses. He noted, however, that there are some in the field, like himself, who think that most of the changes in the immune system, which have been inconsistent, are somewhat predictable in relation to the dysautonomy and hypothalamic-pituitary-adrenal axis (HPA) changes in this spectrum of illness. Dr. Clauw stated that he would reframe the question slightly to ask: "Which effector system is most important?" Are dysautonomy or HPA changes directly causing symptoms or are they doing so through the immune system? He stated that these aren't necessarily mutually exclusive. It could be that in one group of individuals, the most important effector system is the immune system, while in another, the autonomic nervous system is directly driving symptom expression. Dr. Klimas commented that it was fair to say that researchers were subgrouping patients in this way. She stated that her team had been primarily interested in the inflammatory subgroup, which she would probably define larger than Dr. Clauw would. Dr. Klimas noted that her group was also doing autonomic function studies, e.g., tilt table testing, which she had not discussed in her presentation. She did not mean

to imply that the symptoms were not related to autonomic dysfunction, since it could have an important role. However, cytokines might be driving a lot of the autonomic problems and it could be hard to tease out. With respect to the inconsistency of immune findings in different CFS studies, Dr. Steele commented that differences might relate to changing immune or autonomic profiles that occur in patients over time, as indicated by Dr. Klimas's studies.

Dr. Steele thanked Dr. Klimas.

### **Gulf War Exposures and Dysregulation of CNS Inflammatory Processes: Research Previously Considered**

Lea Steele, PhD

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

Before entering the next phase of the meeting's discussion, Dr. Steele provided a brief summary of research previously considered by the Committee indicating that exposures in the Gulf War could stimulate CNS inflammatory processes. ([See Appendix – Presentation 4.](#))

### **Low Level Chemical Toxicity Study of Autonomic Neural Balance**

Mariana Morris, Ph.D.

Professor, Pharmacology & Toxicology, Wright State University Boonshoft School of Medicine

Dr. Morris discussed her team's research on the effects of low-dose sarin exposure on autonomic function and brainstem catecholamines. ([See Appendix – Presentation 5.](#))

Dr. O'Callaghan, a Committee member, asked if Dr. Morris' team had looked for habituation to stress over time in their shaker stress-pyridostigmine bromide (PB) testing. Dr. Morris stated that they had looked at cardiovascular habituation and found continued response over time, but with slight changes that had not been dramatic. Dr. O'Callaghan asked if Dr. Morris had plans to do a rotating stress paradigm. Dr. Morris stated that they had considered it, but thought the current model was a good one because it had produced the changes described. It was always difficult to know how comparable stress intensity is when different models are used.

With regards to the PB-shaker stress test, Dr. Beatrice Golomb, a Committee member, asked how long Dr. Morris' team waited to collect data after the cessation of PB administration and stress. Dr. Morris replied that PB administration had been on-going when measurements were taken, which had been done at the end of the day, after the stressor. Dr. Golomb commented that when there is superphysiological activation of a system, it can lead to long-term depression of that system or vice-versa. The acute effects might enhance heart-rate variability, but the real question is what happens in the aftermath. Dr. Morris stated that they did not have those data for PB, since that project had not included longer-term measures. She believed the most important aspect of their study findings was that sarin had effects on the brain and cardiovascular system at doses that cause no detectable clinical symptoms. Further, these studies document possible health problems associated with low dose, non-symptomatic exposure to sarin. Dr. Golomb concurred and indicated that this raised the question of whether this extended to other acetylcholinesterase inhibitors like PB.

Dr. Haley commented that the biphasic response observed in Dr. Morris' research was an unexpected and provocative finding. He referred to Dr. Rogene Henderson's team finding an initial and persistent upregulation of muscarinic-3 receptors, with a later down-regulation of muscarinic-1 receptors. He asked

if Dr. Morris had connected her team's findings with Dr. Henderson's findings, specifically the implications regarding the change in tyrosine hydroxylase (TH) levels in the locus coeruleus, etc. Dr. Morris stated that they had more to do in this area. The question appeared to be: "What does the messenger (mRNA) mean in terms of physiology?" She stated it would be difficult to say what the answer would be at this point. Dr. Haley stated that this appeared to be a terrific clue with respect to pathogenesis.

Dr. Steele inquired about the implications of having changes in heart rate variability that aren't accompanied by changes in blood pressure variability. Dr. Morris stated that the outflow from the brain to the heart was different from the vascular. Sometimes one will see a change in the blood pressure, but not in the heart rate or vice-versa. She stated that sarin might be involved in the areas of the central nervous system that control the heart.

Dr. Sopori noted that Dr. Morris' team hadn't seen changes in acetylcholinesterase activity. He asked if she might conclude that this was independent of the cholinergic effects of sarin. Dr. Morris stated that there were some studies in acetylcholinesterase knockout mice indicating this might be the case. She, however, wasn't sure if this was true. Although overall changes in the periphery or the brain weren't seen, it is hard to know if there may have been subtle activation in susceptible brain regions. She believed that more early studies were needed to look at the cellular level in order to determine what is going on.

Dr. Meggs noted the Brazilian studies discussed in Dr. Morris' presentation, which used PB to enhance heart rate variability in heart failure patients. These findings showed a cause-and-effect association. Dr. Morris stated that the Brazilian hypothesis was that PB increased variability in the human, which then provided the protective effect. Dr. Golomb commented that PB enhances cholinergic activity peripherally and this can, by itself, improve the pumping of the heart. Dr. Morris agreed, but stated that she believed that the Brazilians thought it was more of a CNS effect. She noted, however, it was not possible to separate the two.

Dr. Bellina Veronesi, an invited scientist who is with the Environmental Protection Agency (EPA), commented that she had thought it might be important to measure something other than anticholinesterase activity following pesticide exposures, but that this idea had not been supported at EPA. She believed, however, that the idea should get out into the open literature so that regulators might be informed about the concept.

Dr. Jau-Shyong Hong, an invited presenter who is with the National Institute of Environmental Health Sciences (NIEHS), noted Dr. Morris' data on delayed TH changes. He asked if she had looked at tissue-organ systems to see if there was an increase in norepinephrine or epinephrine. Dr. Morris stated that they had looked at adrenal tissue in the animals treated with sarin. Long-term sarin exposure produced hyperplasia of the adrenal tissue with reduced levels of norepinephrine. She noted that this suggested that there could be some peripheral effects in addition to the CNS effects.

Dr. Barlow asked if microdialysis experiments could be done, with the aim of looking at neurotransmitter levels (epinephrine, norepinephrine) turn-over. Dr. Morris agreed this would be a good idea.

Chairman Binns thanked Dr. Morris. The meeting recessed at 12:09 p.m. for lunch.

The meeting reconvened at 1:00 p.m.

**Alterations in cholinergic receptors, cytokines, glucocorticoids, and immunity following low-level exposure to cholinergic agents**

Mohan Sopori, PhD

Senior Scientist and Director, Immunology Program, Lovelace Respiratory Research Institute

Dr. Sopori gave an overview of his team's findings relating to immune changes resulting from exposure to nicotine and to low-level sarin and other cholinergic agents. ([See Appendix – Presentation 6.](#)) He noted that Slide 6 of his presentation should read 10 ng IL-beta, not 100 ng.

Dr. Barlow asked Dr. Sopori to hypothesize as to what would happen if his research animals were given chronic administration of an acetylcholinesterase inhibitor, followed by a sarin challenge. Dr. Sopori stated that if small quantities of sarin was administered over time, followed by a massive dose of sarin, the chances would be that the animals would respond better than if there had been no pre-exposure. Dr. Steele asked about the opposite, i.e., giving them acetylcholinesterase inhibitors, such as PB, and then giving them a small dose of sarin or pesticide. Dr. Barlow stated this might be something that helps one build tolerance before being exposed to sarin. Dr. Sopori stated that this would be a beautiful experiment and could be done with another organophosphate, which was a known acetylcholinesterase inhibitor. He had been considering giving the animals nicotine first, and then seeing what sarin would do. Dr. Barlow stated this might be a cleaner experiment.

Dr. Golomb noted, however, that a potential problem with this approach would be the nicotinic alpha-7 receptors, which are neuroprotective when activated. Dr. Sopori stated that these receptors were protective in the terms of the immune system, but may also be in terms of inflammatory diseases. The effects may be indirect. Dr. Golomb noted that another issue was that low doses of cholinesterase inhibitors might also lead to receptor desensitization. This is probably the main mechanism by which PB protects against soman. Dr. Sopori stated that this was a possibility but that they knew, with regards to nicotine, that the receptor does not form a pentamer and could not be desensitized.

Dr. Steele asked Dr. Sopori to discuss how the sensitization process could potentially relate to the effects on glucocorticoids and HPA axis. She noted that this appeared to be a complicated issue. Dr. Sopori agreed and referenced Dr. Sulie Chang's data showing that the changes in the periphery and brain were similar in an initial lipopolysaccharide (LPS) challenge. If sub-lethal dosages of LPS were continued, one doesn't see any more production of TNF-alpha and IL-beta in the periphery. However, TNF-alpha and IL-beta are still produced in the brain. Dr. Sopori stated it would be nice to see if this was mediated through the HPA axis. He stated that if a ganglionic blocker is repeatedly given for five days, followed by sarin, they find the corticosteroid levels to be low twenty-four hours later and that these effects continue for eight weeks. Everything else returns to normal by that time. He commented that he didn't know why glucocorticoid levels stay at this low level. His team plans to do follow-up work on these questions.

Mr. Joel Graves, a Committee member, noted that soldiers were to take atropine if they were exposed to soman or sarin. He asked if atropine would have been a benefit of one received a low-dose exposure to sarin. Dr. Sopori stated that it would be a good thing because it would stop the peripheral response. Dr. Golomb commented that this would also be true for all of the other adverse effects of overstimulated muscarinic activity.

Dr. Baraniuk, an invited scientist who is with Georgetown University School of Medicine, noted that Dr. Sopori was describing the sympathetic cholinergic system, which was different than the parasympathetic system discussed by Dr. Tracey. Dr. Baraniuk stated that it was critical to note the difference in anatomical distribution of sympathetic and parasympathetic nerves, and that two completely different

neural processes had been discussed by Drs. Tracey and Sopori. Dr. Sopori agreed. He stated that it was known that lymphoid tissues were loaded with sympathetic nerves and these are very close to T cell areas. Dr. Baraniuk asked if these were the same nerves discussed by Dr. Tracey, but just attributed to the sympathetic or parasympathetic system. Dr. Sopori stated that experiments had been performed by others showing that immunosuppression occurs with beta agonists and that there are beta agonist receptors on lymphocytes as well as other leukocytes.

Dr. Baraniuk stated that the identified doses in those studies were not usual human doses. Otherwise, all asthmatics would have problems with immunosuppression. Dr. Sopori clarified that he was talking about effects of nicotine and stressed that cigarette smoke should not be equated with nicotine. He stated that tobacco tea was used as a treatment for asthmatics in China. In 1900-1910 America, asthmatics were asked to smoke cigars. His team is preparing a paper, which contains data showing that animals given chronic low-doses of nicotine don't produce IgE in the serum or in the bronchial alveoli. Dr. Baraniuk stated that, from an immunological perspective, it had been known that cigarette smoking enhances suppressor T cell activity and that discontinuing smoking may result in even worse immunity. Dr. Sopori stated this was correct, and that some had proposed that IL-10 producing cells were damaged, although they hadn't seen that in their experiments.

Dr. Baraniuk asked if high doses of nicotine would prevent a response to a later sarin challenge. Dr. Sopori stated that his research had looked at chronic low-dose nicotine treatments and whether this was beneficial with regards to a later sarin challenge. Dr. Baraniuk asked if smokers had a different outcome than non-smokers in the Gulf War. Dr. Sopori stated that he had been trying to find this answer. Dr. Steele commented that there was some published research on this issue and that epidemiologic studies had shown a small increase in GWI risk related to smoking. She stated that, in analyzing her data from a large, diverse population, she gets a similar low or no association with smoking. However, when she looks only at the subgroup of individuals on the frontlines with higher Gulf War illness rates, smoking is a huge risk factor, regardless of what other exposures one looks at or controls for.

Dr. Golomb commented that cigarette smoking experiments were complicated because several things were occurring at the same time. In relation to the introduction of carbon monoxide, she noted that one of the mechanisms of acetylcholinergic toxicity was a mismatch in cell energy supply and demand. As oxygen is a major source of energy, these other mechanisms may be having different effects from the nicotinic effects taking place and that cigarette smoking wasn't really a clean way to evaluate the issue of nicotine. Dr. Sopori agreed that these two factors could not be equated, but at the same time, that it seemed that smoking could have adverse effects. Dr. Barlow inquired whether GWI was associated with the use of chewing tobacco. Dr. Steele stated that, if these data had been collected in the large studies, it had not been reported in the literature.

Dr. Golomb commented that, in relation to the discussion on whether nicotinic stimulation could be beneficial, the data on cholinergic activation, from a mix of nicotinic and muscarinic experiments, indicated that a number of processes might be expected to be protective and could include down-regulation of some receptor subtypes and up-regulation for others. There also might be reduced receptor binding affinity. Some studies also have shown a reduced number of acetylcholine quanta being released by nerves during episodes of stimulation. Dr. Golomb noted Dr. Hermona Soreq's work, which suggests that exposures can produce an increase in acetylcholinesterase, particularly the variant water-soluble form. Dr. Golomb stated that there were a lot of mechanisms that may be working to counteract the superphysiological exposure to cholinergic agonists and suppress these.

Mr. Anthony Hardie, a Committee member, asked if there were any substances similar to nicotine, as pesticides were similar to sarin. Dr. Golomb commented that there was choline, in terms of nicotinic activation.

Dr. Steele noted that Dr. Sopori's low-level sarin studies indicated that, in the short term, there was an up-regulation of IL-1-beta, TNF-alpha and IL-6. Dr. Sopori stated that after two-to-four weeks this up-regulation disappears and thought it could be tied to the reduced levels of glucocorticoid. However, at eight weeks, they found that the glucocorticoid levels, at least the expression of the cytokine mRNA, were still low. Dr. Steele asked whether the low-level sarin exposure generated a reduction in M1 receptors. Dr. Sopori stated that M1, M3 and M5 were up-regulated, while M2 and M4 were the same or down-regulated. More effects were seen when heat stress was used. Dr. Steele thought that their paper had indicated that the M1 receptors were down-regulated, and even more so with heat stress. This would seem to tie in with what Dr. Tracey was saying about M1 receptors mediating the anti-inflammatory processes. This would make a complete picture in the sense of low-level sarin exposure being associated with a proinflammatory response. Dr. Sopori clarified that M1, M3 and M5 receptors had been up-regulated in the lung and that these receptors were involved in bronchial constriction effects. He added that the newer experiments that Dr. Steele was referring to had reported changes in muscarinic receptors in the brain.

Dr. Golomb noted that, in relation to Mr. Hardie's earlier question, that there were other substances than choline that stimulated nicotinic receptors. These include Epidatamine, which was marketed by Abbott. Dr. Sopori stated that Epidatamine reacts with several different receptors, including alpha-3 and alpha-7 receptors. Dr. Golomb stated that the literature that she had read indicated that Epidatamine had 1000x the pain relief of morphine. Dr. Sopori stated that he didn't think Epidatamine had panned out as a pain reliever clinically because there were too many side effects.

Chairman Binns referred to one of the journal articles distributed to the Committee members (Glezer I, Rivest S. Glucocorticoids: protectors of the brain during innate immune response. *Neuroscientist*. Dec 2005; 10(6): 538-552). He noted that it says "too little" glucocorticoid can be like "too much" in terms of having adverse effects. He asked how this related to Dr. Sopori's finding that glucocorticoid levels were low. Dr. Sopori stated that he wasn't sure whether "too little" equated with "too much" but that reduced levels of glucocorticoids would be proinflammatory, along with other effects.

Because a scheduled speaker, Dr. Mohamed Abou-Donia, was not able to attend the meeting, the next presenter was Dr. Jau-Shyong Hong.

**Role of inflammation in the pathogenesis of neurodegenerative diseases: models, mechanisms, and therapeutic interventions.**

Jau-Shyong Hong, PhD

Head, Neuropharmacology Section, Laboratory of Pharmacology and Chemistry, NIEHS/NIH

Dr. Hong discussed his group's research into mechanisms of inflammation-mediated neurodegeneration. This research includes findings that (1) microglia are the predominant cell type involved in inflammation-mediated neurodegeneration, and (2) activity of the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, also known as phagocytic oxidase (PHOX), is an important step in the development of neurotoxicity caused by environmental toxins and reactive microgliosis. He discussed the use of novel anti-inflammatory therapies for neurodegenerative processes associated with microgliosis. ([See Appendix – Presentation 7.](#))

Dr. Barlow noted the effects of the two forms of naloxone were very intriguing, and asked if naltrexone was also active in Dr. Hong's assay. Dr. Hong indicated that naltrexone and naloxone are structurally similar and both are active. Dr. Morris asked if there were any negative side effects associated with these drugs. Dr. Hong stated that naloxone was considered a safe drug, especially when used in a low dose. He indicated that a recent clinical trial of naltrexone had showed minimal side effects.

Dr. Golomb commented that she had a number of patients who were on chronic morphine for pain control. Based upon her observations, she stated that morphine was an amazingly well-tolerated drug for long-term use for most individuals. She stated that it would be interesting to do a pharmacoepidemiologic study to see if patients on long-term morphine had lower rates of Parkinson's disease.

Dr. Sopori asked if it was known why the substantia nigra neurons were more susceptible to inflammation. Dr. Hong stated that there was a two-fold answer: (1) there are more microglia (5x) in the substantia nigra than other brain regions; and (2) there is also evidence that the substantia nigra microglia are more active than other brain-region microglia. He stated that this raises the question why dopaminergic neurons were more susceptible compared to GABA and serotonergic neurons in that region. He suggested that this may be explained by the fact that the antioxidant capacity of dopaminergic neurons is much lower than other neurons.

Dr. Sopori commented that he was surprised that similar findings were made with respect to astrocytes. He asked if there was a difference in the toll-like receptor 4 (TLR4) expression of microglia and astroglia. Dr. Hong replied that they had found the astroglia to be less sensitive to LPS. They also found that there appeared to be an effect related to timing, that is, that activation of astroglia by LPS was secondary to activation of microglia. Dr. Sopori stated that he was surprised that oxidative responses were primary to the generation of TNF-alpha. Dr. Hong stated that his group had published a paper in 2005 addressing this issue. He suggested that Dr. Sopori may have been considering the CD14-TLR4 dependent pathway, but that there is also a CD14-independent site of LPS action. They have evidence that this is through the maternally-expressed gene 1 (MEG1) receptor and that it produces the superoxide. It is the combination of MEG1 and TLR4 that produces this inflammation. He noted that it was much more difficult to produce inflammation in a MEG1 knockout animal.

Dr. Tomás Guilarte, an invited presenter who is with Johns Hopkins School of Public Health, commented on Dr. Hong's finding that there was a loss of neurons 10 months after a single injection of LPS. Dr. Guilarte asked if they had looked at the microglia, and if so, were they activated at 10 months or earlier and became quiescent. Dr. Hong stated that LPS produced TNF-alpha in the liver, which went to the blood and then the brain. The initial TNF-alpha thus came from the blood. The TNF-alpha then activated the microglia to make more TNF-alpha in the brain. He stated that a few hours after the LPS injection,

they saw a tremendous activation of microglia, not only in the substantia nigra, but also the hippocampus and other regions. He stated that this created a cascade of events, which were self propelling, continuing for 10 months. Dr. Guilarte asked what happened to the microglia at 10 months. Dr. Hong stated that they suspected that the initial microglial activation was very fast and then would plateau. The neuron had already been damaged and, after 10 months, they don't see many active microglia.

Dr. Meggs asked if Dr. Hong had any insight into why dopaminergic neurons are selectively targeted by neurotoxins. Dr. Hong stated that this appeared to be the case in relation to exposure to a pesticide (rotenone), a heavy metal, and an infectious agent, which all seem to damage dopaminergic neurons in the substantia nigra first. One of the reasons could be that the oxidative stress isn't selective for the substantia nigra, but that those neurons are extremely sensitive to oxidative stress. Thus any level of stress, which does not substantially impact other types of neurons, may damage dopaminergic neurons because of their low antioxidant capacity. Dr. Guilarte commented that, besides there being more microglia in the substantia nigra and a poorer antioxidant defense mechanism, dopamine autooxidizes, producing reactive oxygen species, and so can be a stressor itself to the neuron. Dr. Hong agreed and added that dopaminergic neurons have varying degrees of oxidative stress susceptibility, even when they are adjacent to each other. Dr. Guilarte noted that there was a recent paper that shows that potassium channels are associated with differential sensitivity between different areas.

With regard to why dopaminergic neurons in the A9 region may be more susceptible than A10, Dr. Malu Tansey noted that her laboratory had found that TNF receptor levels are very high in A9. A little bit of TNF-alpha will go a long way. With relation to Dr. Hong's slide that showed naloxone inhibits NADPH oxidase, Dr. Tansey asked whether it also inhibits TNF-alpha and IL1-beta or if they are regulated differently? In other words, are the neuroprotective effects due to the blocking of superoxides? Or is it also due to the blocking of proinflammatory cytokine production? Dr. Hong stated that it was probably both and added that they had found, in experiments using different knockout mice strains, that two pathways contribute equally to the levels of TNF-alpha generated.

Dr. O'Callaghan agreed that a little bit of TNF-alpha can go a long way in some regions. He reiterated the importance of previous comments that: (1) there are regional differences in the brain with regard to microglia responses to insults, (2) the vulnerability of the nigral-striatal dopamine pathway, and (3) ischemic damage being worse in TNF-alpha knockouts, as well as IL-6 knockouts. He stated that there were recent data showing a difference in microglial characteristics in different brain regions, independent of the fact that there is a higher concentration of microglia in the substantia nigra. He also indicated that toll receptor knockouts are not protected in studies of the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The brain is different than the periphery, and these differences can be teased out with genetic and pharmacologic manipulations. He commented that it was important to figure out how best to look at effects of dextromethorphan. For example, it might be used in an MPTP model and it might work. But it would be important to determine its effects on superoxide and TNF-alpha to see if they had been suppressed. It doesn't mean anything negative about the compound or its suggested target, but it is important to work out the models. We need to know a lot more about region specific effects on microglia. In the long run, we are concerned about developing therapies, and thus, identifying targets.

Ms. Knox commented that, clinically in Parkinson's disease, selegiline and rasagiline supposedly have neuroprotective properties. She noted that this has only been proven in animal models. She stated that it would be interesting to see if this will work in Phase III trials. Dr. Hong stated that the 1<sup>st</sup> International Congress on Parkinson's disease had been held in March 2006 in Washington, D.C. There were discussions about a new FDA-approved Parkinson's disease drug. Dr. Hong understood that this drug provided a neuroprotective effect but the side effects could be problematic with long-term use. Based on discussions with other scientists, he believed that this drug's dosage could be reduced if combined with

dextromethorphan (DM). He stated that DM was generally well-tolerated. He added that, in light of recent findings, he had taken DM himself.

With regard to rotenone in a Parkinson's disease animal model, Dr. Veronesi asked if the effects required mediation by macrophages or if rotenone might directly interfere with mitochondria because it is a soluble molecule. Dr. Hong stated that it wasn't clear yet, but that due to its solubility, rotenone might directly affect PHOX. His group was currently working on this question.

Dr. Steele thanked Dr. Hong.

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

The meeting reconvened at 3:32 p.m.

### **Peripheral Benzodiazepine Receptor (PBR) imaging of central nervous system inflammation and injury**

Tomás Guilarte, PhD

Professor, Department of Environmental Health Sciences, Johns Hopkins School of Public Health

Dr. Guilarte discussed his laboratory's use of peripheral benzodiazepine receptor (PBR) imaging and quantification to study neurodegeneration and microglia activation in rodent and non-human primate brain models. ([See Appendix – Presentation 8.](#))

Dr. Hong stated that he used to think that PBR imaging was selective for microglia, but that it appears from Dr. Guilarte's presentation, that PBRs can also provide a marker for astroglial activation. Dr. Hong asked if this was correct. Dr. Guilarte replied that this was a topic of some controversy, which may be coming to a resolution. He stated that one has to be very careful with regards to the time point in the progression of the disease, since PBRs are in both microglia or astrocytes. If you look early on in the disease, you will probably see microglia. If you look at the mid-point of the progression of the disease, you may see a mixture. If you look late in the disease process, you see it in astrocytes. It really depends on what time point you look at.

Dr. Hong commented that in primates one can see the basal ganglia in the putamen and striatum very well. He asked whether this was also the case with the substantia nigra. Dr. Guilarte indicated that the resolution of current state-of-the-art positron emission tomography (PET) scanners was in the range of about 5.2 mm. The substantia nigra is a very small structure and is difficult to visualize. However, newer PET scanners are providing improved resolution and that his university had acquired a scanner with a resolution of 2.2 mm. They are able to separate the coda from the foramen. There are only about 4-5 of these scanners in the world right now.

Dr. Veronesi said that it was curious that both the astrocyte and microglia, which have different lineages, would have the same receptor. She asked if this was also true for oligodendrocytes. Dr. Guilarte stated that oligodendrocytes have a low expression of PBRs. He added that that they believe the function of PBRs in microglia and astrocyte are different and that they are using subculture systems to try to understand these differences. They believe the function of PBRs in astrocytes may involve protosynthesis of neural cells. However, the function of PBRs in microglia may be related to those cell's ability to migrate to the sites of injury. While there are commonalities, they believe there are also differences. The studies haven't been done to confirm this hypothesis, though.

Dr. Hong noted that Dr. Guilarte's MPTP tests in primates produced images that showed wide-spread activation of the glial cell. He asked if Dr. Guilarte had any ideas as to why this was the case. Dr. Guilarte stated that they didn't have a good handle on this, except to say that in Parkinson's disease, there is a progression of injury. Parkinson's disease starts in the periphery with the basal ganglia affected later. It is possible that MPTP may be affecting Complex 1 in other cells besides dopaminergic cells and it may be that areas other than the substantia nigra are secondary sites of injury. Dr. Guilarte stated what they do know, however, is that this has been reproduced in Parkinson's disease patients. There is wide-spread activation of microglia. They may not have the answer about the specific mechanisms of damage, but they can document the result.

Dr. Tansey commented that several years ago Roche had given a TNF-decoy receptor drug to multiple sclerosis (MS) patients. Nobody knew why these patients got worse, because it was thought that inflammation was part of MS. It turns out through the work of Jenny King and colleagues using the cuprizone model, they now know that TNF is involved in the remyelination step through the R2 receptor. Microglia express R2 receptors more than other cells because they are immunoprivileged. Dr. Tansey stated that it was interesting that even though one sees microglial activation in the demyelination step, the TNF being produced by those cells also has a positive effect. It is important to be careful about overgeneralizing about microglial activation in reference to disease progression. There are many mediators involved, some of which are helpful. More needs to be known about the biology of microglial activation and what the different markers do. Dr. Guilarte agreed. He stated that, while there were some similarities, *in vivo* microglial responses were different than *in vitro* microglial responses. It isn't as easy to observe *in vivo*. The idea that cytokines are all bad is not the case. Some of these cytokines have beneficial effects.

Dr. O'Callaghan commented that Dr. Tansey's point about multiple markers for microglial activation was important. He stated that in terms of multiple markers for these effects, there are some limitations in sensitivity but that neuroglial biology has improved. For example, glucose transporters as well as glutamate (GLAST) transporters, which are localized in astrocytes, have been identified. Dr. O'Callaghan asked Dr. Guilarte about new ligands for these markers, which might provide a better signal. Dr. Guilarte stated that he had been at Johns Hopkins for the past 30 years and that one of the reasons he has wanted to work there is because Johns Hopkins has one of the best PET-radiochemistry program in the world. As he showed, they can dissect the components of the dopamine synapse. They can also measure *in vivo* dopamine release in the brain. Things become more difficult when one looks at, for example, amino acid receptors, probably because the affinity of the ligands is not as high. Also, some ligands don't cross the blood-brain barrier. Thus, a ligand might work in an *in vitro* environment, but a different result may be found in an *in vivo* experiment. This is a big limitation in these studies. Dr. Guilarte stated that there is a new generation of drugs and radioligands being developed. He noted that he had been interested in the N-methyl-d-aspartate (NMDA) receptor protein for awhile but had not identified a good ligand to use. The ligands with a high affinity don't get through the blood-brain barrier. There are a multitude of limitations before one can generate and use a PET scan as shown in his presentation. Although there has been great progress in ligand development, there is still more work to be done.

Dr. Steele asked if there were any laboratories using imaging techniques to look at microglial activation in the spinal cord. Dr. Guilarte stated that resolution was a problem in terms of *in vivo* studies but that *in vitro* studies had been done. If one hypothesized there is low-level or diffuse microglial activation or cytokine dysregulation in the brain associated with multisymptom illnesses, Dr. Steele asked how large an effect would be needed in order for it to be observed using the imaging techniques described by Dr. Guilarte. Dr. Guilarte said that this is one of the questions they have been trying to address. The approach taken was to use different models, producing different degrees of damage/injury. With the new

PET scanners, they should have better sensitivity. The PET scanners that Dr. Guilarte had previously used in his research typically utilized 30 slices per brain whereas the new PET scanners use 125 slices. Dr. Guilarte indicated that there would still be limitations, however. Dr. Steele commented that if GWI was associated with diffuse neuroinflammation throughout the brain that didn't involve a high concentration in any one location, she had been told by Dr. Banati in Australia that these imaging techniques could be used to quantify the total level of inflammation for comparison to healthy controls. This could be done rather than trying to localize specific inflammation in specific areas. Dr. Guilarte stated that this was correct. In some cases, one may be seeing a global response rather than a specific brain response. However, it still reflects the presence of inflammation.

Dr. Veronesi stated that there was some interest at EPA in using PET scanners as a screening tool. She asked if Dr. Guilarte could approximate how much it would cost, per mouse or rat, to do this. Dr. Guilarte stated that it was expensive and depended on the radioligand used. He stated that they had also used this protein (PBR) in another study as a biomarker of inflammation in the lungs. It worked well because PBR is in neutrophils and macrophages. He mentioned this because they were considering using the PBR ligand, but there was also literature showing that fluorodeoxyglucose ligands were specific for neutrophils in lung inflammation. He estimated the cost for PBR imaging was around \$500-\$700 per synthesis but it was only about \$50 for fluorodeoxyglucose. So there is broad cost range based on the particular radioligand, i.e., how much it costs to synthesize it, and the type of imaging system that is needed. PET scanners are more expensive than single photon emission computed tomography (SPECT).

Dr. Roberta White, an invited scientist who is with the Boston University School of Public Health, commented that this methodology seemed to be very sensitive to brain injury and might have potential for picking up abnormalities in individuals before they show a clinical syndrome. She asked how specific this technique was in determining etiologies or causes of the injury. Dr. Guilarte stated that it was nonspecific with respect to etiology. One is looking at real time indicators of glial activation. It doesn't matter if an individual has Parkinson's disease, a viral infection of the brain, or traumatic brain injury, this technique will reflect this damage. That is why they use this technique, because it is global. From an etiology standpoint it is not that useful. But if the purpose is to detect brain inflammation and injury, it is the best approach to use. He stated that they were interested in using it as a tool to screen the effects of chemicals of unknown neurotoxic potential. They didn't need to know beforehand which parts of the brain the chemical affected. However, due to costs, they have not applied the technique to this type of screening research.

Dr. O'Callaghan stated that just because there is "nothing" shown in a specific condition, it doesn't mean that nothing had occurred. Dr. Guilarte agreed. He commented that, two months after injecting MPTP, there was a massive amount of PBR upregulation. At the end stage, when the animals were in a Parkinsonal state, PBR levels were almost back to normal. He stated that it was very important to note the point in the disease progression when measurements are made. This is one of the benefits of PET scanning, i.e., one can examine changes in an individual over time.

Dr. Mary Nettleman, a Committee member, asked whether anything happened to the peripheral PBRs when the central PBR receptors increased. Dr. Guilarte stated that he had raised this specific question in his first grant, but the study section told him that he was crazy to look at it. He stated that the only problem might be that PBRs may already be at high levels in the periphery. How this reflects central nervous system inflammation is not clear. Dr. Guilarte did not think anybody had looked at this particular question. He stated that some researchers have looked at the levels in platelets and other circulating cells in mood disorders. Changes were found, but in terms of correlating peripheral levels with central levels, no data were available. Dr. Steele commented that Dr. Hong had indicated to her that there was some research looking at expression of PBR in peripheral monocytes, as opposed to other circulating white

blood cells, that may provide a peripheral marker of neuroinflammation. Dr. Hong stated this was an interesting question for discussion. However, with reference to Dr. Guilarte's finding that there was little microglial activation but massive symptoms resembling Parkinson's disease two months after MPTP injection, Dr. Hong stated that his group may have a partial explanation. They have found that when large numbers of microglia are activated, they can resemble neutrophils that regress and die of apoptosis. His group is currently studying this phenomenon. He thinks it is possible that there is a maximum amount of damage to neurons inflicted by microglial activation and that after that point, there are few microglia present at the end stages of this disease. Dr. Guilarte agreed. He stated that his group has the post-mortem brains of these test animals and are working to confirm the *in vivo* findings. They are doing an extensive neuropathological assessment of these brains, looking at microglial immunohistochemistry, glial fibrillary acidic protein (GFAP) immunostaining for astrocytes, PBR immunohistochemistry, colocalization, etc. They have confirmed the changes in the dopamine transporters and vesicular monoamine transporters. They are looking to confirm whether there are immunoreactive microglia as well. Dr. Hong speculated that Dr. Guilarte would probably see less microglia, while the astrogliosis will probably be persistent. Dr. Guilarte stated that they see a lot of glial cells and astrocytes by immunohistochemistry, but very few microglia, post-mortem.

Chairman Binns thanked Dr. Guilarte.

### **Committee Discussion**

Dr. Steele began the discussion by asking the Committee and visiting scientists to provide their general impressions of the idea that Gulf War illnesses may result from persistent CNS inflammatory processes. She stated that the discussion would then address what the major outstanding questions were with regards to this idea and what types of studies were needed to address these questions.

Dr. Klimas stated that there was a lot of research looking at the blood-brain barrier, where there are glial cells that extend into the periphery and where things happening in the periphery can have a lasting effect on the CNS. She stated that she thinks this is an important area to consider, since sometimes these effects outlast the initiating condition. Dr. Golomb commented that this may be important and wondered if it was essential to hypothesize because there are many exposures that will have peripheral consequences that will then be central, even if the original agent doesn't cross the blood-brain barrier. One example is that blood pressure lowering drugs don't need to cross into the CNS to protect against stroke, which is a central consequence. She stated that this might seem obvious, but illustrated her broader general point. If, for example, peripheral cytokines can cross the blood-brain barrier and the chemical alters peripheral cytokines, this in turn can have central consequences. There has been a lot of discussion over the history of the Committee about the blood-brain barrier and factors that affect it, e.g., heat, stress, etc. This issue, along with the one brought up by Dr. Klimas, is important and should be addressed at some point. However, one does not have to hypothesize that the blood brain barrier has been breached in order for there to be important CNS consequences. Dr. Klimas agreed, but stated that, within these models, it takes such a tiny amount of antigen to create an inflammatory response in the brain. But that the antigen doesn't have to be in the brain. This is what is exciting about the concept. There is also a sensitization booster effect, so that one size of "hit" is required the first time, but the second time a lower dose is needed for a bigger "hit."

Chairman Binns asked the Committee and visiting scientists to share their thoughts on this topic. Dr. Barlow stated that the Committee needed to frame all of the fascinating science heard that day into a research question to pose to Dr. Haley's group. This question would focus on the specific experiment, in

a defined animal model, that would address an acetylcholinesterase inhibitor and already available agents, such as morphinins, e.g., naloxone/naltrexone, to see if these would have a beneficial effect.

Dr. Steele stated that she thought there was another question that needed to be answered first, and that is whether it was likely that these processes are going on in ill Gulf War veterans. Dr. Barlow stated that it was her impression that, based on what she has seen and read, this is a reasonable assumption and there is enough evidence to try to do something in the clinic if one could find the appropriate and safe drug and demonstrate a change in the appropriate marker. Dr. Nettleman stated that this is the key missing question, i.e., what is the biomarker. Even though PET scanning is available, a more specific assessment is needed because the therapy should be targeted to the subpopulation that is likely to benefit from it. Treatment assessment could also be done the old-fashioned way, i.e., do people feel better or not. However, she would like to see if this group could target something more specific.

Dr. Steele stated that she had found one clinical trial on DM in fibromyalgia patients. These researchers were able to determine which patients would have the best response to the treatment based on a preliminary screening test. Dr. Hong stated that he wasn't a clinician and wasn't in a position to discuss this type of study. However, in terms of a biomarker, he thought that there was some excellent work done that could be informative. He discussed research looking at circulating lymphocytes in Alzheimer's patients for markers of what is happening in the brain, using genomic and proteomic techniques. One of the advantages is that they are white cells. Dr. Hong is working on a promising collaborative project where they are looking at monocytes in an animal model. Another line of research is in the cardiovascular field, where researchers are using C-reactive protein (CRP) as an index for whole body inflammation. Dr. Hong stated that there was a clinical trial in Taiwan that was investigating the treatment of heavy smokers with DM. They are finding a reduction in the smokers' cough, as well as a reduction in CRP. There is an increase in blood flow, which reduces atherosclerosis. Dr. Hong stated that there could be some general or easy-to-measure chemical that could be used as biomarker for whole body or brain inflammation. Dr. Steele wondered whether, if the inflammation was going on preferentially in the central nervous system, it wouldn't be picked up on the CRP test. Dr. Hong stated that he was talking in general and using this as an analogy. For the brain, the monocyte would probably be used. Dr. Hong also clarified that they had not looked at PBR receptors on monocytes, but a different kind of index of the white cell. However, he wouldn't be surprised if one could see PBR. Dr. Steele stated that the fibromyalgia study she had referenced had looked at this question and found PBR receptors significantly elevated on the monocytes but not other lymphocytes.

Dr. Golomb commented that it was not practical to do PET scans on all patients in a clinical setting. However, if the goal is to have an objective marker and evaluate whether treatment would demonstrate benefit using that marker, this could be done in a research setting and then potentially be applied to treatment more broadly later. Dr. Steele stated that it sounded like Drs. Golomb and Barlow were suggesting that it would be worth moving ahead clinically with this type of study. However, Dr. Steele thought a primary question to be addressed was whether there were differences in microglial and/or astrocyte activation in ill and healthy Gulf War veterans. Dr. Golomb stated that the reality is, as Dr. Barlow suggested, that it might be equally inexpensive to proceed directly to treatment trials. This also has the advantage of determining if there are clinical benefits, even if you are wrong about the mechanisms. Dr. Golomb also agreed with Dr. Nettleman that clinical trials are often expensive. Dr. Golomb noted that, if this is an effect that goes away when you discontinue the drug, cross-over trials are often an extremely efficient way to identify significant effects at a low cost.

Dr. Nettleman commented that, based upon the day's presentations and discussion, she understood that microglial activation was an acute response to an insult to the brain. She noted that it would be similar to finding a fever and that cytokines caused the fever, but missed the fact that there is an infectious agent.

She stated that there was tantalizing evidence that there is some long-term, chronic process happening that may not show up on PET scans after the damage has been done. The most interesting thing to her, in relation to basic science questions, is in the latent phase. She doesn't believe in the perpetual motion machine. There has to be some sort of pathway to cause continued degeneration, whether it be an aging process, failure of repair mechanisms, etc. However, if we could stop the effect or mechanism acutely, perhaps with naloxone or naltrexone, this doesn't mean it will work in the chronic process. She stated that this was a doable experiment before a clinical trial. She stated that clinical trials had been done successfully before based upon symptoms alone. Dr. Barlow agreed, stating that if one could determine what the end point would be in a clinical trial, the question would be what needed to be done first to prove that one would get something in an animal model. She stated that these two approaches could be done simultaneously so one could design the best experiment in the clinic. Dr. Clauw asked what animal model would be used for Gulf War illness. Dr. Barlow stated that this was simply her general impression of how to approach the problem and that the details would need to be worked out.

Dr. Guilarte stated that there doesn't appear to be an animal model, nor do we know what is going on in the CNS. There are just symptoms. Thus, how can one treat? It is like giving antibiotics to someone who has a cold. One must have some idea of what is happening in the central and/or peripheral nervous system. Dr. Golomb disagreed with this and stated that the response to treatment often drives our understanding of the basic biology. She noted that often the hypothesized mechanism is wrong when treatment is first proposed. She noted that it was originally thought that statin drugs worked via inhibition of the mevalonate pathway. However, it now appears that the effects relates more to low-density lipoprotein (LDL) receptor downregulation, antiinflammation and antioxidant effects. There are a variety of drugs that have been discovered by accident and for which the mechanism is still not known, e.g., colchicine for familial Mediterranean fever. If it helps the patient, it should be used. Based upon what she had heard during the day's presentations and discussion, she stated that there appeared to be a relatively safe-sounding drug, i.e., DM, that might be readily tested in a small group in a cross-over trial design. If the results were negative, it wouldn't be proof that one should stop there. But if it showed a significant positive signal, it would be an indication that further research should progress.

Dr. Hugh Tilson voiced a note of caution, stating that empirical experimentation was part of the grand tradition of bad medicine, as well as good. Dr. Golomb agreed. Dr. Tilson further noted that "n of 1" self-experiments and conclusions that a drug must be safe because the investigator took it were also part of the scandal of American pharmaceutical development. If there are potential treatments that might work or if someone makes an unsubstantiated claim, the right thing to do would be a proper study. Dr. Golomb stated that is what she was recommending. She stated that she wasn't recommending that the Committee accept Dr. Hong's personal experience with DM and generalize it to all Gulf War veterans. Dr. Tilson stated that even then it was very risky to experiment with human subjects until more was known about the intervention. He stated it wasn't appropriate to say that something is safe, when it hasn't been properly tested for long-term use. The right thing to do, if there is any promise here, is a proper course of early investigations. However, he indicated that it wasn't clear that there was a theoretical link to propose even this. He reiterated that extreme caution must be taken when contemplating treating humans with bioactive substances for a hypothesis that has yet to be posited. He stated that he had nothing against empirical medicine, but that one could not make assumptions about safety. Dr. Guilarte commented that these were the concerns that he had been trying to convey.

Dr. Clauw voiced concern about being on "a slippery slope." He stated that he had enjoyed the basic science presentations given during the day, and found it to be terrific science about the role of glial cells, etc. However, there is a huge disconnect between this research and the clinical picture of Gulf War illness. He thought it was relatively easy to create an agenda that leads people in one direction. He understood that in this situation, this had been done to provide a hypothesis that the Committee would

discuss. However, at the end of the day, the Committee needs to step back and examine whether there is real evidence that Gulf War illness is a neurodegenerative disease. He stated that only a handful of the thousands of clinicians who had been treating Gulf War veterans might think this. Dr. Steele interjected that the purpose of the day's presentations was not to present Gulf War illness as a neurodegenerative disease. Dr. Clauw noted that a number of individuals had stated during the meeting that Gulf War illness was neurodegenerative and inflammatory. He believed there were serious gaps in knowledge about whether this is either a neurodegenerative disease or inflammatory disease. Dr. Steele stated that the purpose of the day's presentations was to take information from research in other areas that may be pertinent to GWI. The assumption is not that GWI is the same thing as the other conditions discussed, e.g. traumatic injury, neurodegenerative diseases, or cytokine infusions. The idea was that these different realms can inform us about what might be going on in Gulf War veterans and lead research in a direction that may help identify markers and treatments. She reiterated that she wasn't saying Gulf War illness is the same as early Parkinson's Disease, for example. Clearly, individuals with Parkinson's disease don't have Gulf War syndrome before they develop tremors and the symptom profiles are not the same. Dr. Clauw commented that the day's presentations, however, had focused on animal models for Parkinson's disease and Alzheimer's disease. Dr. Steele stated that this information was presented to provide information on understanding and studying neuro-inflammatory processes that persist chronically. In the case of GWI, this might involve neuronal cell death that then feeds back to microglial activation in a cycle of a persistent inflammation. Alternatively, other models suggested that the process might not require neuronal cell death, relying on persistent stimulation of microglia and neuroinflammatory processes that were unrelated to neuronal cell death.

Dr. Clauw stated that, at the end of the day, one needed to go back and say whether there is evidence in the Gulf War illness patients that they have a disease like Alzheimer's disease, etc. Dr. Steele said she did not agree with the comment "like Alzheimer's" and referenced studies discussed earlier in which : (1) Hepatitis C patients, who were infused with interferon, developed chronic fatigue syndrome, or (2) test animals developed widespread chronic pain after a localized injury. She stated that the mechanism for this was the sensitization of the glial cells. Dr. Clauw replied that he was a pain doctor and that he respected Linda Watkin's work and agreed that glial cells were playing a role in that animal model of pain. However, this was not the case more globally in all pain conditions. Dr. Steele asked if Dr. Clauw was familiar with Dr. Schwartzman's study showing elevated levels of proinflammatory cytokines in the spinal fluid of patients with complex regional pain syndrome. She stated that she had been puzzled about why these types of CNS processes had not been examined more carefully in studies of multisymptom illness patients. Dr. Clauw stated that they had, but these studies often didn't get published in a journal because they had negative results. He stated that they had looked at a number of cytokines in the cerebrospinal fluid samples they had and didn't find anything. Just as in the peripheral blood tests, they didn't find any indication of inflammatory disease in Gulf War illness or fibromyalgia. Dr. Steele noted that the hypothesis wasn't that it was in the periphery, but rather the central nervous system. She asked if he had looked in the CNS in any way. Dr. Clauw stated that they could look more aggressively because they had spinal fluid samples. This was simple enough to do.

Dr. Clauw stated that if one were considering the things that could be tested in randomized, controlled trials in this spectrum of illness, there is a huge universe of drugs that are known to work in individuals with fibromyalgia, chronic fatigue syndrome, irritable bowel syndrome and other illnesses that clearly occur at increased frequency in Gulf War veterans. However, it was a large leap to consider entirely different categories of disease, like neurodegenerative and inflammatory diseases, and use these to inform as to what should be done for the clinical condition of Gulf War veterans. Dr. Steele stated that some believed that this was a reasonable hypothesis, and may answer more questions than might be addressed by consideration of, for example, serotonin dysregulation. She stated that a central inflammatory process could tie together a number of isolated pieces of this puzzle.

Dr. Golomb referenced Dr. Tracey's presentation about cholinergic dysregulation associated with particular exposures and evidence of a central muscarinic relationship to peripheral immune indicators, which could be tested as suggested markers. By definition a cross-over study means that you can't be doing long-term therapy. One is looking at short-term trials of drugs that have good safety records in clinical practice, e.g. DM, naloxone, naltrexone. She stated that she, personally, didn't believe that these drugs would be highly effective. However, she believed that the harm side of the equation was relatively modest and there was a substantial potential that something interesting could emerge.

Dr. Meggs stated that the question seems to be whether it is a reasonable hypothesis that low-grade chronic brain inflammation plays a role in Gulf War illnesses, particularly in those with neurocognitive problems. Could this hypothesis be tested by looking at the peripheral benzodiazepine receptors in PET scans in veterans with neurocognitive problems? It seemed possible and very similar to the situation raised by Dr. Soreq's hypothesis about two variants of acetylcholine receptors. This is something that can be quickly and easily testable. It didn't pan out in Dr. Soreq's case, but it could have been a major breakthrough. This avenue of research could also be a major breakthrough if this is what is going on in these patients.

Dr. Haley agreed. He thought this should be tested first. He reminded members of the "big green bubble" from his presentation in May 2006. Ill Gulf War veterans probably have lots of things, and there could be some "bubbles" in there that might be neuroinflammation. The problem with the trials conducted in the last decade is that they had to be huge trials to evidence an effect. Most effects would have been washed out by all the veterans included as GWI "cases" who didn't have the disease. He stated that the approach that should be taken is: (1) determine what types of clinical profiles are positive with PBR marker, (2) select a group of people who are positive for this marker, and (3) conduct a short-term cross-over trial with dextromethorphan, etc.

Dr. Guilarte stated that it seemed to him, whether it is PBR, etc., there were a lot of studies that could be done with educated hypotheses. One could look at the function of the neurotransmitters that are thought to be dysregulated using radioimaging techniques, e.g. behavioral manifestations of Parkinson's disease in magnesium-exposed primates and lack of dopamine release. He stated that there didn't have to be neurological degeneration to have dysfunction. He didn't think these questions had been addressed with PET, SPECT, or other imaging modality in ill Gulf War veterans. If educated hypotheses were made as to what was happening in these veterans, e.g. what systems should be examined, the technology and methodology is available to look at it and get a definitive answer as to whether the cholinergic system, serotonergic system, glial cells, etc., is/are involved. He would propose that this approach be taken. He commented that he, personally, wasn't looking for money to do these studies since he was already committed to multiple projects. However, he believed that one needed to take the available information, make educated hypotheses, and then test the hypotheses.

Dr. Veronesi stated that she agreed with the day's discussion, i.e., maybe it doesn't have to be one thing or another. We understand that we have varying profiles of cytokines, but nobody is mentioning polymorphisms. Someone else mentioned that if you do a lateral study, you see these disparate profiles mimic each other. Maybe we are looking at groups whose symptoms are moving in tandem. This needs to be addressed. She went on to say that the "black hole" is the neuro-immuno-endocrine interaction driving the symptoms. Neurodegeneration is the most drastic endpoint. Many of the things happening earlier, for example when an individual becomes middle-aged, can be addressed by addressing different components of neuro/immuno-dysfunction. It is a relatively new field, but we do have access to some of the experts and have heard some of the end points they consider important. She stated that it shouldn't be viewed as "either/or" and that it is worthwhile to take advantage of this expertise.

Mr. Hardie commented that chemical sensitivity had not been discussed that day, and wondered if there might be some tie-in. He noted that, early on, veterans were experiencing fatigue, pain, etc. when exposed to various chemicals, e.g., paints, pesticides, perfumes, etc. He wondered if the differences might be that there are different variables being introduced as individual Gulf War veterans are being exposed to these chemicals. Dr. Meggs stated that we don't know the brain mechanism by which people get cognitive dysfunction from chemical sensitivity. The science is very good in the airway, and it may be related. However, this definitely does need to be studied, as many veterans report chemical sensitivities and their exposures wax and wane as they are exposed to common chemical inhalants.

### **Public Comment – Day 1**

Chairman Binns invited the public to ask questions or make specific comments about the day's scientific presentations. He asked that these be kept brief.

Ms. Alison Johnson commented that dextromethorphan had been suggested as a treatment for multiple chemical sensitivity ten years ago. She stated that there were rather negative outcomes in those who tried it, e.g. patients becoming manic. She stated that she would be glad to share more about these findings.

Ms. Denise Nichols commented that Gulf War veterans wanted answers, effective diagnostic testing and some type of treatment. She warned that the Committee should avoid in-fighting among disciplines. She stated that she was seeing some of this during the day's discussion, and this would not help Gulf War veterans. She stated that the veterans did not want to be researched until they died. She added that the PET scan option might be worth looking at. She commented that the veterans studied in San Francisco with Dr. Weimer haven't had nuclear magnetic resonance imaging spectroscopy (NMRIS). She reiterated that we need to move forward and asked that more be done to produce results for Gulf War veterans.

Chairman Binns stated that there would be time provided for general public comments on the following day. He thanked everyone for their participation. He stated that he was sorry to have to abbreviate this discussion, because it was an important one. He noted that while there were some divergent ideas, there was still a lot of common ground that was worthy of pursuit.

The meeting recessed at 5:16 p.m. for the day

## Day 2

The meeting reconvened on Tuesday, August 15, 2006, at 8:31 a.m. in Room 230, VA Headquarters, 810 Vermont, N.W., Washington, DC. Dr. Barlow was not present for the second day of the meeting.

Chairman Binns stated that some Committee members and visiting scientists would have to leave early and asked those individuals to comment on the previous day's presentations and/or provide suggestions as to what research questions or studies should be pursued in relation to the information discussed.

Dr. White stated that the previous day's presentations had been interesting; noting the one on heart rate variability and sarin was congruent with much of the literature on toxicant and particulate exposures. She stated that, in terms of one single theory or biomarker, which she believed was the ultimate goal, we are still some distance from achieving this. She thought that some of the discussion may lead to studies that could identify sensitive objective markers of disease, but would not necessarily be specific to the Gulf War situation. However, having some objective outcomes would really help in our ability to document that something happened and that Gulf War veterans should be taken seriously, which seems to have been a battle since 1992. In terms of the inflammatory response idea, she thought that it was part of the picture related to neurotoxicants. She stated that the more that is known about effects of exposures, the more that will be known about what all these things mean. As an environmental health researcher, she tended to focus on exposure-outcome relationships. She thought the previous day's presentations may have provided insights regarding more markers to look at, along with another way to look at mechanisms in the broader picture of what happened. She thought the previous day's presentations were nicely given and cogent.

Dr. Hong asked to clarify his presentation concerning DM. He stated that there was a considerable amount of research being done on DM in Taiwan. He had been going back to Taiwan for the past few years for extended times to work with his colleagues there. He had become interested in this as a result of the naltrexone findings, which is a safe and prescribed drug in the United States. He believed that DM may be as good, if not better, than naltrexone. Three to four years ago, some physicians in Taiwan began to use DM off label for fibromyalgia, Parkinson's, etc. Two years ago, a multi-center, double-blind clinical trial was initiated involving multiple conditions. They just broke the code for two of the groups, one of which contained the heavy smokers. When they looked at the data, it was clear-cut that DM benefited the smokers. There is also an interest in treating alcoholics with naltrexone and DM. A by-product of the use of these drugs appears to be improved liver function. Chairman Binns asked what other diseases were the subject of clinical trials. Dr. Hong stated that Parkinson's disease was the one of greatest interest for his group. He also indicated that depression, schizophrenia, arthritis, multiple sclerosis, fibromyalgia, liver function, and carotid artery stenosis were of interest, since these conditions were inflammation-related.

Dr. Clauw clarified that DM was an NMDA antagonist. It is the only NMDA antagonist being used clinically because the more potent NMDA antagonists are too toxic to be used in humans. He stated it was very dangerous to infer that improvement while receiving DM is due to the treatment of an underlying inflammatory disease. Several of the diseases listed by Dr. Hong may also involve an excitatory amino acid component, especially in the pain component of the disease. Dr. Hong stated that this was a major interest, and that they had a project studying the NMDA/anti-inflammatory effect. He added that if he had been asked this question two years ago, he would have agreed with the point made by Dr. Clauw. However, he had done additional work with colleagues who had found that the anti-inflammatory effects were unrelated to the NMDA receptor. These findings will be published soon. Dr. Clauw disputed this by stating that it had been clearly established that the beneficial effect of DM in fibromyalgia was due to NMDA receptor antagonism. He referenced studies where ketamine infusions

were given. Ketamine is only a NMDA antagonist, not an anti-inflammatory drug. Through these studies, researchers were able to predict which individuals obtained an analgesic effect with DM. Chairman Binns asked that further discussion of these details be put on hold, in the interest of time. Dr. Hong asked to clarify one point, though, and reiterated that, with respect to his group's LPS and Parkinson's disease research, they were looking at the most extreme endpoint of neurodegeneration.

Chairman Binns then introduced Lord Alfred Morris of Manchester. Chairman Binns noted that Lord Morris had served 43 years in the British Parliament: 33 years in the House of Commons and 10 years in the House of Lords. Lord Morris has been a minister in several British governments, including Minister for War Pensions and the first Minister for the Disabled. He authored much of the basic legislation involving disability in the United Kingdom (UK), and given that record he was recently awarded the prestigious Kessler Award for inspired leadership and services to disabled people around the world. This award is conferred by the Rehabilitation International Foundation, which represents disability organizations in 124 countries. Chairman Binns further noted that Lord Morris was also known as an active supporter of veterans. Lord Morris has been an advisor to the British Royal Legion since 1985 and has been one of the most vocal supporters of ill UK Gulf War veterans since the war.

Lord Morris thanked Chairman Binns and expressed his delight to join the Committee, which he stated was held in the highest regard by British veterans of 1990-1991 Gulf conflict. He noted the Committee's 2004 findings, and stated that these were widely quoted with approval and strongly influential in the continued debate on the still unresolved problems of Gulf veterans in broken health. His interest and involvement in this area has been informed by a long parliamentary experience. He joked, that as the third or fourth most senior member of Parliament, he would deduce that he was beginning to get the hang of it. He went on to state that his involvement in this issue was informed by his work for four years as chairman of the highly prestigious Parliamentary and Scientific Committee at Westminster, as well as his work with the inter-parliamentary Gulf War committee, set up by the British Royal Legion, which is the UK sister organization of the American Legion. The group's principle concern is to focus parliamentary and public opinion on the urgency of the need now, fifteen years after the conflict, for closure of the fallacy that has been going on for way too long. The Gulf War group's membership is comprised of parliamentarians from the UK's main political parties, distinguished medical specialists and surgeons, representatives from the principal ex-service charities, as well as service men and women who fought in the Gulf War. This group is administered by Ms. Sue Freeth, who is Director of Welfare for the British Royal Legion. Lord Morris noted that Ms. Freeth was in the audience. He went on to say that representatives of the inter-parliamentary group had visited the U.S. twice for briefings by government departments, including the Department of Defense (DoD) and VA. Lord Morris' participation in the Congressional oversight hearings on Gulf War veterans' illnesses had provided further opportunities for the group to engage in dialogue with the U.S. Congress, including hearings to access the status of research into and the treatment of Gulf War veterans' illnesses in the U.S.

Lord Morris commented that the Gulf War was the biggest conflict faced by UK troops since the Korean War. It was the also the first time since 1918 that war was waged against an enemy known to have chemical weapons readily available for deployment. The Ministry of Defence (MoD) had prepared for the conflict on the assumption that these weapons would be used. In fact, millions of people in Britain and the United States had seen televised reports of Saddam Hussein's startling use of chemical weapons. These weapons were used against their own subjects in Hallabja and then against the civilian population in Iran in 1990, only months before Hussein's invasion of Kuwait. In light of the range of weapons facing the troops deploying to the Gulf, both the MoD and DoD gave high priority to do all they could to safeguard the troops against the effects of the use of these weapons. Steps taken by the MoD correctly assessed the threat faced by British troops, but not all the health risks of the measures taken to protect from those threats, which are now more fully understood. There is still much more to do, but the pace of

progress is much quicker than before. It is for parliamentarians in both the US and UK to do all they can to support those who work on the front line.

Lord Morris stated that he had been asked on several occasions the question “What do British Gulf veterans want most?” Is it better pensions? More and better medical care? The answer is that what they want most of all is to have their illnesses recognized for what they are – Gulf War-related illnesses. These veterans are often told that it is all in their minds. The implications of this are that these veterans are malingering, were not great heroes in 1991, and were now seeking help that wasn’t theirs by right. British Gulf veterans simply want the truth told about their suffering since the conflict. The MoD has constantly said there was no use talking about Gulf War syndrome, because Gulf War syndrome isn’t a discrete, pathological entity. Lord Morris stated, however, that nobody ever said it was, noting that a nephritic syndrome is comprised of over 40 pathological entities. Lord Morris stated that there are endless propositions raised whenever there have been important developments in research into the health problems of Gulf War veterans. He noted that MoD published the mortality figures more often than they published details of their engagements. Lord Morris noted that advocates had never said that far more people have died because of the Gulf War. However, what has been stated and can be documented is that there is a great deal of suffering and a most urgent need for more research of the kind that is discussed by the Committee.

Lord Morris commented that he had been the architect of the Lloyd Report. Having failed to persuade the executive government in Britain that there should be an independent public inquiry, he decided to support an independent nongovernmental inquiry. He did this on behalf of a vast range of opinion in the UK, lead by the British Royal Legion. The Royal British Legion had repeatedly asked for an independent public inquiry and was told that there had been in-house inquiries, which, according to Lord Morris, have the nasty habit of finding in favor of the government. They were asking for an independent inquiry, at the highest judicial level, into the problems and needs of Gulf War veterans. Lord Lloyd of Berwick, who is the most senior former high court judge in the UK, akin to a justice of the U.S. Supreme Court, agreed to head this inquiry with the condition that he would not be paid one single cent. He was helped by Dr. Norman Jones, one of the UK’s most distinguished physicians, and Sir Michael Davies, who just retired as Clerk of Parliaments, the most senior official of British Parliament. They had the strict understanding that their participation was voluntary. Lord Morris recommended that Committee members read the Lloyd Report if they hadn’t already. The report stated that if it is accepted that Gulf War syndrome is the best way forward, it can’t be argued that the term shouldn’t be used. If a child asks what is wrong with his father or why he died, it would not mean much to tell him that he had died of symptoms and signs and ill-defined conditions. It is surely better to tell him that his father died of Gulf War syndrome. Lord Morris concluded by saying that there was no more compelling priority then to act justly to those citizens who are prepared to lay down their lives for their country. He noted that there was no delay in the response of the UK or US troops to report to duty in 1990-1991, and nor should there be any delay in discharging in full our debt of honor to them.

Chairman Binns thanked Lord Morris.

Chairman Binns introduced Dr. Michael Kussman, the newly-appointed VA Under Secretary for Health. Dr. Kussman is a board-certified internist and served as Chief of Internal Medicine at Tripler Army Medical Center in Honolulu and as Chief of Medicine at Brook Army Medical Center in San Antonio. Dr. Kussman is a veteran, rising to the rank of Brigadier General and the position of Commander of Walter Reed Health Care System. Prior to his new position at VA, he served as Deputy Under Secretary for Health and Principal Deputy Under Secretary for Health.

Dr. Kussman thanked Chairman Binns and stated it was a privilege to address the Committee on the important work it does. He noted that Secretary Nicholson would have been there, but he had just left for Iraq and Landstuhl Medical Center in Germany, following the path of the evacuation of the service members in Operation Iraqi Freedom (OIF). He passed on the Secretary's appreciation for the group, its work, perspectives and suggestions. While he did not serve in the Gulf War, he has been to the theater twice. As he retired from the service in 2000 and then joined the VA, he stated that he has watched this puzzle unfold. From a personal perspective, as a retiree and veteran, he thanked the Committee for what it was doing. Under Dr. Joel Kupersmith's, VA Chief Research and Development Officer (CRADO), and Dr. Joe Francis's, Deputy CRADO, leadership, Dr. Kussman hoped that there would continue to be a positive relationship with the Committee as time moves forward, with the VA taking the Committee's advice and molding it into actions that will improve Gulf War research.

Dr. Kussman presented certificates of appointment to Mr. Hardie and to Drs. Nettleman, Tilson, Clauw and O'Callaghan for their service on the Committee.

Dr. Kussman offered to take any questions. Dr. Klimas asked about the future of funding for Gulf War research at VA. Dr. Kussman stated that VA was working with Congress and was committed to allocating 15 million dollars to the University of Texas Southwestern Medical School and that this funding would be ongoing. He stated that one of the questions he is frequently asked is: "What about other research?" It was his understanding that there was 13 million additional dollars committed in fiscal year (FY) 2006. He didn't know what the budget would be in FY2007, but the intent was to continue this funding as best they could.

Dr. Baraniuk asked Dr. Kussman to outline the scope of his job and the scope of issues that he deals with other than Gulf War illnesses. Dr. Kussman stated that when he retired from the service, he still wanted to serve and was happy that VA offered him a position. He noted that VA was the second largest government agency with an annual budget of 35 billion dollars. His job is to orchestrate the spending of this 35 billion dollars, as well as take care of 200,000 employees running the largest integrated health system in the U.S. The volume of paperwork is overwhelming, and in an organization of 200,000, there are a lot of stories on a daily basis. One of his frustrations with the job are the demands of being reactive. It is hard to be proactive when you are "swatting at alligators" all day. VA is the premier health care delivery system in the country, but isn't perfect. A balance is required to make sure the people in the trenches who are giving care to veterans have the resources and money to do their job, without worrying whether policy, standards and procedures are in place. He noted that the organization was very politicized, with 535 board of directors on the east end of Pennsylvania Avenue. Everybody in Congress has some presence and influence on what VA does. Issues that he deals with from the current war include seamless transition and patients with post traumatic stress disorder (PTSD) and complicated and horrific wounds, including traumatic brain injuries. He discussed the level of care given in the field and noted that if a soldier survived the initial onslaught, he or she has a 98.7% survival rate. He went on to discuss the budgetary challenges, making sure that there is enough money for research, working with graduate medical education affiliates, etc.

Dr. Steele asked if Dr. Kussman had personally interacted with veterans affected by Gulf War illness. Dr. Kussman stated that he had and that it was something that had always concerned him. The challenge is to figure out what is going on by thinking outside of the box.

Chairman Binns noted that the Committee was focused on providing research advice, but as many veterans had been saying over the years, the Committee is the only forum that exists for general comments and complaints. He noted that Dr. Kussman was in a position to oversee the clinical side of VA, and thus he wished to bring concerns about the VA's clinical guidelines to Dr. Kussman's attention.

Chairman Binns stated that the VA guidelines for treating Gulf War veterans were based on research that is 5-6 years out of date. Requests had been repeatedly made to revise the guidelines in light of new and emerging research. Dr. Kussman stated that if there was a better way, they certainly would want to do this. He would be more than happy to work with the Committee if it had suggestions as to how to fix the guidelines.

Dr. Haley stated that Dr. Perlin's administration, which had included Drs. Kussman, Goldberg, Kupersmith, and others, had evidenced a tremendous improvement in communication and support. He expressed his appreciation for this and looked forward to it continuing.

Chairman Binns thanked Dr. Kussman.

### **Gulf War illnesses and chronic fatigue syndrome comparative studies – The Miami experience**

Nancy Klimas, MD

Professor, University of Miami Miller School of Medicine

Director, Chronic Fatigue Syndrome and Gulf War Illnesses Center, Miami VA Medical Center

Dr. Klimas discussed her ongoing genomic and immune function studies of Gulf War veterans and chronic fatigue syndrome patients. ([See Appendix – Presentation 9.](#)) Her presentation included preliminary information from an ongoing data collection that are not included in the appendix, pending finalization of the study and analyses.

During the discussion of natural killer (NK) cell activity in Gulf War veterans pre- and post-exercise, Dr. Golomb asked what happened to the function of these cells, e.g., whether the activity normalized, went supernormal, etc. Dr. Klimas stated that the function stayed low.

Dr. Haley asked what was known about the physiology of the NK cell, noting that with exercise one normally gets an increase in white cell count. Dr. Klimas stated that NK cells were the most primitive and most CNS-responsive cell. She noted that NK cells phylogenetically evolved with dinosaurs and were the only immune system that a shark had. She stated that NK cells were hardwired and softwired to the fight-or-flight response and were really autonomically sensitive. She went on to say that she was not sure why they would shift compartments but had been assuming that it is an autonomic message. Dr. Haley asked where the NK cells were coming from. Dr. Klimas stated that this was a good question that she probably couldn't answer very well, but could say that 5% of an individual's cells circulate, while 95% are within the spleen and lymph system. The second largest lymphatic distribution is in the gut, with the spleen being the largest. Dr. Haley asked about the length of exercise intervals. Dr. Klimas replied that it was about 30 minutes, depending on the patient's performance.

Dr. Clauw commented that a lot of this effect was probably due to catecholamines, to which NK cells are extremely sensitive. Dr. Klimas stated that the question then is why do they change compartments in response to exercise. Dr. Clauw stated that this was because the NK cells demarginate. He had been part of a study eight or ten years before that involved infusing epinephrine into fibromyalgia patients with low NK cells. They showed a very clear dose-response, with an increase in NK cell number that went up transiently when epinephrine was infused. He indicated that the response was very linear and that he didn't find Dr. Klimas's results surprising.

Dr. Klimas stated that they would continue these studies, but that the bigger question related to the physiological effects. She wondered why an active cell line would be released into the periphery post-exercise in patients with an illness commonly associated with exercise relapse. She commented that it

raised autonomic-immune interaction questions, which are important, and that the inflammatory autonomic interchange is a critical component to understanding the mediators of this illness. Dr. Golomb commented that they were looking at cell lines that have variable activity in these populations, raising questions about what the net effect might be. We don't know if this is part of exercise that is ultimately beneficial or harmful. Dr. Klimas agreed, and stated it might be that NK cells are a flag of everything else going on, which is what she suspects.

Dr. Sopori noted that the NK cell activity standard deviations appeared quite high. Dr. Klimas said that those were ranges, not standard deviations. He asked if the differences were statistically significant and Dr. Klimas indicated that they were highly significant. With regards to the number of individuals in the study, Dr. Klimas stated that there were 18 Gulf War patients, 36 chronic fatigue syndrome patients and approximately 90 controls. In relation to an earlier question, Dr. Sopori stated that an increase of NK cells comes from the bone marrow in response to cytokines. He asked if Dr. Klimas thought it was possible that, because there would be differences in the cytokine milieu in patients, that NK cells are being thrown out of the bone marrow during exercise instead of coming from the other areas she referenced. Dr. Klimas stated that this was possible, and it shouldn't be hard to track. Bone marrow is home to more naïve cells, i.e., not a mature cell line. Dr. Sopori noted that they can also mature outside, but many times they mature within the bone marrow right before they are released. Dr. Klimas stated that she would not know, then, how to sort that out. Dr. Sopori asked whether they saw perforin in the T cell compartment, because the cytotoxic T cells should also have the same low perforin. Dr. Klimas said that she had presented data on this the previous day in her chronic fatigue presentation, and the cytotoxic T cells had the same low perforin. She commented that they are still in the midst of doing these analyses.

Dr. Morris said that she found Dr. Klimas' findings interesting, and wondered if she had looked at the effects of stress or had more information on autonomic changes, e.g., related to heart rate. Dr. Klimas stated that they were measuring all of this and that is why there had been an exercise component to the study. For CFS, she had done an autonomic study with tilt table testing and have beautiful data from this study. One of things they looked at was the cytokine cascade, post-tilt table. The results were very interesting and there were definitely differences between controls and the chronic fatigue group. There was release of proinflammatory cytokines, but differences related more to timing of when the cytokines were released. It was very hard to capture because the blood draws had to be timed just right. They used exercise challenge in the present study because it was a more traditional look at something known to relapse, and they wanted to look at mediators of relapse.

Mr. Graves noted that one of Dr. Klimas' first slides stated that they were recruiting deployed, healthy Gulf War veterans. Dr. Klimas stated this was the case. They were mixed into the control population. In the final analyses they will have the deployed/healthy, deployed/sick and chronic fatigue groups matched.

Dr. Haley asked if the chronic fatigue syndrome patients were veterans. Dr. Klimas stated that they were a "mixed bag." Dr. Haley asked if there were lots of veterans with chronic fatigue syndrome that were not Gulf War veterans. He asked because, in VA studies, there is an implied obligation that veterans be recruited. Dr. Klimas stated that she tried to recruit every Gulf War veteran that she could. Dr. Haley said it sounds like it might have been difficult to find veterans with chronic fatigue that were not Gulf War veterans. She said that there was somewhat of a bias because chronic fatigue syndrome patients seek her out. While she now has a VA chronic fatigue syndrome clinic, she didn't think many veterans knew about it yet.

Chairman Binns thanked Dr. Klimas.

**Public Comment – Day 2**

Chairman Binns noted that Dr. Nass and Ret. Capt. Julia Dykman had signed up to provide public comments to the Committee. He asked the individuals who had not spoken the previous day to limit their comments to four minutes. Those who had spoken previously were asked to limit their comments to two minutes. Dr. Nass indicated that she did not wish to provide comment.

Ret. Capt. Julia Dykman spoke to the Committee. She stated she had served in Vietnam and the Gulf War as a nurse. She had been stationed at Al Jubayl, Saudi Arabia, and was in charge of the clinic and casualty area. She developed problems with open sores while in the Gulf. Since then, she had been diagnosed as having autonomic dysfunction through Dr. Goldman's National Institutes of Health (NIH) study. She was pleased to hear all of the information on autonomic dysfunction because there was not much out there, even in the civilian population. She wondered if they were still getting Gulf War veterans sixteen years after the war. She stated that access to VA originally required veterans to have symptoms within two years of coming back from the Gulf. She noted that there were few places to be tested for autonomic dysfunction unless one went to a research center and that it was difficult to access studies through VA. She stated that they had a Gulf War-assigned doctor at her VA, who works a half-day, two times a month. She didn't think anyone was looking at newly-diagnosed Gulf War veterans, because they could not access the system. It was good to see all these studies, but veterans needed to know how to get into these studies. She stated that autonomic dysfunction seemed to be present in a lot of conditions and was a progressive thing for Gulf War veterans and wondered whether researchers were really seeing the Gulf War population with autonomic dysfunction.

Ms. Denise Nichols spoke to the Committee. She noted that the interest level during the previous day's presentation was very good. She reiterated that Gulf War veterans were still waiting for nuclear magnetic resonance (NMR), SPECT, and other diagnostic tests. She stated that these veterans were being lost, because they were giving up and going quiet, and that some were dying in car accidents, etc. She thanked Chairman Binns for bringing up the clinical guidelines and agreed that they need to be updated. However, where is this going? What benefit will the veteran see? She encouraged the Committee to put out an invitation to VA doctors that deal with Gulf War veterans to come forward with any clinical findings that they may have. Finally, she noted that there was a lot of video equipment in the room and asked why the Committee meeting was not being broadcast on CSPAN. She stated that the veterans and doctors out there could tune in, and that she had previously brought up the idea of video conferencing. She thought it was a waste to not get this information out to the public and researchers at large.

Ms. Alison Johnson spoke to the Committee. She noted Dr. Klimas' comments about not being able to find certain study participants whose addresses had changed. She stated that in her 30 years of work with chemically sensitive people, the individuals who drift or "lose their addresses" are the sickest. She asked that the Committee push for an environmental unit where individuals with chemical sensitivities can be isolated. She acknowledged that it was expensive and that not everybody could be tested in a clean environment. She said that the basic science was needed, and money shouldn't be preventing this from occurring. She commented that some people in New York didn't want to know how polluted the World Trade Center site was because we can't afford to clean it up. She believed that one needed to go with the science, i.e., find out what is there and then deal with it. As an example, she commented that she didn't think Dr. Haley would be able to use neuroimaging to see how the brains of individuals with multiple chemical sensitivities would react to perfume exposures. This was because his neuroimaging equipment was located in a clinic and the patients would face many other exposures in the process. She stated that, sooner or later, a clean facility would be needed.

The meeting recessed at 10:16 a.m. for a break.

The meeting reconvened at 10:34 a.m.

### **Gulf War-related Research at Wright State University**

Mariana Morris, Ph.D.

Professor, Pharmacology & Toxicology, Wright State University Boonshoft School of Medicine

Dr. Morris discussed certain research being conducted at Wright State University, including her research dealing with the effects of low-dose sarin and stress on the autonomic function and genomic expression and Dr. Gerry Alter's work looking at chemical metabolism in humans with chemical sensitivity. ([See Appendix – Presentation 10.](#))

Dr. Guilarte stated that there appeared to be a dramatic effect related to fear conditioning and asked if Dr. Morris' group had followed this up. Dr. Morris stated that they had not yet. Dr. Guilarte asked what they were measuring and Dr. Morris replied that they were testing response when light and sound were paired together and that sarin-treated animals didn't respond as well as controls. Dr. Guilarte stated that the normal response is for the animal to freeze. Dr. Morris stated that the sarin-exposed animals do not seem to freeze. Dr. Guilarte stated that this was an extremely important finding from a neurobiological perspective, because the neurobiology of the fear position is fairly well known. The hippocampus and amygdala are involved, as are NMDA receptors. He thought this would be important to follow up. Dr. Morris agreed. She stated that they had data on dopamine levels in the amygdala that showed changes in the sarin-treated animals. Dr. Haley inquired about the direction of these changes. Dr. Morris stated that the levels were increased. She said a journal article covering these findings was currently under review and offered to send a copy once it was published to anyone who was interested.

Dr. Clauw asked if they had a chance to look at pain behavior in the sarin-treated animals. Dr. Morris indicated that they had not. Dr. Clauw noted that both the autonomic and HPA profiles were almost identical to that seen in fibromyalgia. Dr. Clauw stated that they may have stumbled across the first animal model for fibromyalgia, but that posed another problem in that the exact same changes were occurring in people who were clearly not exposed to sarin.

Dr. Clauw then asked about controls in the enzyme experiments and how samples were collected, since enzyme levels are very sensitive to changes in temperatures, freeze-thaw, etc. Dr. Morris stated that the samples had been collected locally and that as far as she knew they were controlled under the same environment as the cases at the Rea Clinic in Dallas. They used the same protocols. Dr. Haley commented that paraoxonase and arylesterase had been shown to be very stable in freeze-thawed samples, but he wasn't sure though about alcohol dehydrogenase. Dr. Morris stated that she didn't think there was a problem. She commented that the Rea Clinic didn't have many control samples and that most of the individuals who go there are patients who go to be tested. Dr. Morris asked if Dr. Haley had worked with them. He indicated that he hadn't, but may be looking to in the future.

Dr. Golomb noted that she found two of Dr. Morris' findings to be very interesting: (1) a very low dose of sarin leads to alterations in gene expression and it would be very interesting to look at what happened long-term; and (2) a major subset of those alterations were reductions in enzymes, a phenomenon also linked to chemical sensitivity. She thought it would be great to unite these findings and determine whether there are reductions in the enzymes involved in chemical sensitivity. This could be done with sarin, but even better would be the use of pesticides. She noted that there was a Gulf War study that showed chemical sensitivity to be strongly associated with reported pesticide use. Dr. Morris agreed and thought it would be appropriate to look at pesticides because of the number of people affected by them each year.

Dr. Haley commented that the scatter plots showed arylesterase/alcohol dehydrogenase and PON/alcohol dehydrogenase. He asked what if they had compared arylesterase with PON. He stated that a critical graph would be to place arylesterase on the vertical axis and PON on the horizontal. This would define the phenotypes and he thought it would be useful to analyze the data in this way. Clearly alcohol dehydrogenase was important in the graph Dr. Morris had shown, while PON didn't appear to be. Arylesterase was important, too. He suggested putting PON on the horizontal axis, which would show three groups, reflecting the phenotypes. Dr. Morris stated that she would take this suggestion back to Dr. Alter.

Dr. Haley commented that these findings were quite dramatic and Ms. Knox asked him to explain. Dr. Haley said that the findings meant that people with low arylesterase activity, who are poor metabolizers of organophosphates, are more likely to be the sick individuals, while those who are better metabolizers are more like the healthy controls. Dr. Haley asked about the range of substrates for alcohol dehydrogenase, i.e., what else besides alcohol is metabolized by alcohol dehydrogenase. Dr. Meggs said that ethylene glycol, along with other glycols and alcohols, are metabolized by alcohol dehydrogenase. Dr. Haley asked why they had looked at aldehyde dehydrogenase for the study. Dr. Morris commented that there was a lot of formaldehyde sensitivity among those with chemical sensitivity.

Dr. Meggs asked whether the activity level would be different from phenotype, because you may have more than one phenotype associated with the activity and quantity of enzymes, which are all different. Dr. Morris stated that they only had a small sample of blood and this is what they had looked at initially. She agreed it would be useful to look at the quantity of enzyme.

Dr. Tansey commented that Mr. Hardie had indicated that his tolerance for alcohol had dropped considerably and that this phenomenon might relate to the correlation shown in the graph. Chemical sensitivity is associated with alcohol intolerance. She also commented that, having worked on glial cell derived neurotrophic factor (GDNF), artemin and neurturin in Gene Johnson's lab as a postdoc, she was shocked to see an increase in GDNF and a decrease in artemin in the hypothalamus. This is because both are from the same GDNF family and are very critical neurotrophic factors for different reasons. GDNF clinical trials had been stopped because the recombinant protein traveled too far into the hypothalamus and suppressed appetite. Neurturin did not do this and is in clinical trials for Parkinson's disease. If sarin decreases neurturin levels, one might be able to restore the levels with adenoviral gene therapy, which has already been FDA-approved in humans. Artemin is also a big surprise because not much is known about what it does. She commented that it was really nice to be able to identify promising therapeutics from these kinds of studies.

Chairman Binns thanked Dr. Morris.

**Overview of the Congressionally Directed Medical Research Programs (CDMRP)**

COL Janet Harris, PhD, RN

Director, Department of Defense Congressionally Directed Medical Research Programs

COL Harris gave an overview of the DoD's Congressionally Directed Medical Research Programs, including the development of the FY06 Gulf War Veterans' Illnesses Research Program (GWVIRP) ([See Appendix – Presentation 11.](#))

Dr. Meggs noted that one of the Committee's first recommendations was to open up Gulf War illnesses research monies to competitive bidding from all sources, because the person who has the high risk/high gain idea might not be eligible to receive VA funding. He stated that DoD's GWVIRP solved that problem, and applauded the initiative. Dr. Harris reiterated that the program was open to any researcher, including international ones, and that the majority of the funding in her program goes to extra-mural investigators. Only 10% goes to researchers within DoD.

Mr. Smithson, a Committee member, stated that funding for FY2007 was currently in appropriations and going into conference. He asked whether there were any Gulf War illnesses research initiatives included in the FY2007 budget. COL Harris stated that she hadn't seen a "line item" for this yet. Chairman Binns commented that he would address this in a minute.

Dr. Morris asked when the deadline for proposals was expected to be. COL Harris stated that she couldn't say yet, but noted the general timeline outlined in her presentation. Dr. Steele asked about the usual length of time allowed for proposals after a program announcement is released. COL Harris stated that it was typically two-four months, depending on several factors. Exploration/hypothesis development awards involve new ideas and a short application, so researchers usually didn't need a lot of time to prepare their proposals. If it was a clinical trial or consortium, which wasn't the case with this program, the time allowed for submitting proposals would be longer.

Chairman Binns thanked COL Harris.

Chairman Binns introduced Vic Edgerton, legislative staff member for Congressman Dennis Kucinich. Congressman Kucinich serves on the House subcommittee with Congressmen Shays and Sanders, who had all been strong supporters of Gulf War illness research over the years. As COL Harris noted, Gulf War research money only gets to COL Harris if Congress appropriates it. And Congress only appropriates it if there are staff members willing to work on these projects. Chairman Binns stated that Vic Edgerton was the one to thank for doing the work for the FY2006 monies. He added that they were hopeful there would be a line item for the same amount of money in the FY2007 budget.

Chairman Binns asked Dr. Haley to speak about the Gulf War Illness research program being developed at the University of Texas Southwestern Medical School. Dr. Haley first wanted to say that this meeting was a turning point in this field. The last meeting foreshadowed it, but this meeting had been phenomenal. For years, we have been laboring in an ambiguous position. It has been difficult and frustrating, hoping to get to the point of knowing where to direct basic science. Until basic science is focused on this and looking at mechanisms of disease and biomarkers at the cellular level, we haven't made a lot of progress. Dr. Haley stated that he had seen a major shift that day with tremendous preliminary data and likely hypotheses, not just pie-in-the-sky hypotheses. This needed to be communicated to Congress because now is the time to put money into this research. We didn't have the tools to do this research 10-15 years ago. Now we not only have the tools, but they are paying off. So now is the moment that investment will make a big difference.

With regards to the program at UT-Southwestern, Dr. Haley stated that the program had not yet begun. They were still developing the template for it, which was very important. VA has never done something of this nature before and it was important to get it right. UT Southwestern was working with VA Central Office, Dallas VA Medical Center and VISN 17 personnel to develop a mechanism for this funding that will serve the future of the VA, but also make it possible to do creative work. The funding was initially announced in December 2005, and a memorandum of understanding was negotiated and signed at a news conference in April 2006. Since then, VA Central Office legal staff had been developing a contract, which UT Southwestern had received three weeks prior and were in the process of reviewing. The memorandum of understanding was just a general overview of intent, but it wasn't a legal document. Once the contract is negotiated, they will then know what the structure is and what they will be able to do. Dr. Haley stated that everybody had agreed that they wanted a creative atmosphere where exciting hypotheses and tools were used to develop multiple projects, including animal studies, human brain imaging, nuclear imaging, and epidemiologic studies, in a way that would allow the various fields of research to communicate amongst each other. This would entail basic scientists talking to epidemiologists and brain imaging scientists, which would make the most of these opportunities.

Dr. Haley stated that projects would be reviewed by a merit review committee that reported to Dr. Al Gilman, Nobel Prize laureate and Dean of UT Southwestern Medical School. Dr. Gillman will chair this blue-ribbon committee. They are in the midst of establishing the criteria by which the committee will review the proposals.

Dr. Haley noted that another key thing making all of this possible was the fact that UT-Southwestern had a lengthy track record of working with Ft. Detrick to develop preliminary data, survey mechanisms that are unique for Gulf War veterans, a brain imaging center, and basic science protocols. While this particular funding was from the VA, it was built on the basis of a long relationship with DoD, which has become more productive over the years. He stated that they were ending their relationship with DoD with mixed feelings because the collaboration had become so productive. They would build on this platform to make the new program creative and productive.

Dr. Klimas asked if UT-Southwestern would be collaborating with other institutions and VA facilities. Dr. Haley indicated that they probably would be, but the rules and procedures for this type of collaboration had not been developed yet.

Dr. Clauw commented that this sounded a little different than what was presented at the May 2006 meeting. He stated that he had the impression then that there would be more of a clinical program associated with the project. Dr. Haley stated that this had not changed, but there were no rules yet. Dr. Clauw asked whether a treatment program would also be a part of the program and that treatments and trials were what Gulf War veterans were continually asking for. Dr. Haley stated that he did not know yet.

Chairman Binns invited Dr. Leda Cummings, who was an audience member and with the Sameuli Institute, if she would like to pose any questions to the Committee or visiting scientists. Dr. Cummings asked Dr. Morris about the low-dose sarin exposures and the absence of alterations in gene expression in neurons *in vitro*. She asked what might explain alterations *in vivo*, but not *in vitro*. Dr. Morris stated that Dr. Steven Berberich had done these studies. All she could say was that he used multiple doses and found nothing different, at least with the protocol he used, acutely, i.e., 24 hours after exposure. Dr. Morris offered to provide Dr. Berberich's email to Dr. Cummings so that they could communicate directly. Dr. Sopori stated that it was possible that it was an indirect effect because we know that sarin causes neurological changes very fast. Dr. Golomb agreed, noting that you may need the *in vivo* preparations for the initial effect, which then leads to the subsequent effect.

The meeting recessed at 12:00 p.m. for lunch.

The meeting reconvened at 1:04 p.m.

Upon returning, Chairman Binns complimented the Committee staff on the meeting preparations. He then introduced Dr. Bill Goldberg, Portfolio Manager of VA's Gulf War Research Portfolio, and Dr. Joe Francis, Deputy CRADO.

Dr. Francis conveyed the apologies of Dr. Joel Kupersmith, CRADO, who was not able to be present at the meeting. He indicated that Dr. Goldberg was the continuity to the Committee as a neuroscientist while he himself was a health services researcher. He was fascinated and impressed with the quality of scientific presentations over the past two days, and extended his thanks to Dr. Steele, the Committee's support staff and all of the visiting scientists. He discussed his background in studying acute confusional states, i.e., delirium, both from a clinical epidemiologic perspective as well as with outcomes research. The cytokine hypothesis is the number one mechanistic hypothesis in this field now. His group, as well as several others, had found that acute confusional states lead to more permanent sequelae than previously thought, and can be very dangerous to the functional status of older individuals. Feedback loops that occur in the neuroimmune system are very important. Dr. Francis stated his belief that we are on the cusp of really understanding the mechanisms; and it was this understanding that helps drive relevant and rational therapies. He stated that VA ORD firmly believed that the care of veterans was a sacred trust, and their mission was not purely the generation of knowledge, but to improve the health of veterans. Their secondary mission was to improve the health of the nation. He noted several discoveries made by VA that had benefited the general population, e.g., that the currently-accepted tuberculosis treatment is based on findings from a VA Cooperative Studies project. However, veterans are different and VA has a higher bar in terms of respecting human rights and following the fundamental principle of medicine, that is, "to do no harm." VA was the first to establish a human rights committee, which predated institutional review boards, to review scientific merit as well as societal and individual risks. These committees were among the first to involve veteran stakeholders and advocates, in addition to the scientific community. Dr. Francis stated that this was why VA ORD was pleased to support the current initiative. He felt that the figures speak for themselves, and that VA ORD had clearly made a major commitment in the field of Gulf War illnesses research. He said that they are in this for the "long haul." He added that the "long haul" also had to be defined by the political process, as COL Harris mentioned. There were a lot of things about FY2007 and beyond that could not be predicted and surmised. However, VA's commitment to this issue was there, and he didn't anticipate it going away.

Before Dr. Francis turned the proceedings over to Dr. Goldberg, he noted that Dr. Haley had given an excellent summary of the current state of the VA/UT-Southwestern initiative. He stated that this was a collaboration and mechanism to bring in the best science from across the nation in this endeavor. He noted there were many federal acquisition regulations that must be adhered to, which prevented them from discussing the contract negotiations in more detail.

### **Gulf War Update**

William J. Goldberg, PhD

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

Dr. Goldberg conveyed the apologies of Dr. Timothy O'Leary, Director, VA Biomedical Laboratory Research and Development, who was not able to be at the meeting. Dr. Goldberg went on to review VA's

Gulf War Biorepository Trust initiative and VA's FY2006 Gulf War illnesses research portfolio. ([See Appendix – Presentation 12.](#))

During Dr. Goldberg's discussion about the Gulf War Biorepository, Dr. Steele asked, for clarification, whether tissues from Gulf War veterans without amyotrophic lateral sclerosis (ALS) would be collected. Dr. Goldberg indicated that this was the case. Dr. Steele asked if DNA would also be collected. Dr. Goldberg indicated that they would be collecting DNA from the post-mortem blood and serum samples. Dr. Steele asked if there were any plans to collect DNA samples from living veterans too. Dr. Goldberg stated that this would be determined by the individuals who were running the tissue bank. Dr. Francis interjected that one of the VA's key initiatives was to establish a genomic medicine project. This will take advantage of VA's defined patient population that is tracked longitudinally with electronic health records and the capability VA has already built in research and development to look at conditions of particular importance to veterans. While it would not be the sole mission, he believed that Gulf War illness would be a part of this project. The federally chartered advisory committee that will help define the genomic medicine project was scheduled to meet in September 2006. The first issues addressed by this committee would be ethical concerns, identification/deidentification of information, etc. They have had a slight setback in terms of the information breach that happened at VA in May 2006. This breach did not involve VA ORD, but they are in a situation of rebuilding Congress' and veterans' trust that VA will be able to handle this very sensitive information. No one can guarantee 100% security, but VA is working to establish enough checks and balances to protect veterans. VA is looking at a number of solutions specifically for genetic information, because if it is released inappropriately, it could be very damaging to an individual veteran. This is the year to lay groundwork for the project.

Dr. Golomb asked if the VA was looking to move to a veteran identification system that didn't use Social Security numbers. Dr. Francis stated that he was leading VA ORD's effort to look at the issue of data security. If the nation can come up with a better personal identifier than a Social Security number, VA ORD would certainly use it. Dr. Francis noted that a lot of what was done in VA research was linked to non-VA databases for which Social Security numbers were critical. Two-thirds of veterans over the age of 65 use Medicare. In order to link with Medicare data, VA must use Social Security numbers. However, VA ORD can have the researcher use other identifiers that have been "stripped" of personal information. In current research data systems, they scramble the Social Security numbers. However, it isn't difficult, especially in rare illnesses like ALS, to trace the data back to the individual. The other approach being used is encryption. VA advocates universal precautions. Datasets must be encrypted whether the researcher thinks it contains sensitive data or not. This is a habit the research community needs to get into. Dr. Francis noted that Dr. Haley was nodding in agreement, because one of the many interesting aspects of the contract discussions involved data security. It is important to build this correctly so we can sustain the effort for the long haul.

Upon concluding his discussion of the Gulf War biorepository, Dr. Goldberg stated that it was his understanding that the biorepository officials anticipated being able to consent potential donors in about three-four weeks. Dr. Baraniuk asked if cerebral spinal fluid could be added to the list of collected samples. Dr. Goldberg stated that, as the focus would now be on a central nervous system disease, VA would add cerebral spinal fluid to the list. Dr. Sopori asked if spleen or liver would be added, because the hypothesis being discussed over the course of the meeting was that something happens to inflammatory cytokines. Dr. Goldberg stated that he would discuss this with the individuals running the tissue bank and Dr. O'Leary. If at all possible, they will make every effort to add these samples to the list too.

Ms. Knox asked how consents would be obtained. Dr. Goldberg indicated that he didn't know the particulars, but would find out the answer for her. Dr. Francis commented that it was VA's practice to not rely on blanket consents. VA also uses an opt-in approach; this provides a higher level of protection for

the veteran. VA has a national research advisory council that helps determine these issues. Ms. Knox noted that it would be appropriate to broach this specific issue, as is done with living wills for example, when a veteran entered a VA facility. She noted that deidentified tissue could be used for a cancer repository. Dr. Francis agreed, and noted that there were some universities that were instituting some very innovative encryption and other techniques that would allow this. Dr. Francis noted that the consent for this program would also be on a separate form and would be explained to the individual clearly. He stated that the biggest cost related to the genomic initiative is the clinical cost of consenting.

Mr. Smithson asked about the manner in which VA would be publicizing the Gulf War biorepository to Gulf War veterans. Dr. Francis stated that they would like to use every mechanism at their disposal. They would appreciate the help of Mr. Smithson's organization, the American Legion. Dr. Francis stated that Mr. Reuben Wright, a Gulf War veteran and VA ORD staff member, worked with all four branches of the military and did a lot of outreach work and communication in building communication lines. Dr. Francis noted that whenever VA ORD advertised a research project, the advertising must be approved by an institutional review board. This was to ensure that the veterans understand the protocols and purpose of the research.

Dr. Steele asked if the tissue bank would accept samples from veterans who were not receiving most, or any, of their care from VA. Dr. Goldberg stated that this would be an issue that would have to be worked out when it came up. There are financial and legal liability issues that must be addressed. Dr. Goldberg noted that the one sample that had been collected from a veteran outside the VA had involved a Herculean effort by many individuals. He wasn't sure if this would be possible on a routine basis. Ms. Knox commented that they should be applauded for making that effort.

Dr. Goldberg distributed the most current Gulf War research portfolio listing to the Committee members and the public. Copies of the "2004 and 2005 Annual Reports to Congress: Federally Sponsored Research on Gulf War Veterans' Illnesses" were also distributed to Committee members.

During his discussion of funding trends, Dr. Goldberg noted that chronic multisymptom illnesses were categorized under the "General Health" category not in the "nervous system" category. Dr. Francis commented that there clearly had been a major initiative around brain and nervous system research. He noted that when they classify a portfolio, a project can sometimes fall into more than one category, so the proportions should be taken with a grain of salt. He also noted that some of the work looking at environmental exposures and trends happened through VA's Public Health and Environmental Hazards office, headed by Dr. Lawrence Deyton. Much of the work alluded to today, such as Dr. Han Kang's research, was supported through this office. Dr. Francis was glad to see that VA ORD was now spending more under the category of immune and infectious diseases. He hoped that the cytokine research here would pay off. He noted that genomics was not listed as a category, but probably cuts across all of the listed categories.

Dr. Golomb asked if it would be possible to separate the brain and nervous system category into psychological and non-psychological studies. She noted that this division had been of considerable interest to the Committee. Dr. Francis stated that, because of the imperfections of the categories, the full listing of the protocols had been provided. He felt that the summaries of these projects provided a good sense of how they are categorized. He commented that there were several ways to "slice and dice this," and they would certainly like to accommodate any suggestions about how to do this. Dr. Goldberg noted that the funding trends, which were graphed in the last slide of his presentation, were a cross-service slice of the Federal Gulf War research portfolio. This included all of the projects at DoD, VA and Health and Human Services (HHS). Any effort to "reslice and dice" would have to be reviewed by three additional groups, and coding is a very time-consuming issue. There are sub-divisions within these categories, but it

is difficult to create a clear bar graph showing them all. He stated that the graph presented showed the general trend, in terms of categories, as to where the research focus was going and coming from. Dr. Goldberg stated that it wasn't perfect, but did provide some sense of whether particular areas are on the upswing or downswing. This allowed the portfolio managers to get some feedback from the Committee as to whether this was appropriate or not and give direction on priority research. Dr. Goldberg stated that the middle appendix in the Annual Reports had the complete breakdown of federally funded Gulf War research studies. He further noted that these trends were only for the primary assignment of the study. Many projects have more than one area to which they have been assigned. There are many more nervous system projects, for example, buried in the environmental exposures category. This was because the primary focus of the study was on the environmental exposure. He noted that there were no "double counts" in the graphed data.

Mr. Graves asked if this meant that the psychological and stress-related studies were listed under "General Health." Dr. Goldberg stated that a lot of the cytokine-related work was probably in symptoms and general health, but the assignments were determined on a project by project basis. He stated that he couldn't give a "hard and fast" answer on that.

Chairman Binns stated that, as in the past, the Committee's staff would take a look at the individual studies and categorize them for the benefit of the Committee. This would allow the Committee to give its feedback to VA ORD. Chairman Binns stated that, historically, the brain and nervous system had included the psychological studies. He noted that there was also the issue of how many ALS studies were included. However, he didn't think this needed to be teased out at that time. Dr. Goldberg offered to provide Dr. Steele with any study abstracts that she might not have.

Mr. Hardie commented that there was a line item in the FY07 National Defense Authorization Act that would authorize a new cancer research institute to study the prevalence of cancers in current members of the Armed Forces, as well as veterans. He asked if VA ORD was familiar with this, and if so, if there was tie-in to this work. Dr. Goldberg stated that the cancer prevalence study was most likely Dr. Han Kang's project. He wasn't sure, however, about the bigger picture and didn't think there was a direct link with what VA ORD has been doing. Most of the identified studies have been funded through VA ORD initiatives, rather than any national study.

Dr. Tilson noted that VA had a clinical database, which could allow one to look at how the syndrome was evolving over time. This database could also help identify particular therapies that were being used empirically. This information should allow us to come up with better ways of approaching therapy. Dr. Tilson asked if there was strategy to capture the population level data in the VA's electronic medical records. Dr. Francis stated that there was a major effort to try and capture different populations for longitudinal tracking using electronic health records. However, it was not easy to do. As powerful as the VA's records are, it really is a front-end effort that draws from 128 separate databases. VA is in the midst of converting the information structure to a unified database known as the "Health Data Repository." This is running in parallel with the VA rehosting its electronic medical records that will allow these things to be computationally easier to do, and marrying them with the critical databases, e.g. Tricare, Medicare, other corporate data systems, and death and beneficiary data from Veterans Benefit Administration. It was taking longer than they anticipated because of discussions among VA's "Board of Directors" regarding how the VA's information technology system should be created. Dr. Francis stated that an upcoming paper in the November 2006 issue of *Health Affairs* provided a case study of how VA ORD built this type of system for diabetes. It took many years and is quite complicated. But they would like to emulate this approach for other conditions. Dr. Tilson thanked Dr. Francis for acknowledging that this work is all about the veterans and the need to balance the urgency of doing something with protections of veterans' dignity and the rigor of science. However, use of these data is important, particularly in the

situation where there is a population data resource that can help unlock the complex puzzles of veterans' chronic multisymptom illnesses.

Dr. Golomb noted that, from her experience, Dr. Tilson's proposal to use the VA electronic health records to follow Gulf War illnesses was more complicated than one might imagine. She stated that several of her colleagues had sent patients to her clinic for a one-time Gulf War visit, and there is no way to ascertain from their charts that they have Gulf War illness. You would not even know from their charts if they have subcomponent symptoms associated with Gulf War illnesses, much less what treatments they received. Dr. Tilson recognized this as a thoughtful clinician's input, but it shouldn't dampen the enthusiasm for getting on with utilizing this resource. Dr. Francis referenced Dr. Morris' discussion on microarray analysis and how the genetic problem became a computational problem. One of the underpinnings of their efforts was trying to understand how to mine the textual data from the medical records. They have to use artificial intelligence and fuzzy logic to find and use these data. VA is having discussions with leading informational technology companies to understand how to do some of these things.

Chairman Binns noted that the Committee had recommended the establishment of a treatment development center that would collect information, as suggested by Dr. Tilson, from patient records and from clinicians inside and outside the VA system, looking to identify potential therapies for future research. He asked if this center was still being pursued. He noted that this was something uniquely within VA ORD's hands and would be difficult to hand over to UT Southwestern. He stated that the Committee would love to see this center be included on the portfolio listing next year. Dr. Steele commented that a research funding announcement (RFA) had been prepared to solicit proposals for a center. Chairman Binns agreed, noting that the RFA had been ready for release for the past seven or eight months. Dr. Francis stated that they had not forgotten about the concept. He stated that he couldn't provide much information, but could say that dialogue was occurring with UT Southwestern about this matter. He stated that there would be a VA on-site component to that, as well as information sharing. They will execute whatever mechanism is necessary to continue the initiative.

Dr. Steele asked if VA ORD intended to issue another RFA on Gulf War illness. She stated that one had been on the table for awhile, even before the Dallas initiative. Dr. Goldberg stated that since there was not yet a FY2007 budget, it would be premature to commit to what they could or could not do next year. Dr. Francis reiterated that VA ORD was in this for the "long haul." Their commitment was to see that there was interest, good science, and momentum in this area of research.

### **Committee Business and Discussion**

Dr. Steele stated that that the Committee report was still being drafted. She indicated that it would be useful to schedule the 2007 meeting dates as soon as possible and that members would be contacted in the next few weeks to schedule these dates. She went on to say that the next scheduled meeting (November 2006) would be held in Dallas, TX. Dr. Haley and his colleagues at UT Southwestern would be sharing the Gulf War research projects being planned.

Mr. Smithson stated that the presumptive period for undiagnosed illnesses for compensation purposes would expire at the end of 2006. The American Legion had been speaking with VA about the matter. Mr. Smithson believed that VA intended to extend the period and that the last extension had been for five years. The American Legion's position is that it should be extended indefinitely because there are so many unanswered questions. Mr. Smithson thought it would be a good idea for the Committee to weigh in on this matter by sending a letter to the Secretary. He acknowledged that this wasn't a research issue

per se, but research does drive it. With ongoing research and a lot of unanswered questions, we can not allow the benefits door to be closed for individuals developing symptoms in the future. Chairman Binns stated that the Committee could extend a comment that in light of the advancements being made in research, an extension of the time would be order. He stated that he could suggest this to the Secretary in his monthly email. The Committee agreed.

With no other general business, Chairman Binns asked that the discussion return to aspects of the scientific presentations and possible research recommendations.

Dr. O'Callaghan offered his perspective on the discussions, first pointing out that if the Gulf War illness problem was easy, it would already have been solved. He stated that we were dealing with an event that occurred many years ago involving multiple exposures and causes that led to multiple constellations of symptoms, many of which could have neuro-immune linkages. This seemed pretty clear, all the way down to several different molecular levels, based on talks given at the meeting. He noted Dr Tracey's presentation about peripheral-brain feedback control and the various discussions about imaging possibilities. He stated, however, that there hadn't been a lot of discussion about definitions. He also noted that several different end points had been discussed but that it was hard to know if changes from baseline were good, bad, or don't matter. The information presented should be put into context of what we already know. He noted that Dr. Guilarte was looking at glial activation associated with pretty nasty pathology in terms of the models used. This didn't necessarily tell us if glial activation associated with a plethora of other cytokines and chemokines represented adverse effects or were linked to overt cytopathology and dysregulation of the peripheral immune system. The science is no longer in its infancy in terms of basic neurobiology, and we are at the point where we are getting good definitions of these dysregulated phenomena. We need to take the information from basic neuroscience and let it guide us to targets that are related to the effects seen in veterans.

Mr. Graves commented that the focus should be on veterans, their treatments and how to translate that to the clinic. He commented that the previous day's discussion had become elevated and when he was thinking about it afterwards, he thought that both parties had been right. There is a sense that we have to be responsible in moving forward with the science and treatments. However, there is also a sense of urgency as more time lapses and people are dying. Mr. Graves was also concerned that, as more time goes by, more things were going to start "popping up" due to delayed onset of conditions from earlier exposures, e.g., brain cancer. There has to be a balance. Some things can be moved forward quickly, e.g., studies looking at DM, while other things might require a more methodical approach. These can be examined on a case-by-case basis.

Dr. Golomb stated that she wanted to clarify her position from the previous day's discussion. She thought that a clinical trial would be appropriate to initiate, but never intended that any of the protections be circumvented, nor did she intend it to be based on anything other than an approved, sufficiently-defended protocol. She wished to defend her suggestion that a double-blind, placebo-controlled trial could be done with an over-the-counter drug that people take all the time. Chairman Binns stated that he respected her position.

Mr. Hardie commented that he was deeply impressed with the Committee and the level of discussion was exciting. He stated that it was encouraging and exciting to see others learning and getting excited about the prospects. He wished to reemphasize, however, the sense of urgency. He referred to his own chronic health conditions, and stated that he wasn't the only Gulf War veteran who had a sense of concern that it wasn't related to PTSD or being hyperaware. He said that you can learn a lot about the medical system by way of journeys through every sort of medical test available. He concluded by thanking the scientists and their support staff for their hard work.

Ms. Knox stated that it was eye-opening to sit at the table and hear Dr. Haley say that he has mixed emotions about not being able to work with DoD. She commented that, looking back 10 years, it was very difficult to work with DoD. DoD denied that there were any low-level chemical exposures for years, so the science couldn't be done. It is rewarding to see the full circle come around, and see the science finally catching up. She was excited about the meeting and about what was going to happen at UT-Southwestern. She commented that she thought that researchers would find genetic predispositions. She didn't want this to result in DoD or VA saying to a veteran that they were predisposed to this illness and, therefore would not be compensated. She stated that this was a real concern for veterans because they already have a difficult time getting compensation. She noted that this was a concern for the genome project too, and we need to be aware of it. She concluded by commenting that "the soldier is waiting."

Dr. Sopori stated that he had learned a lot at the meeting in terms of science and other issues. He was impressed by the hypothesis that there was a dysregulation of the immune system, particularly involving proinflammatory cytokines. If you look at other diseases for which we don't know the causative agents, e.g., stress disorders, fibromyalgia, delirium, one also sees dysregulation in the proinflammatory cytokines. The question is what caused it. We don't know. We know that a lot of people were exposed to various insecticides, etc. However, it could not be something that was in a limited area, given the number of individuals affected. A lot of veterans were exposed. Some developed the conditions while others didn't. When one gives a mouse an infection, it gives a response but then recovers. However, you may have a particular mouse that responds but doesn't recover. This often involved loss of regulation or feedback mechanisms. Animal strains may be developed that are more likely to develop this kind of phenomenon. Dr. Sopori went on to say that it is also important to look at chronic effects. Looking at things early on may not tell you anything. He cited lung cancer and the effort to look for genetic predispositions. It may take 20 years for the symptoms to develop. Then you have to look back to see what caused it. One has to be careful when one defines early changes.

Dr. Tansey thanked the Committee for the opportunity to be there and learn from all the researchers presenting. She stated that when they first heard about this at UT Southwestern, they all thought it was a controversial theory and there were a lot of politics involved. However, as she listened more to the science and to the patients, she doesn't believe it is that controversial. She thought it was clear that there was neuroimmune and neuroendocrine dysregulation involved. These are tough, complex systems to grapple with. However, UT Southwestern has a unique opportunity to learn from what has gone on so far and design the best experiments possible, using both unbiased and candidate gene approaches. They know a lot about the gene families that are probably involved. She looked forward to working with Dr. Haley and others present to find biomarkers and treatments.

Dr. Nettleman stated that those present have accepted and taken for granted that Gulf War illness is an illness. She felt that any Committee report had to reaffirm this repeatedly, because there are still people out there who don't get it. She also thought there were many opportunities raised during the course of the meeting. She noted the discussions about neurodegenerative disease studies and that these studies could be repeated in Gulf War veterans. She respected the points about not circumventing any of the research safeguards, but acknowledged that we are running out of time. Science has fallen on its face many times, and modern medicine is far from perfect. So we end up being cautious about causing harm. We don't want to take it to the point where we fail to do good. Her final point, as a women's health researcher, was that a big genetic difference in chronic fatigue syndrome and Gulf War illness was the Y chromosome in the two populations. Thus, Gulf War illness is not going to be equal to chronic fatigue syndrome, even though it will have similarities.

Dr. Clauw commented that he thought the Committee should strongly recommend that the VA treatment guidelines be modified. He stated that he played a large role in creating the first set, but noting that much has been learned about fibromyalgia and chronic fatigue syndrome since then. If people with chronic multisymptom illnesses were managed according to what we now know, the Gulf War veterans would be doing a lot better than they were now. This is the case with fibromyalgia. Even though there are thousands of published articles, what happens in clinical practice is not in accordance with the literature. He stressed the need for randomized clinical trials. He expressed skepticism that any more epidemiological or basic science studies were going to have major and quick impact on the health of Gulf War veterans. Based upon how the VA portfolio is set-up, he didn't see how randomized clinical trials could be done. The grants being offered were small. We have to think about ways to take the new ideas into clinical practice and do randomized clinical trials, which would inform the VA about what might have happened with the Gulf War and how to take care of this population with chronic multisymptom illnesses, which are common in the VA and general population.

Dr. Haley stated that his take from the meeting was a real enthusiasm for the mechanisms discussed. We can't assume these are true but have to show whether they are true or not in Gulf War veterans. If we were to show neuroinflammatory changes are important, or even perhaps the principle cause of this disease, this was only a start. It would show us the direction to take research, but then we need to know why and what is sustaining it. This is where animal studies are critical. Dr. Haley did want to comment on Dr. Clauw's statement about basic science studies. Dr. Haley stated that well-directed animal studies, looking at mechanisms of neuroinflammation, may define some of the critical areas in short order. Bringing to bear the genomics, proteomics, etc., we can get enough clues to allow a fairly quick strike and direct therapy at critical areas. We need to quickly determine whether neuroinflammation is the problem, in whole or part. If it is, the "full court press" is on what are the mechanisms and what is sustaining them, leading to the critical treatment that will make these veterans well. Dr. Haley commented that it would be a real coup if researchers and clinicians were able to make veterans well from this effort. He stated that his group would be presenting UT Southwestern's overall research plan to the Committee in November 2006. This plan would include projects that would provide insights into this hypothesis.

Dr. O'Callaghan agreed with Dr. Haley. He stated that, in context of the talks and discussion over the past two meetings, there was a reasonable consensus that there was a neuroimmune component to Gulf War illness. This begged the question as to whether there was enough information to go ahead with some attempt at therapies without going after mechanisms first. He noted the need for immediacy. Dr. Haley stated that this point echoed what had been discussed in Committee meetings for the last four years regarding treatment. There are two general approaches to identifying treatments. The first is the empirical approach. The closer we get to knowing what the mechanism is, we can guess what treatments might work and go ahead and try them. The other is the rational approach, which is learning more and more about the mechanisms. Thus, if the empirical approach hasn't worked, researchers continue digging into the mechanisms and this might provide insight into a potential treatment. Dr. Steele commented that, from her perspective, determining if there is a neuroinflammatory component to this disease would allow researchers to start looking at treatments right away. In a way, it would provide the best of both approaches, and provide a point that allows investigators to move forward and backward.

Dr. Golomb seconded what Dr. Haley had said. Everything that had generated the hypotheses heard today was based on new basic science. Without this basic science, we would make no additional progress. The idea of doing no more basic science means we are stuck with the treatments that we have, and they have not proved to be greatly effective. She stated that she had additional ideas about the genesis of the neuroinflammatory process and believed that neuroinflammation was an extremely promising avenue for future study. If someone wanted to propose to DoD or VA a sufficiently-justified

treatment trial, she didn't see why it couldn't be done at this point. If they can defend it, it should be subject to the peer review process.

Dr. Tilson stated that he liked everything that he had heard and would "second" it all. He stated that he was all for basic science, rational drug development, empiricism, and peer review. He would only add that this has been a dazzling overview. He wondered if we had a terrain map of the field yet. However, he wasn't sure where the Centers for Disease Control (CDC), NIOSH, NIH, and the research portfolios for other multisymptom syndromes stood on this hypothesis. He noted that one of the big funding sources, i.e., pharmaceutical companies, hadn't been mentioned either and wondered if they were working on promising leads.

Dr. Steele asked the Committee and visiting scientists to provide suggestions concerning specific studies related to the meeting's discussions. She began by suggesting studies that might be done in the short term, such as looking for proinflammatory cytokines in cerebrospinal fluid. Another option would be to conduct imaging studies of the type described in the meeting to see if they would show any indication of neuroinflammation. If neuroinflammation is underlying veterans' multisymptom illnesses, how can we find out? If there are leads about peripheral markers that reflect CNS inflammatory responses, this would also be an attractive next step. She asked if any of the visiting scientists knew if elevated levels of proinflammatory cytokines in the cerebrospinal fluid could be measured. Dr. Tansey stated that this was possible using very sensitive, quantitative ELISA tests. The problem is proinflammatory cytokine levels change depending on the particular challenge. Therefore, the study must be designed well, so that you aren't looking at a static picture but at responses to a particular stimulus. These would need to be longitudinal studies. Dr. Tansey noted that one needs to be very aware that estrogen can be protective against neuroinflammation. Researchers tend to stick with male mice because of these compounding issues. She stressed though that this research could technically be done.

Chairman Binns stated that, substantively, he had nothing new to add. However, he wanted to talk about this process. He felt that this meeting had been a great event. He stated that a tremendous debt of gratitude was owed to Drs. Steele and O'Callaghan for bringing these researchers together and presenting something that had coherence. Chairman Binns stated that he had an experience a couple of years ago, while visiting with the head of neuroscience at Stanford. This researcher told Chairman Binns that there was, at any point of time, 10 years of research that had already been done, but it hadn't been linked yet. Chairman Binns believed this intuitively at the time, but felt now we are proving that we can shortcut this process by bringing together the people with the right expertise. He thanked the Committee and visiting scientists for sharing their expertise. He also thought that the sense of urgency in this matter had been conveyed.

Dr. Tilson thanked Chairman Binns for providing his time, vision, and courage to proceed in this endeavor. The Committee concurred.

The meeting adjourned at 2:27 p.m.

**Appendix**

**Presentation 1 – Lea Steele**

**Gulf War Illnesses, CNS Pro-Inflammatory Processes, and Autonomic Dysregulation**

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**Lea Steele, Ph.D.**  
**August 14, 2006**

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**Consideration of CNS Proinflammatory Processes in Relation to Gulf War Illnesses**

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- **The Gulf War and Gulf War illnesses**
- **Innate immunity, CNS cytokines, and "sickness response" symptoms**
- **Today's presentations and discussions**

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**The Gulf War and Gulf War Illnesses:  
A Brief Overview of the Research**



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

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Aug 2, 1990 - Iraq invaded Kuwait

Jan 16, 1991 - Air strikes began

Feb 24, 1991 - Ground combat began

Feb 28, 1991 - Cease fire declared



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

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~ 700,000 U.S. troops deployed

Relatively few casualties

After the war:

low rate of dx psychiatric illness

high rate of "multisymptom" illness



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

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



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

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- **"Gulf War illness" diverse symptoms in multiple systems, with few objective diagnostic markers**
- **Why were veterans ill?**
  - > Etiology/causes?
  - > Nature of the illnesses/pathophysiology?



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## **Large number of Gulf War-related exposures of potential concern**

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- Chemical weapons
- Oil well fires
- Depleted uranium
- Heavy use of insecticides/repellants
- NAPP pills (pyridostigmine bromide)
- Vaccines
- Infectious diseases
- Tent heaters
- Particulates
- Fuel exposures
- Solvents, CARC paint



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## **Large Amount and Diverse Sources of Information on Gulf War-related Exposures**

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- *Government, special panel reports*
- *Reports assessing exposures types and levels*
- *Research studies*
  - ✓ *Epidemiologic, clinical studies of Gulf War veterans*
  - ✓ *Occupational health studies related to exposures*
  - ✓ *Animal, in vitro studies related to exposures*



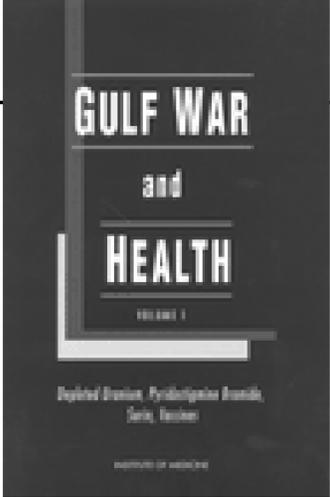
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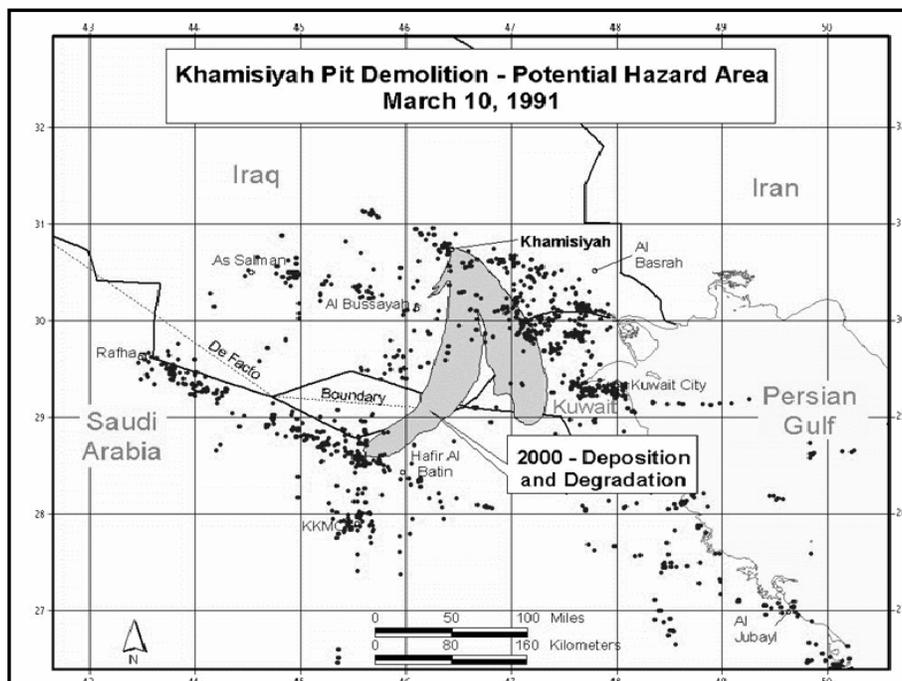
**Summary/Panel Reports**

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



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

Study	Year	Animal Model	Major Finding
Burchfiel <sup>44</sup>	1976	monkey	Persistent effects on electroencephalograph readings
Husain <sup>120</sup>	1993	mouse	Delayed development of spinal cord lesions
Jones <sup>49</sup>	2000	rat	Chronic reduction in nicotinic ACh receptor binding in cerebral cortex
Kassa <sup>165</sup>	2000	rat	Chronic alteration in immune function (lymphocyte proliferation, bactericidal activity of macrophages)
Kassa <sup>167</sup>	2000	rat	Persistent changes in DNA and protein metabolism in liver tissues
Kassa <sup>166</sup>	2001	rat	Subtle chronic signs of neurotoxicity and immunotoxicity with repeated exposures
Kassa <sup>161</sup>	2001	rat	Impaired spatial memory
Conn <sup>57</sup>	2002	rat	No persistent effects on reported indices of temperature regulation and motor activity
Henderson <sup>13</sup>	2002	rat	Delayed, persistent changes in cholinergic receptors in brain areas associated with memory loss and cognitive changes
Hulet <sup>126</sup>	2002	guinea pig	Persistent failure to habituate on functional test battery
Scremin <sup>263</sup>	2002	rat	Persistent increase in cerebral blood flow in specific areas
Kalra <sup>151</sup>	2002	rat	Suppression of immune response (antibody-forming cells and T cell responses) mediated by the autonomic nervous system
Roberson <sup>264</sup>	2002	guinea pig	Chronic depression of AChE activity, persistent behavioral changes (disordered activity, increased rearing behavior)
Husain <sup>127</sup>	2003	mouse	Persistent reductions in respiratory exchange, blood AChE activity and BChE activity, NTE activity in various tissues
Scremin <sup>262</sup>	2003	rat	Down-regulation of muscarinic receptors in hippocampus, decreased habituation
Kassa <sup>162-164</sup>	2003 2004 2004	mouse	Chronic alteration in immune function (increase in CD19 cells, decrease in CD4 cells, decrease in mitogen-induced lymphoproliferation, increased NK cell activity)

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**April 2003 Report from DOD Special Assistant for Gulf War Illnesses**

# Environmental Exposure Report

## Pesticides

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

**1-800-497-6261**

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

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## Information Synthesis/Analysis

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**What does all this tell us about  
Gulf War illnesses?**



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## Epidemiologic Studies: General Findings

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- **Mortality:** no overall increase in disease-related mortality;  
higher rate of brain cancer mortality in relation to Khamisiyah
- **Diagnosed medical conditions**
  - Excess rate of ALS
  - Excess rates of chronic fatigue syndrome (40x), fibromyalgia
- **Psychiatric conditions**
  - Overall rates of psych conditions low (e.g. PTSD: 2 – 10%)
  - Higher PTSD rates associated with combat, other psych stressors during deployment



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### Epidemiologic Studies: General Findings

- All studies show significantly elevated rates of symptoms, symptom complexes, “Gulf War illness”



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

Group Studied	Case Definition Used	Prevalence in Gulf War Veterans	Prevalence in Non-Gulf Veterans	Excess Illness in Gulf Veterans
Pennsylvania Air Guard <sup>85</sup>	CMI	45%	15%	30%
U.K. male veterans <sup>349</sup>	CMI (modified)	62%	36%	26%
Kansas veterans <sup>265</sup>	KS Gulf War Illness	34%	8%	26%
Kansas veterans <sup>265</sup>	CMI (modified)	47%	20%	27%
New England Army veterans <sup>243</sup>	CMI (modified)	65%	33%	32%

CMI: chronic multisymptom illness, as defined by Fukuda et al.<sup>85</sup>

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**Gulf War Illnesses**

Studies consistently indicate 25-30% of Gulf War veterans affected by Gulf War illness symptom complex

Complex of multiple symptom types:

- *Neuro/cognitive/mood*
- *Pain*
- *Fatigue/sleep disturbances*
- *Gastrointestinal*
- *Skin*
- *Respiratory*

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**Gulf War Illnesses**

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**Gulf War Illnesses**

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

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

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

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

---

<b>Psych stressors</b>	Evidence consistently indicates <u>no</u> association
<b>Pesticides</b>	Consistent, significantly elevated associations, indication of dose/response effect
<b>NAPP/PB pills</b>	Consistent, significantly elevated associations, indication of dose/response effect

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

---

<b>Chemical weapons</b>	Two studies support sign association
<b>DU</b>	Almost no useful information
<b>Oil well fires</b>	Results inconsistent, may relate to proximity/duration
<b>Vaccines, individual</b>	Very little clear information
<b>Number of vaccines</b>	1 strong study suggests association



### **Gulf War illness: Etiologic Factors**

---

- **Neurotoxic exposures: Strongest epidemiologic evidence supports pesticides and PB as etiologic factors in GWI**
  - *Information from other sources (exposure patterns, occupational and animal studies, etc) supports plausibility of association*



## Gulf War illness: Etiologic Factors

---

- **Epidemiologic studies consistently indicate that psych stressors during deployment not associated with higher rates of GWI**
  - *Animal studies suggest possible synergism w/exposures*
  - *Consistent association of psych stressors with PTSD, other psych diagnoses*



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## Clinical Studies in Ill Gulf War Veterans: Objective Indicators of Pathology

---

- **Neuroimaging: 3 MRS studies indicate reduced brain cell mass in brainstem, basal ganglia, hippocampus**
  - 1 study: elevated dopamine in veterans with left basal ganglia damage
- **Autonomic dysfunction: 4 studies indicate abnormalities**
  - Orthostatic intolerance to tilt
  - Blunted heart rate variability responses to stressors, tilt
  - Reduced circadian variation in heart rate variability
- **Neuropsychological testing: indicators of cognitive deficits (attention, visual-spatial skills, memory)**
- **Abnormalities on audiovestibular measures, postural sway tests**



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## Clinical Studies in Ill Gulf War Veterans: Objective Indicators of Pathology

---

### Immune measures:

- Skowera/Peakman:  
Elevated IFN- $\gamma$ , IL-2 (unstimulated CD4);  
elevated IL-10 (stimulated CD4)
- Zhang/Natelson:  
Elevated IL-2, IL-10, IFN- $\gamma$  in PBLs



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## Extensive Amount of Information Available

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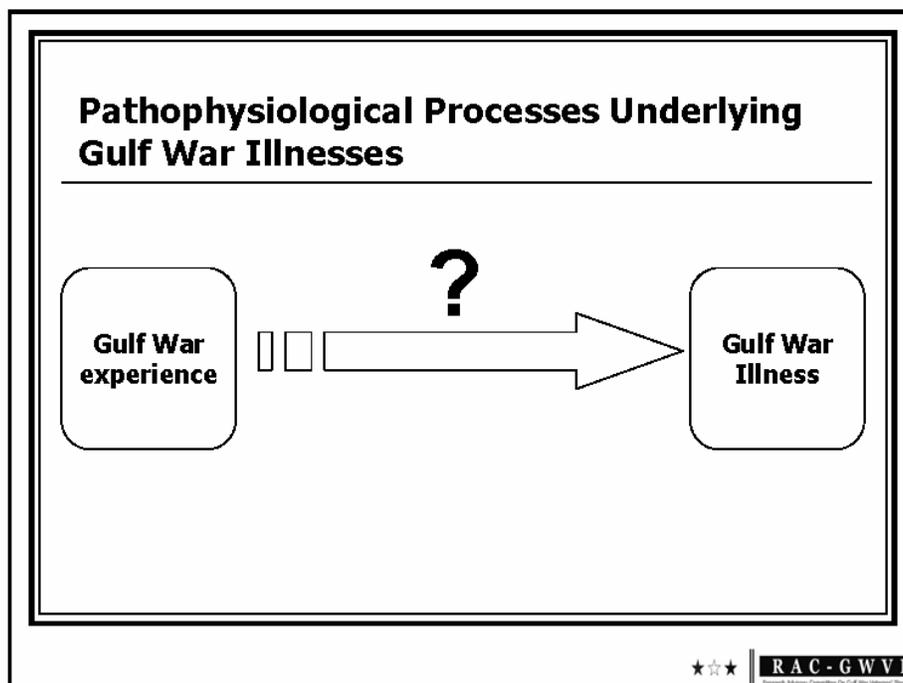
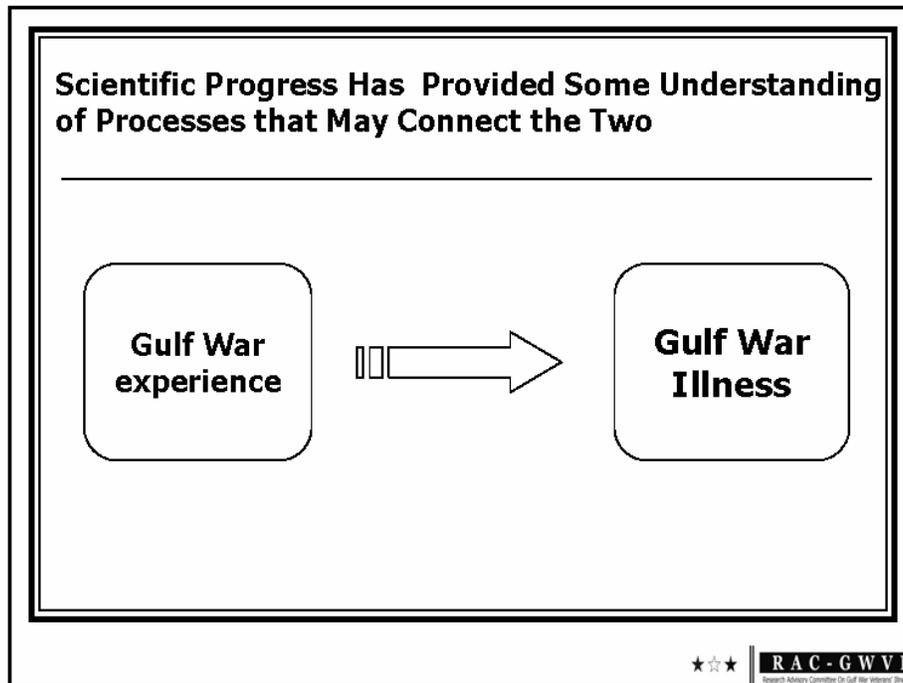
Gulf War  
experiences  
and  
exposures

Gulf War  
Illnesses



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**Why Are Veterans Ill? GWI Pathophysiology:**

**Major Questions Remain**

---

- *What pathophysiological process(es) underlie this complex of multiple types of symptoms in multiple systems?*
- *How might these processes have been triggered by experiences/exposures in the Gulf War?*
- *Why have these symptoms persisted for so long?*
- *Why are there few objective indicators of disease in symptomatic veterans?*



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**Why Are Veterans Ill? GWI Pathophysiology:**

**Major Questions Remain**

---

- *Pathophysiological process(es) that explain diverse types of symptoms in multiple systems?*
- *How might these processes have been triggered by experiences/exposures in the Gulf War?*
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- *What pathophysiological process(es) underlie this complex of multiple types of symptoms in multiple systems?*
- ***How linked to experiences/exposures in the Gulf War?***
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- *Why have these symptoms persisted for so long?*
- ***Why so few objective indicators of disease?***



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## CNS Immune Activation, Cytokines, and "Sickness Response" Symptoms



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**Straightforward Observations: Symptom Complex**

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**Gulf War  
Illness  
Symptom  
Complex**

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**Straightforward Observations**

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**Neuro/Cognitive  
Widespread Pain  
Fatigue  
Misc other**

- Individually, symptoms are common
- Symptom complex?

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**Resembles "Sickness Response" Symptom Complex**

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**Neuro/Cognitive  
Widespread Pain  
Fatigue  
Misc other**

- Fairly common symptom complex
- Associated with familiar conditions

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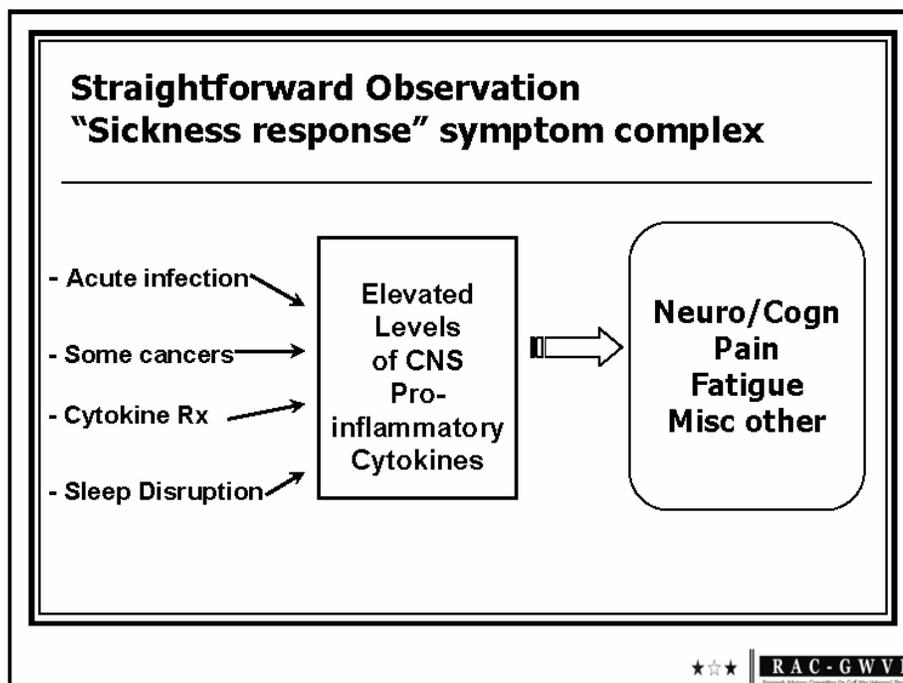
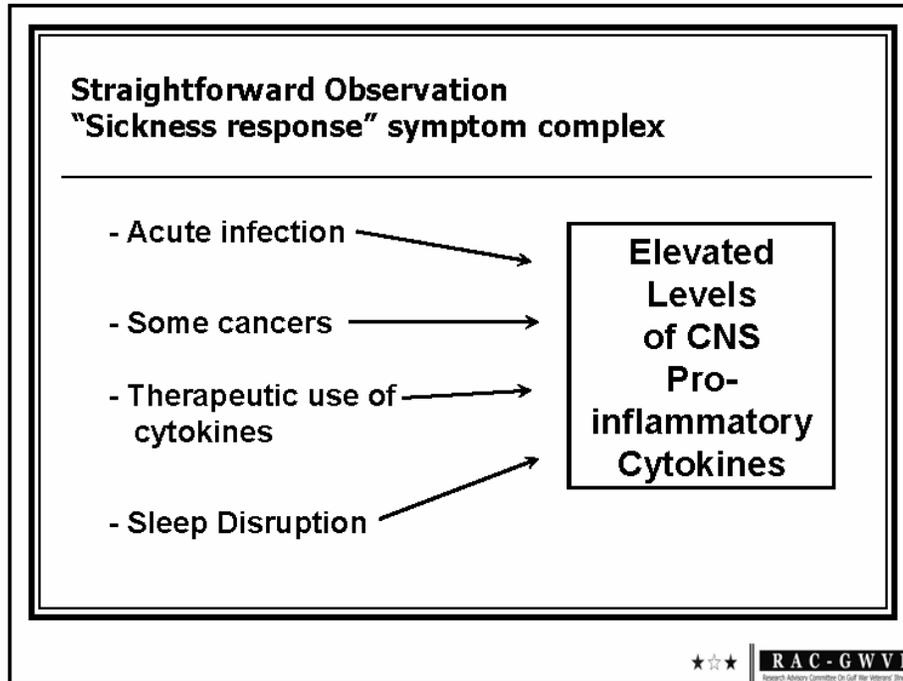
**"Sickness Response" Symptom Complex Associated with Familiar Conditions**

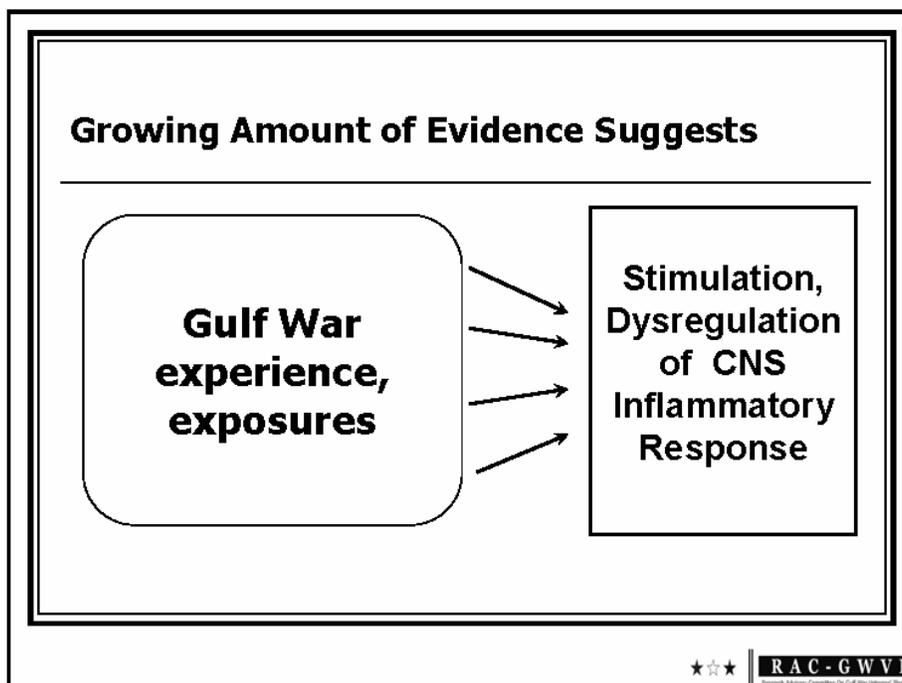
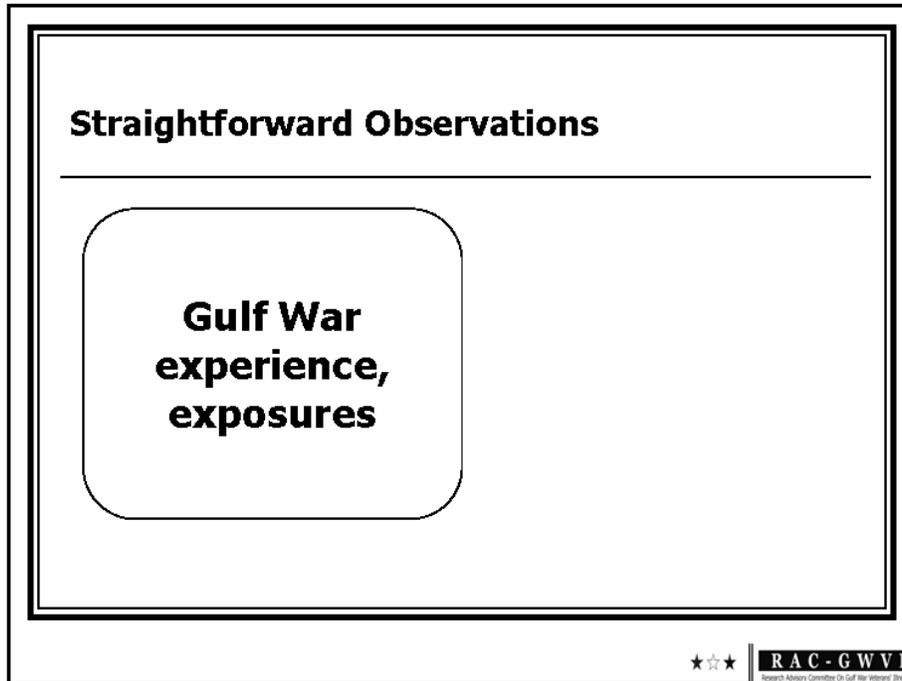
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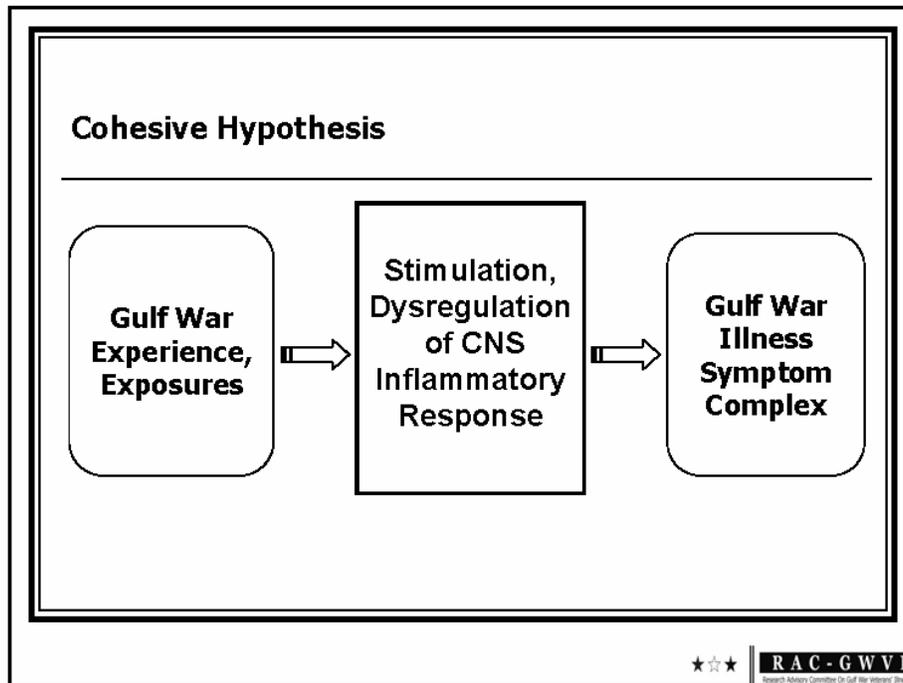
**Neuro/Cognitive  
Widespread Pain  
Fatigue  
Misc other**

- Acute infection
- Some cancers
- Therapeutic use of cytokines
- Sleep Disruption

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**Innate immunity, Cytokines, and  
Neuroinflammation**

**101**

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## Cytokines

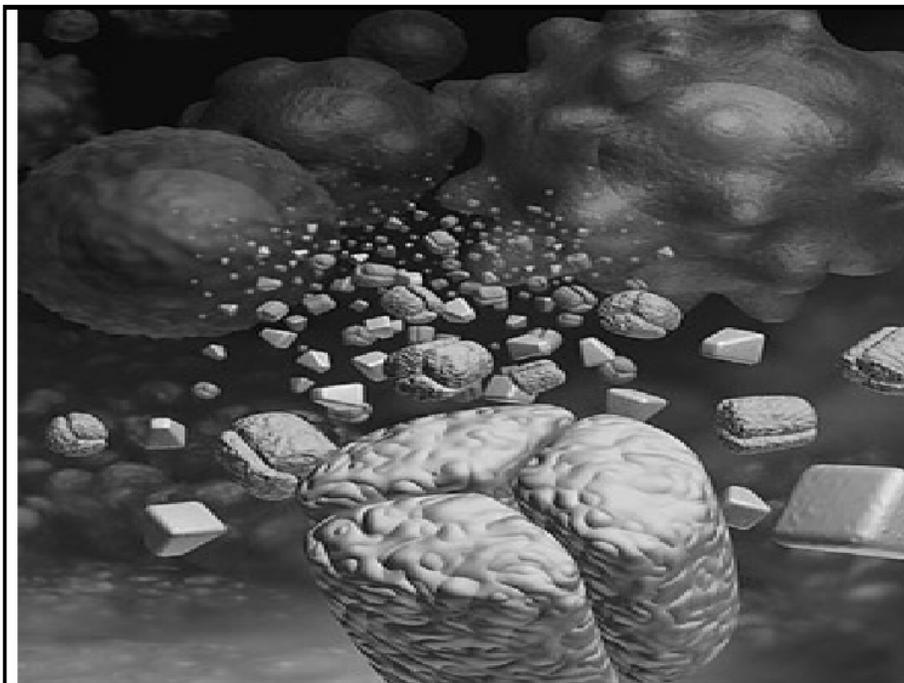
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- **Cytokines are proteins, produced primarily by immune cells; play multiple roles in intracellular communication**
- **Orchestrate host defense in activating, regulating innate and adaptive immune response**
- **Over 200 cytokines and receptors identified; multiple grouping schemes (e.g. proinflammatory: TNF, IL-1, IL-6)**
- **Insult/infection stimulates cytokine "burst"; signaling cascade**



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## Cytokines

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- Usually produced transiently; prolonged action can be harmful
- Pleiotropic, redundant
- Can act synergistically, antagonistically; generally part of a "cocktail"



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## Immunity in the Central Nervous System

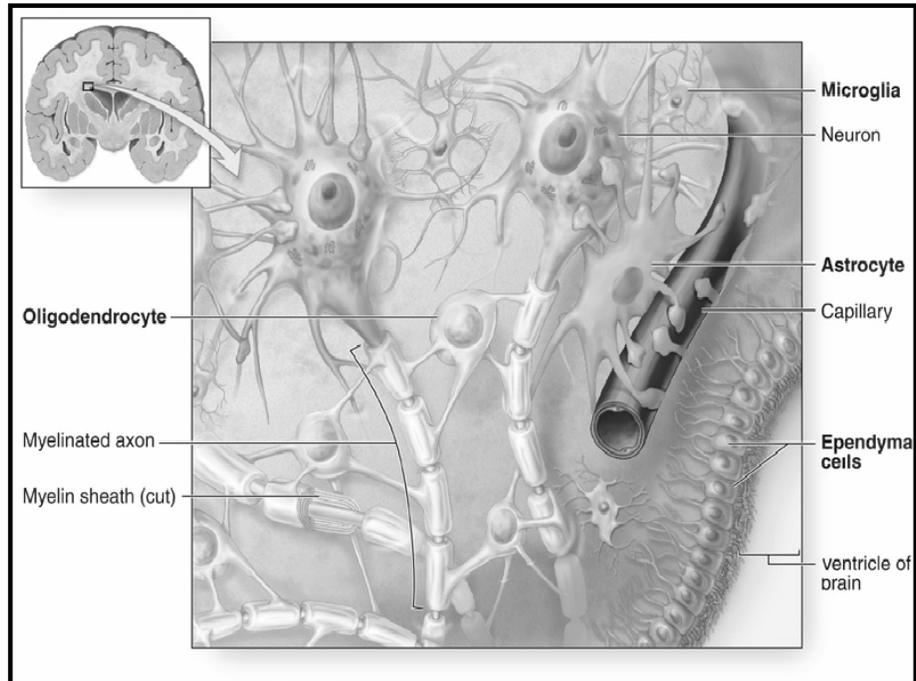
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- CNS used to be considered "immunoprivileged"; glial cells considered structural support for neurons
- Many more glial cells (microglia, astrocytes) than neurons; respond to changes in CNS microenvironment
- CNS immune response primarily "innate": neuroinflammation
  - *Lymphocytes, cytokines can infiltrate if BBB breached*
  - *CNS inflammatory response can be triggered by peripheral immune activation*



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## CNS insult → Microglia activation

### Microglia change shape, mobilize

- Changes in surface markers and receptors
- "Burst" of soluble compounds → signaling cascade
  - *Cytokines*
  - *ROS, enzymes*
  - *Prostaglandins, neurotrophic factors*
- Response profile varies with nature of insult  
(*infection, injury, cell infiltration, etc*)

## **Regulation of inflammation**

---

- **Anti-inflammatory cytokines**
- **Other factors associated with inflammatory response (e.g. NF Kappa B)**
- **HPA axis**
- **Neuronal "contact inhibition" in CNS**
- **Periphery: cholinergic anti-inflammatory pathway**



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## **Cytokines and the Expression of "Sickness Response" Symptom Complex: Diverse Sources of Info**

---

- **Animal "sickness response" research**
- **Infectious disease**
- **Chronic pain models**
- **Symptoms associated with cancer, chemotherapy**
- **IFN therapy for hepatitis, cancer**
- **Sleep, psych conditions, other conditions**



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## **Cytokines and "Sickness Response" Symptoms Animal research**

---

- **Peripheral, CNS infusion of microbes, LPS, or specific cytokines elicits fever, increased pain sensitivity, reduced activity, memory impairments, etc**
- **Response may vary with specific organism, cytokines, combos**
- **Symptoms elicited by cytokines in the CNS; peripheral cytokines stimulate CNS cytokines**
- **Peripheral cytokine levels not indicative of CNS cytokine levels**
- **Extremely low CNS cytokine levels required to elicit symptoms**



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## **Cytokines and "Sickness Response" Symptoms: Infectious Disease**

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- **In humans, acute infection associated with peripheral elevations in variety of cytokines**
- **Cytokine levels highly correlated with severity of "acute phase" symptoms: fatigue, myalgia, poor concentration, etc**
- **Chronic symptoms develop in a subset of individuals following infection**
  - **Appear to be differences in patterns of cytokine expression between those who do/don't develop chronic sequelae**



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## **Persistence of Symptoms: Chronic Pain Models**

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- **Animal models of chronic pain following peripheral injury**
  - Pain develops at sites distant from initial injury
  - Pain persists after damage of initial injury is resolved
  - Exaggerated pain response: hyperalgesia, allodynia
- **Persistent pain results from glial activation in the spinal cord**
  - Initiated in microglia, sustained by astrocytes
  - Model: glial "sensitization" process: elevated inflammatory response after multiple "hits"; persists after threshold reached
- **Preliminary indications that process extends to brain**
- **Schwartzman: CRPS associated with elevated cytokines in cerebrospinal fluid**



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## **Cytokines and "Sickness Response" Symptoms: Cancer and Chemotherapy**

---

- Various cancers associated with complex of chronic pain, fatigue, cognitive impairment
- Symptoms can be associated with the disease itself, or precipitated/worsened by some types of chemotherapy ("chemobrain"), radiation
- Symptom severity correlated with cytokine levels
- Fatigue, cognitive difficulties, sleep disturbances persist in subset of patients who are cancer free



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### **Cytokines and "Sickness Response" Symptoms: Interferon Therapy**

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- **IFN-alpha used to treat hepatitis C, cancers**
- **About 1/3 meet criteria for CFS during treatment**
- **IFN treatment stimulates other types of cytokines**
- **2-phase response**
  - *"neurovegetative"; fatigue, pain, sleep disturbances, gastrointestinal problems, anorexia*
  - *"neurocognitive"; depressed mood, cognitive impairment*



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### **Cytokines and "Sickness Response" Symptoms: Diverse Sources of Information**

---

- **Animal "sickness response" research**
- **Infectious disease**
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- **Symptoms associated with cancer, chemotherapy**
- **IFN therapy for hepatitis, cancer**
- **Sleep, psych conditions**



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### **Cytokines and "Sickness Response" Symptoms: Diverse Sources of Information**

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- **Animal findings indicate (and models used in human studies generally assume) that chronic symptoms are likely the result of persistent CNS proinflammatory processes**
- **Most of these research areas involve basic research and drug development efforts to counter adverse effects of elevated CNS proinflammatory processes**



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### **Persistence of Inflammatory Processes in the Central Nervous System:**

#### **Diverse Sources of Research Information**

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- **Neurological, neurodegenerative diseases**
  - Parkinsons Disease
  - ALS
  - Alzheimers Disease
  - Multiple Sclerosis
- **Chronic infection (e.g. AIDS, prion disease, herpes viruses)**
- **Autism**
- **Other....**
- **Little info from research on patients with CFS, FM, MCS**



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## Today's Presentations and Discussions

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- **Dr. Tracey: Autonomic/cholinergic regulation of the inflammatory response**
- **Dr. Klimas: Immune parameters associated with chronic multisymptom illness in the general population**



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## Today's Presentations and Discussions

---

### **Research on Gulf War-related exposures that provide insights into their possible role in the stimulation or dysregulation of CNS inflammatory processes**

- **Dr. Morris: ANS dysregulation following low-level sarin**
- **Dr. Sopori: Effects of sarin, other cholinergic compounds on cholinergic receptors, immune measures, glucocorticoid levels**
- **Dr. Abou-Donia: Indicators of glial activation, elevated ROS, and neuronal cell death following sarin, combined Gulf War-related exposures**



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## Today's Presentations and Discussions

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- **Dr. Hong: Research relating neurotoxic exposures to microglial activation, persistent CNS inflammatory processes associated with neurodegeneration**
- **Dr. Guilarte: Methods for studying CNS inflammatory processes in vivo**



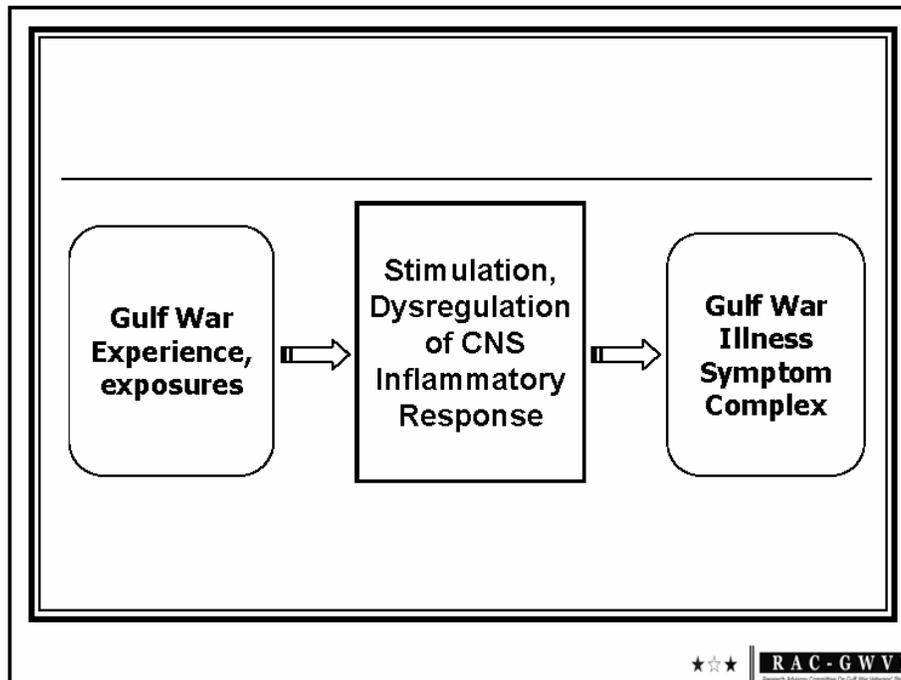
## Today's Presentations and Discussions

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### **Discussion:**

- **General impressions**
- **Priority research questions related to CNS inflammatory hypothesis of GWI**
- **Types of studies needed**



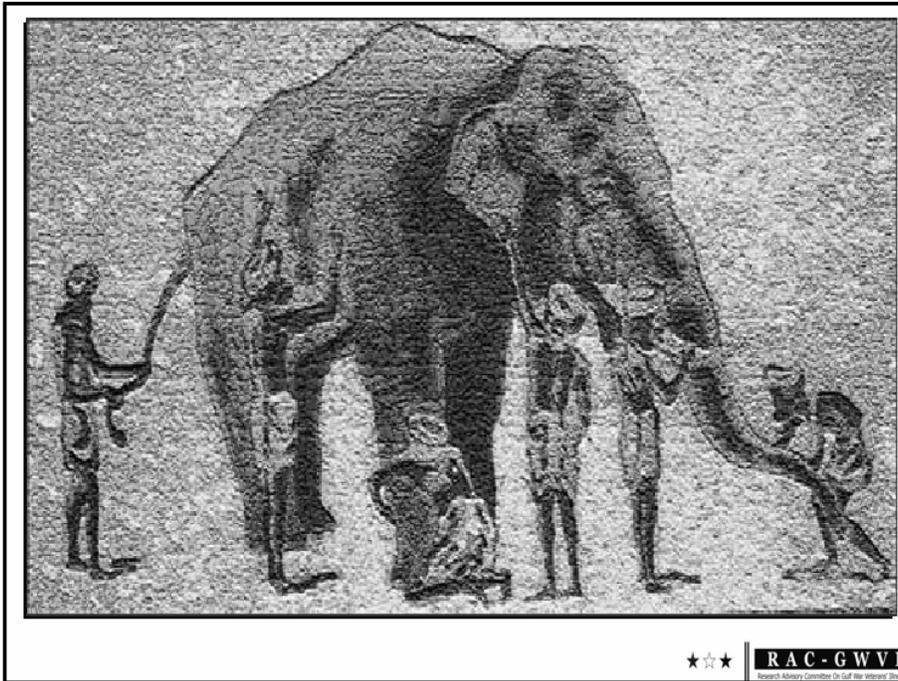


### Hypothesis.....

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- **Potential to address many of the "mysteries" of GWI**
  - *Diverse symptoms in multiple systems*
  - *Few objective markers of disease*
  - *Persistence of symptoms over time*
  - *Linkage with characteristics of Gulf War service*
- **Possible targets for markers, clinical assessment, animal models**
- **May provide targets for treatment interventions**
  - Research already underway in related fields

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## Presentation 2 - Kevin Tracey

*The Feinstein Institute  
for Medical Research*   
North Shore-Long Island Jewish Health System

***The Cholinergic Anti-inflammatory Pathway  
In the Inflammatory Reflex***

**14 August 2006**

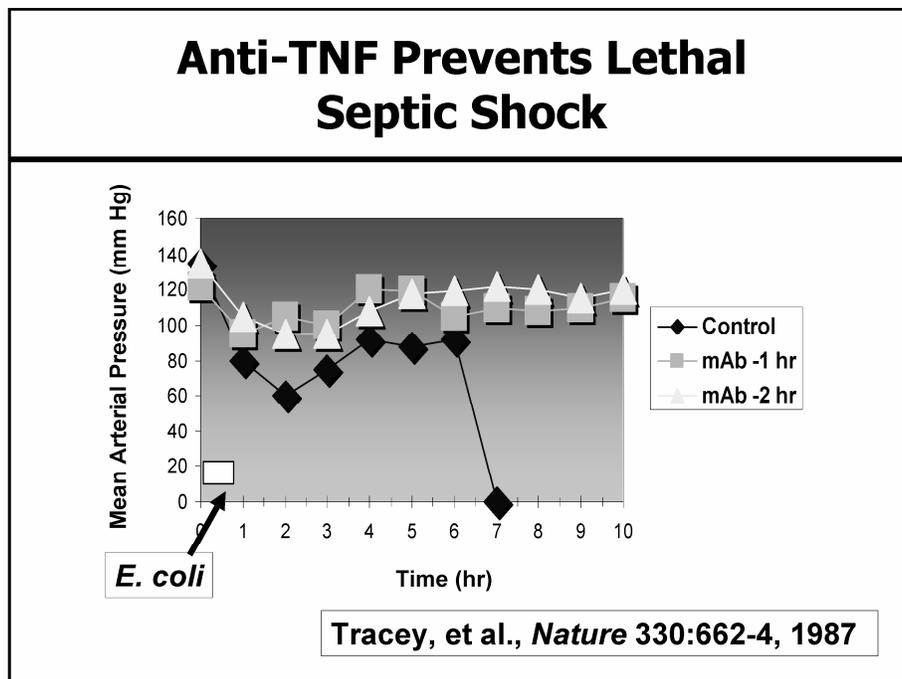
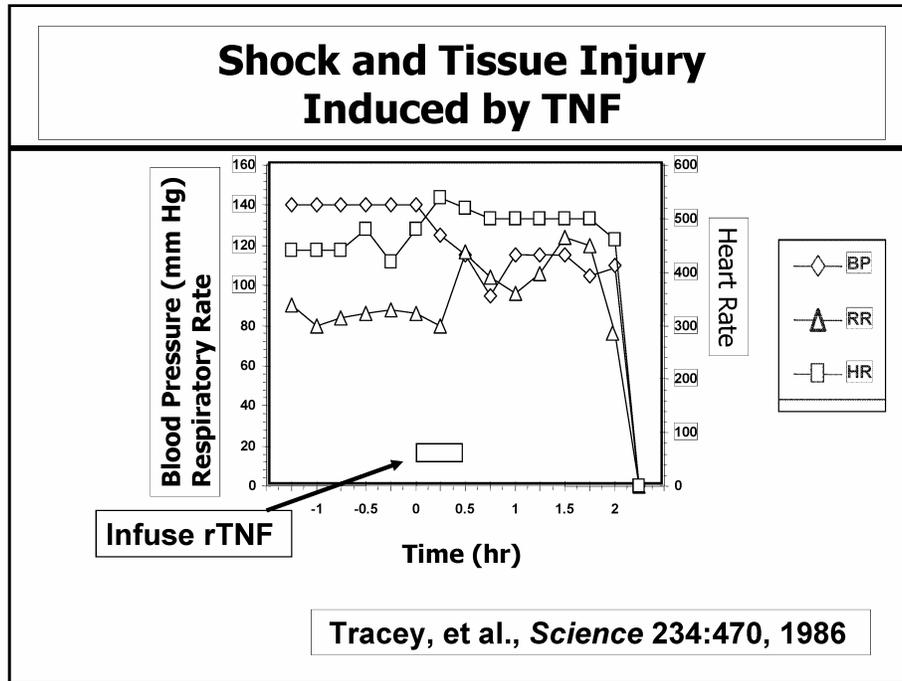
**Kevin J. Tracey, MD**

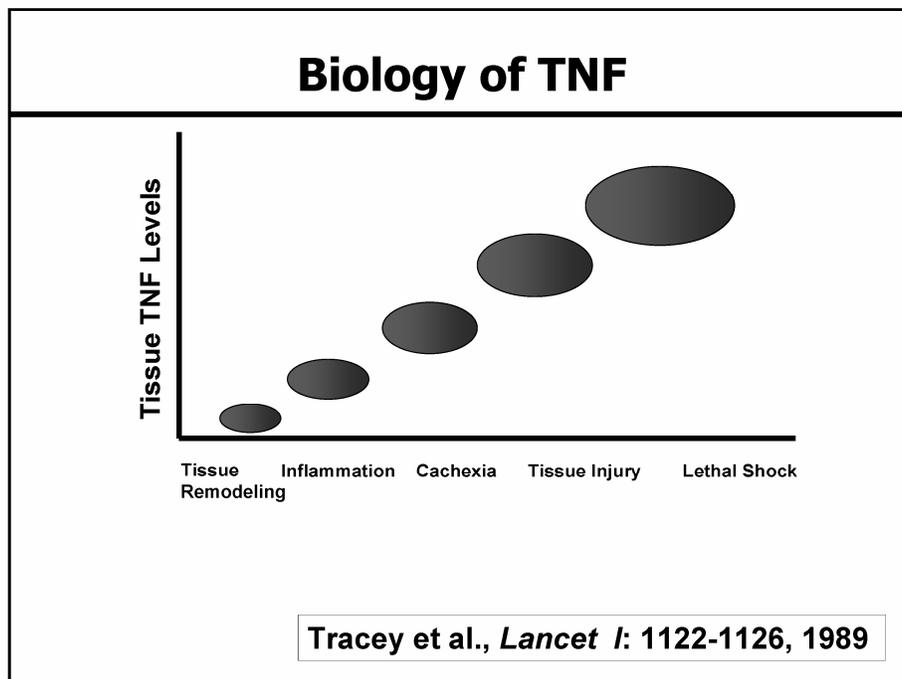
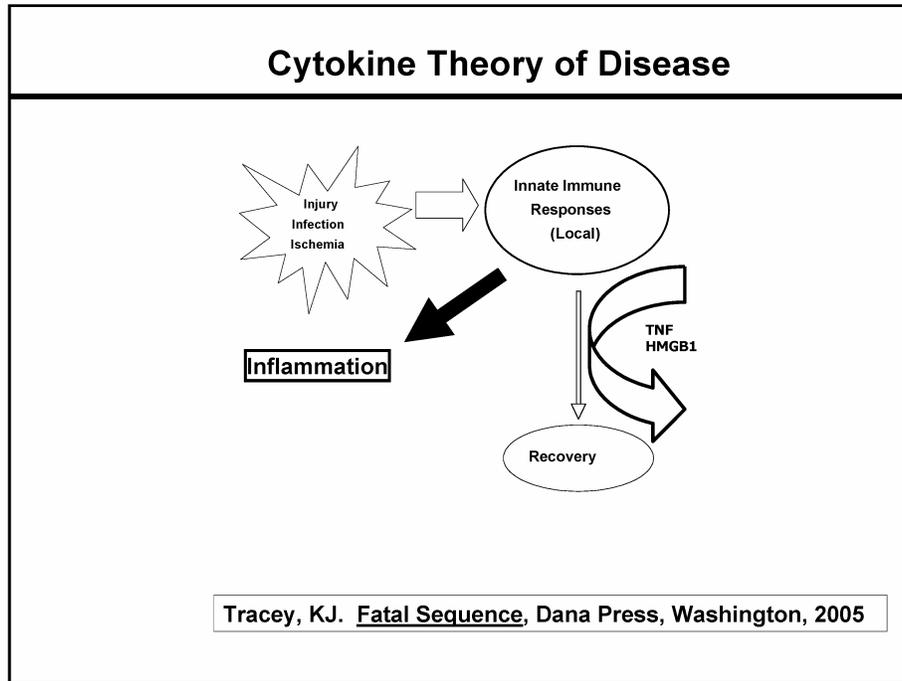
**CEO, Feinstein Institute  
Vice President, Research, NSLIJHS**

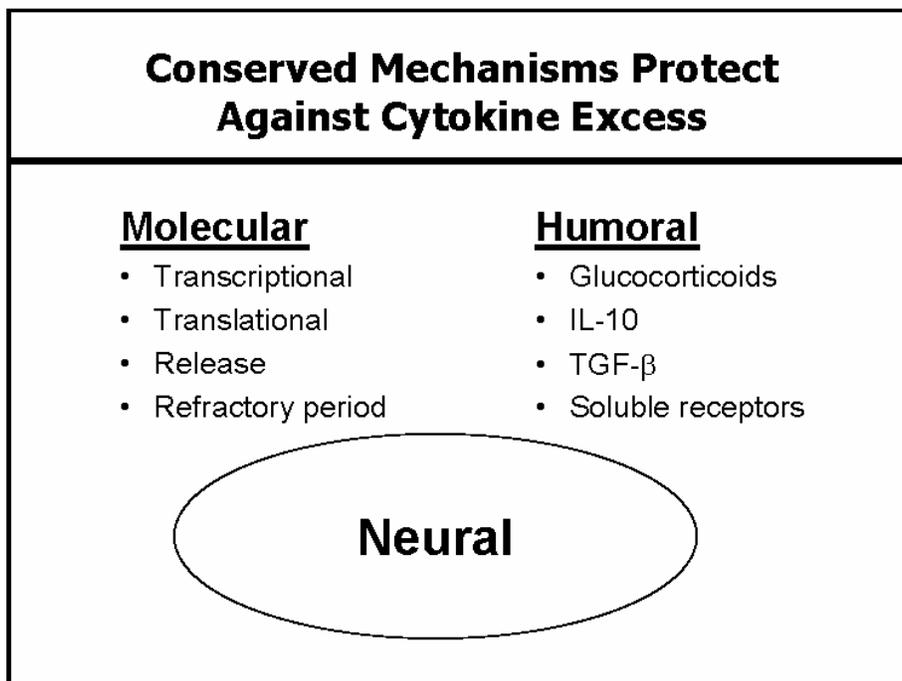
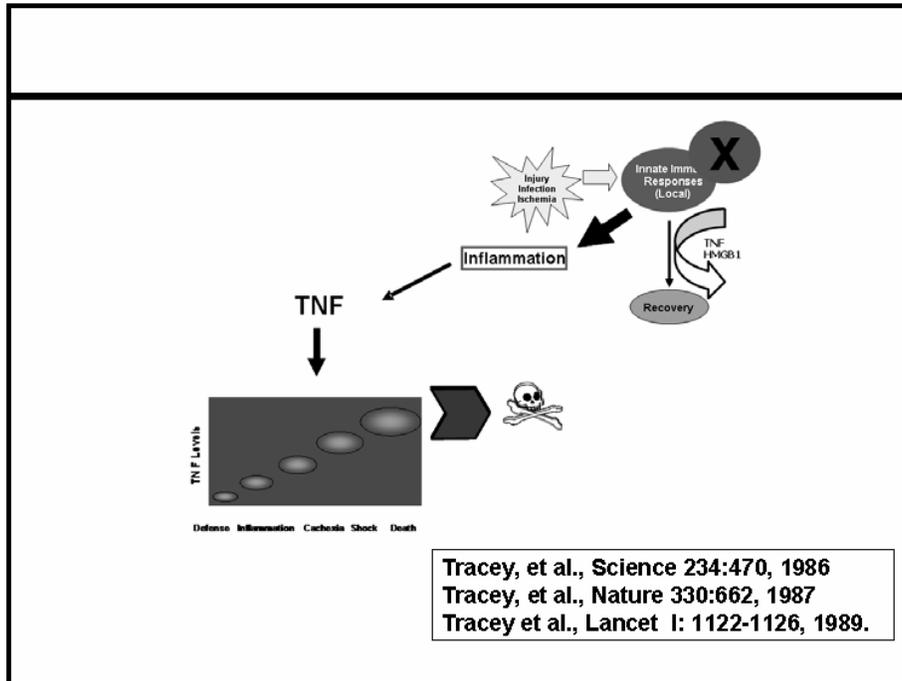
## Janice

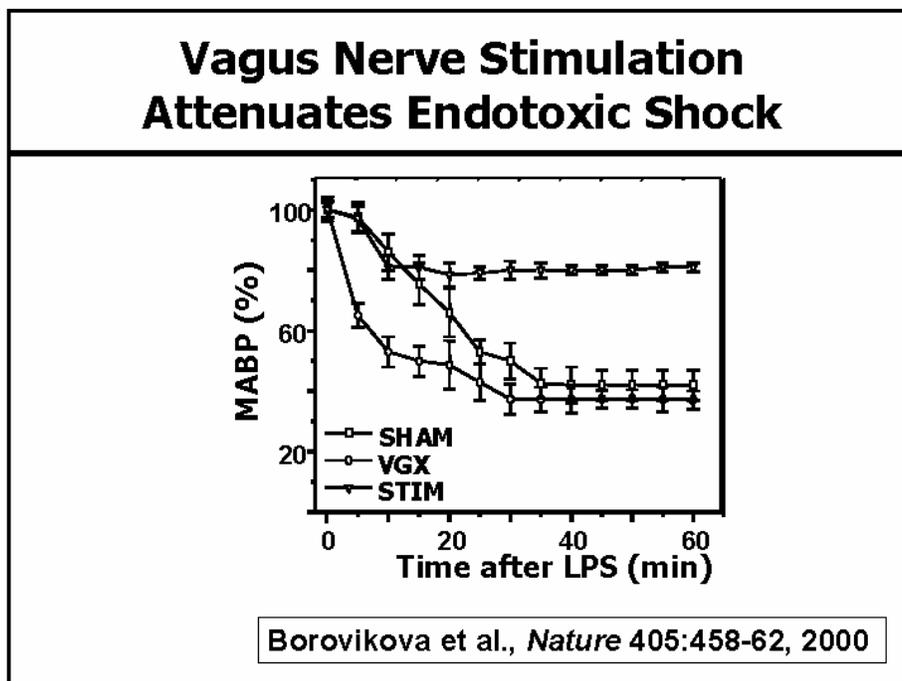
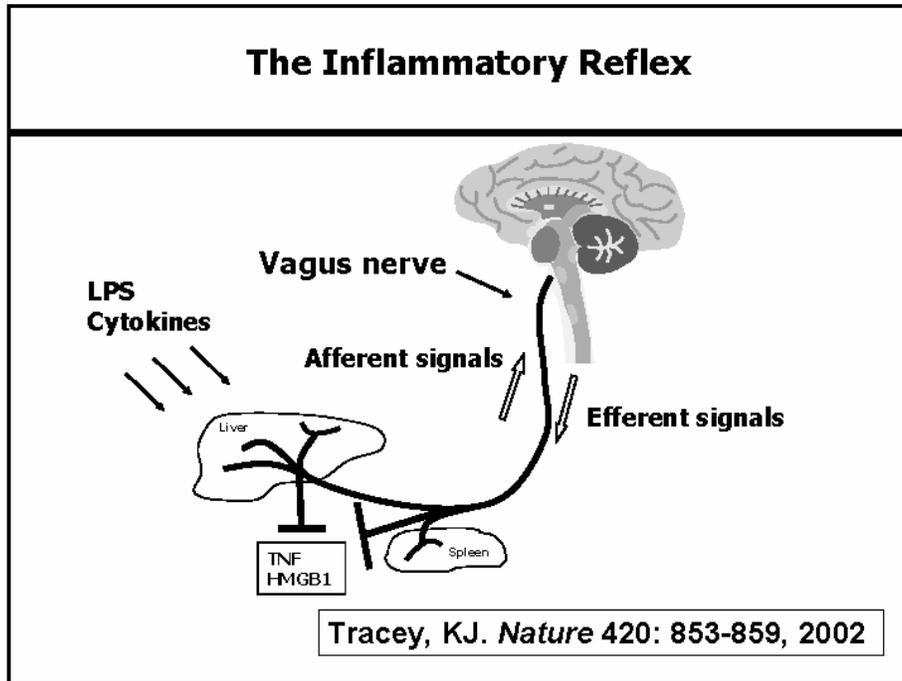
- Friday, May 3, 1985, 5:35 pm
  - 11 month old girl transported to ER via ambulance
  - Crying and clutching her pink teddy-bear blanket
  - 75% of her total skin surface injured with full and partial thickness scald
- Monday, May 6, 1985
  - Tangential excision and skin grafting
- Tuesday, May 7th, 1985, 9:45 PM
  - Acute septic shock
  - Renal failure
  - Respiratory failure

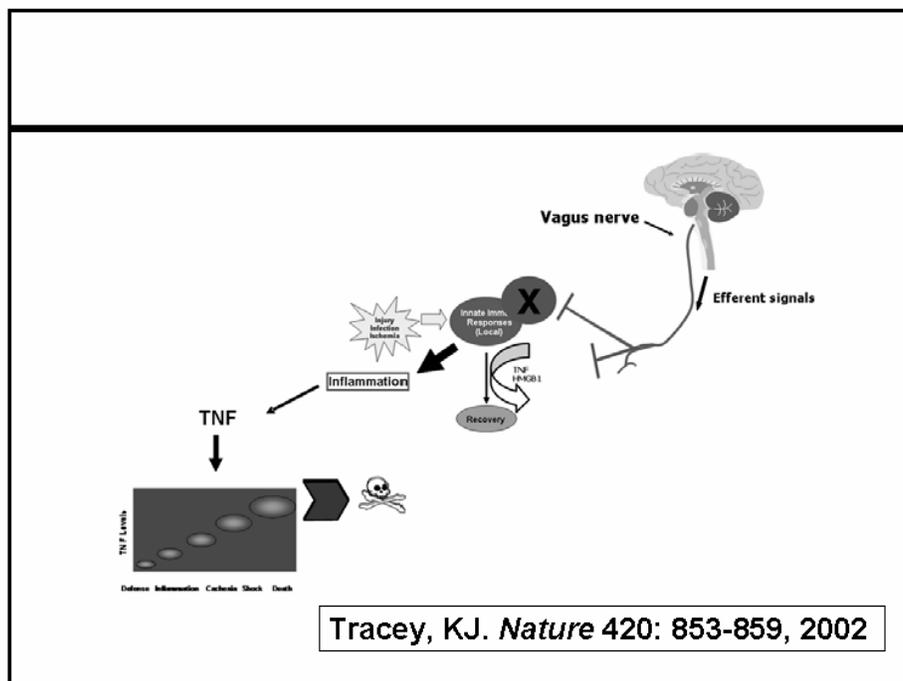
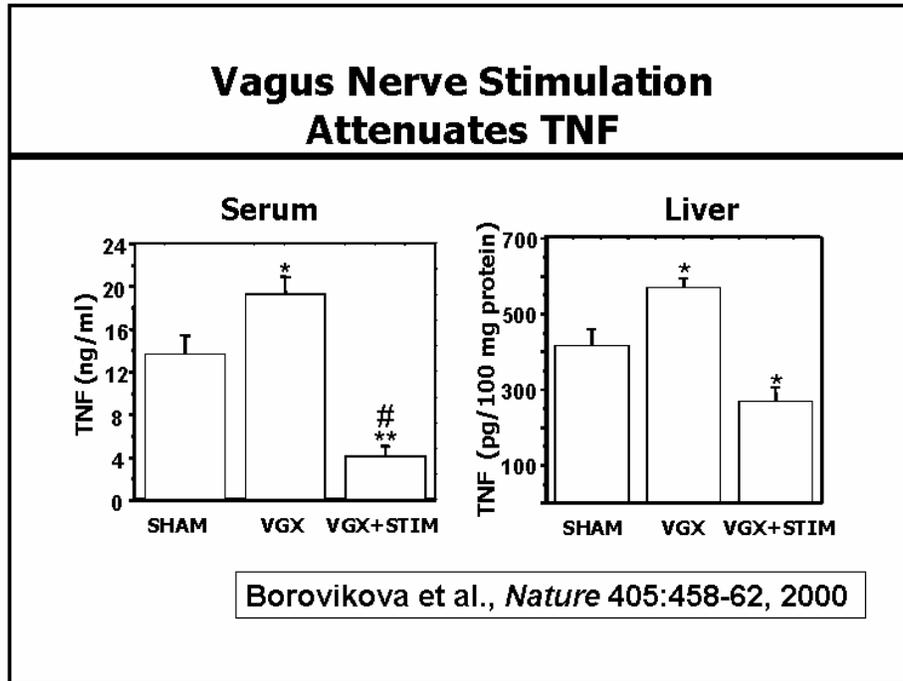
Tracey, K.J. Fatal Sequence, Dana Press, Washington, 2005



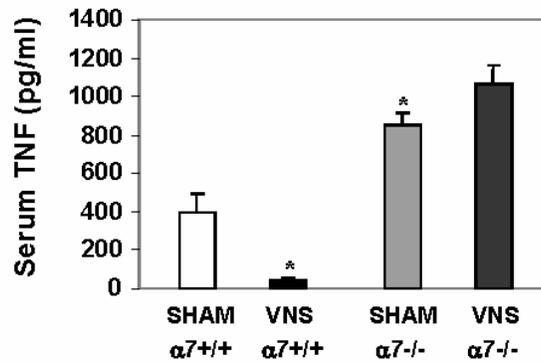






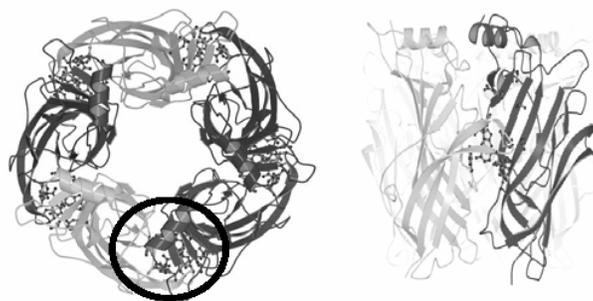


### Nicotinic Acetylcholine Receptor $\alpha 7$ Subunit is an Essential Regulator of Inflammation



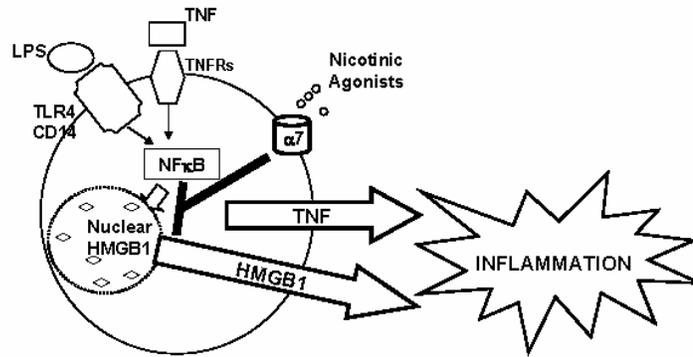
Wang et al., *Nature*, 421: 384-388, 2003

### Pentameric Structure of Nicotinic Acetylcholine Receptor

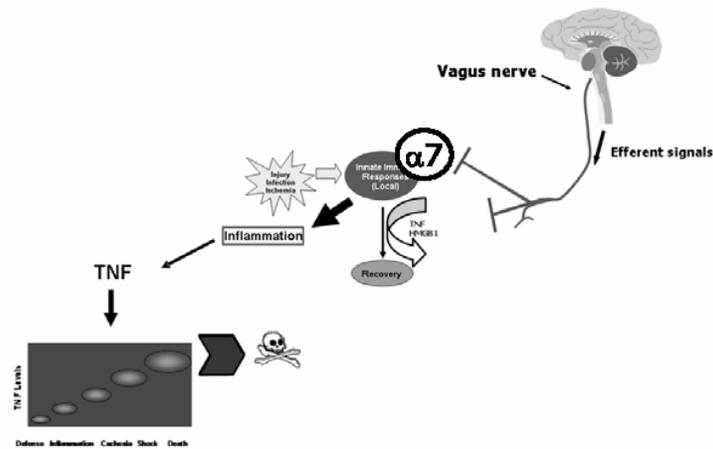


Brejcs, K. *Nature* 411: 269-276, 2001

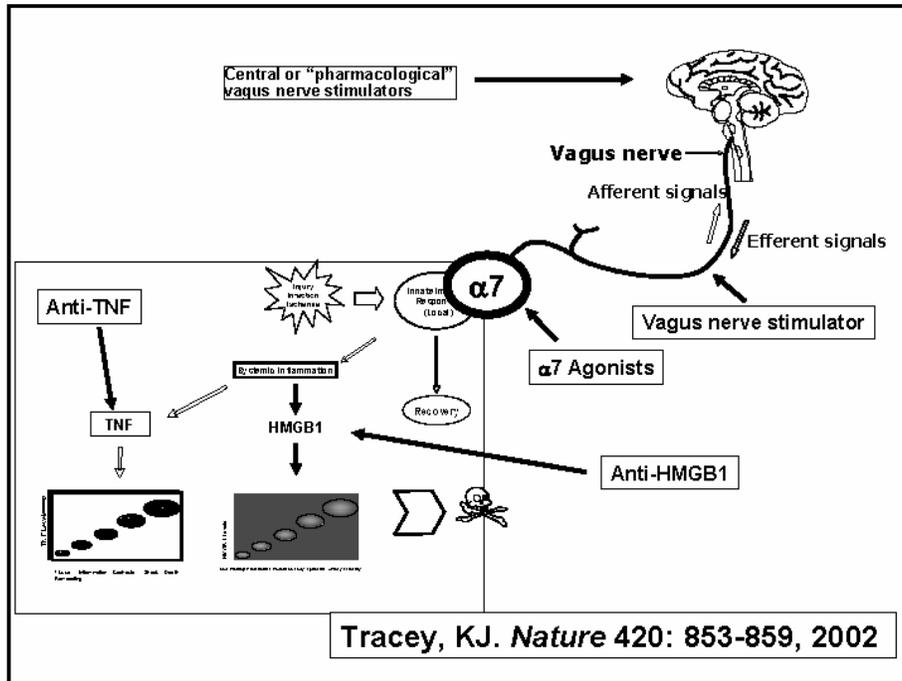
### $\alpha 7$ Inhibition of Cytokine Release in Macrophages



Wang et al, *Nat Med*, 10:1216-21, 2004



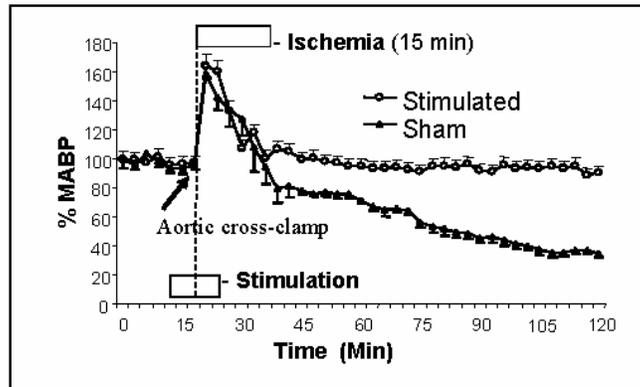
Tracey, KJ. *Nature* 420: 853-859, 2002



### Efficacy of VNS and $\alpha 7$ Agonists

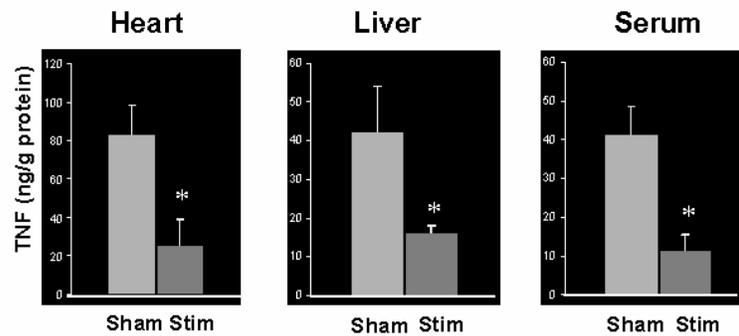
Disease	VNS	$\alpha 7$
Endotoxemia	X	X
Sepsis	X	X
Arthritis	X	X
Hemorrhagic Shock	X	X
Ischemia-reperfusion	X	X
Subcutaneous inflammation	X	X
Pancreatitis	X	X
Autoimmune diabetes		X
Ileus	X	X
Colitis		X

## Vagus Nerve Stimulation Attenuates Ischemia Reperfusion



Bernik et al., *J. Exp. Med.* 195:781-788, 2002  
Bernik et al., *J. Vasc. Surg.* 36:1231-6, 2002

## Vagus Nerve Stimulation Attenuates TNF in Ischemia Reperfusion



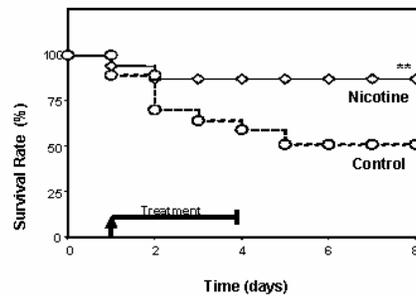
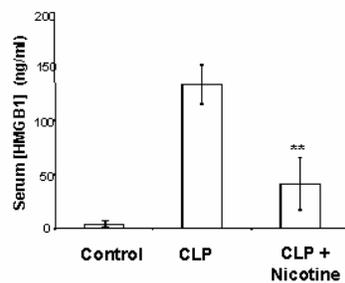
Bernik et al., *J. Exp. Med.* 195:781-788, 2002  
Bernik et al., *J. Vasc. Surg.* 36:1231-6, 2002

### Development of $\alpha 7$ Agonists as Anti-inflammatory Agents: Inhibition of TNF Release

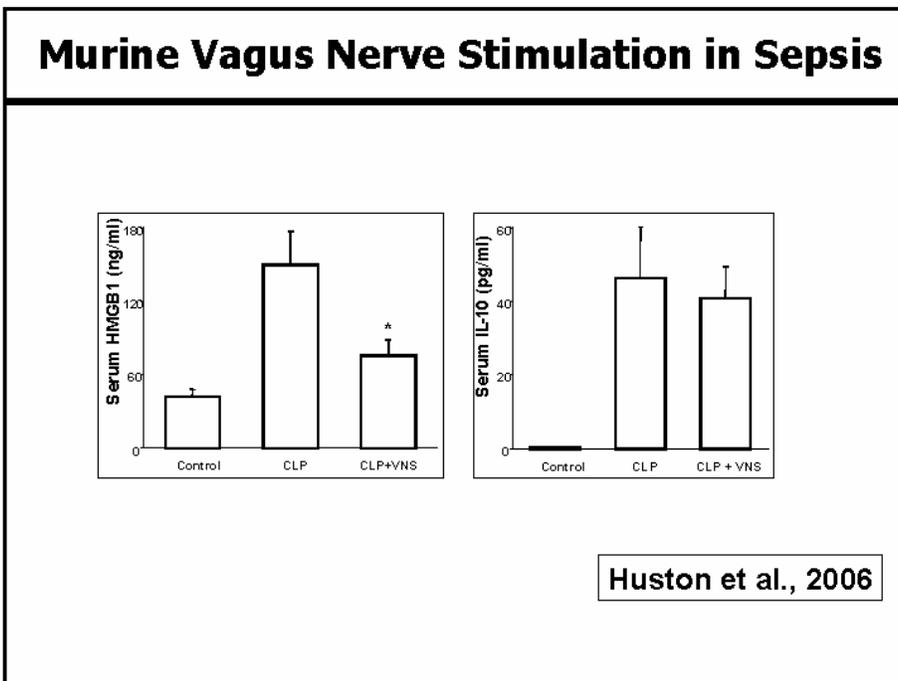
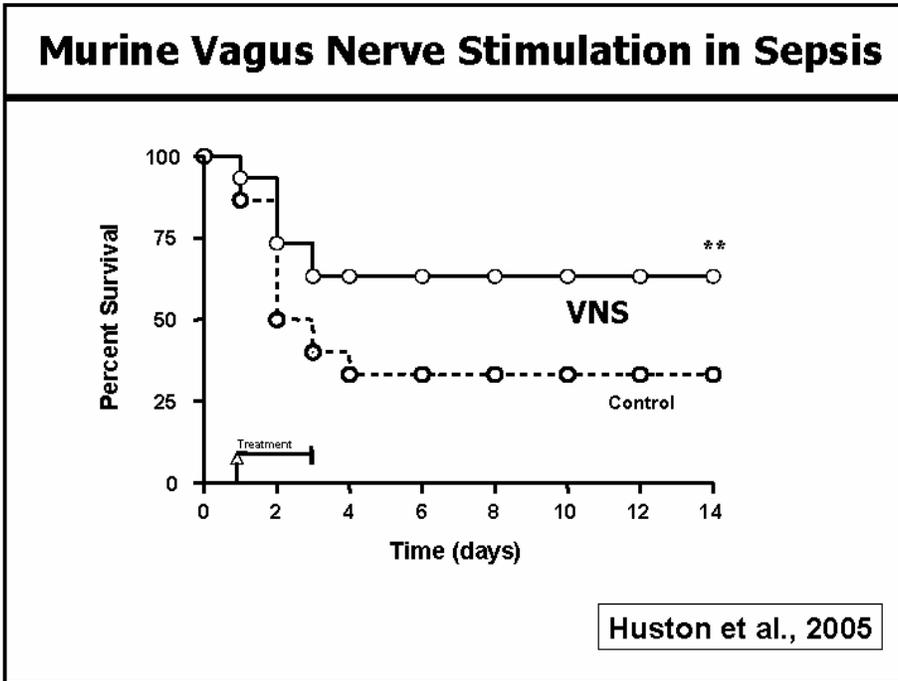
Compound	EC <sub>50</sub> ( $\mu$ M)
CAP13	< 1
CAP15	< 1
CAP22	~ 1
CAP17	< 10
CAP19	< 10
CAP25	< 10
CAP26	< 10
CAP02	< 25
CAP11	< 25
CAP06	< 50
CAP03	Inactive
CAP12	Inactive
CAP27	Inactive
CAP23	Inactive

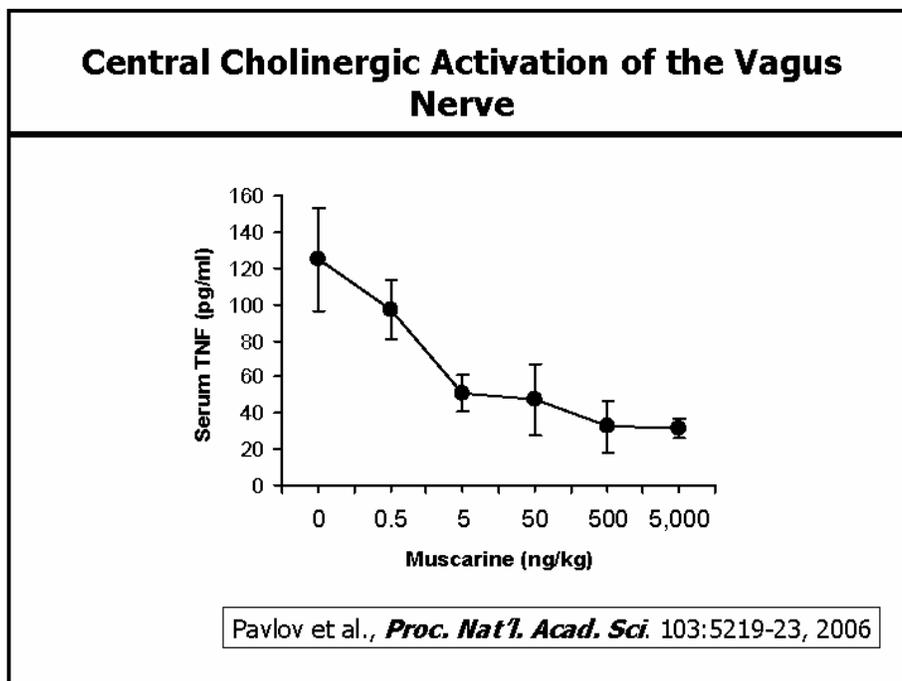
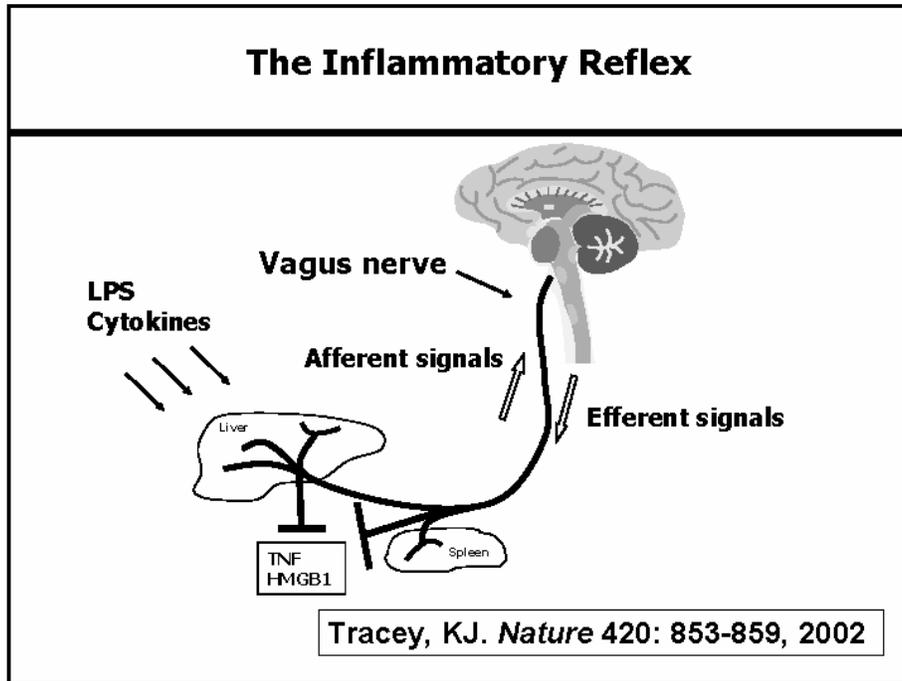
Y. Al-Abed, 2002-5

### Cholinergic Agonists Suppress HMGB1 During Severe Sepsis (CLP)

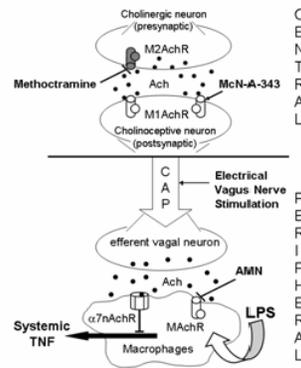


*Nature Medicine*, 10:1216-21, 2004



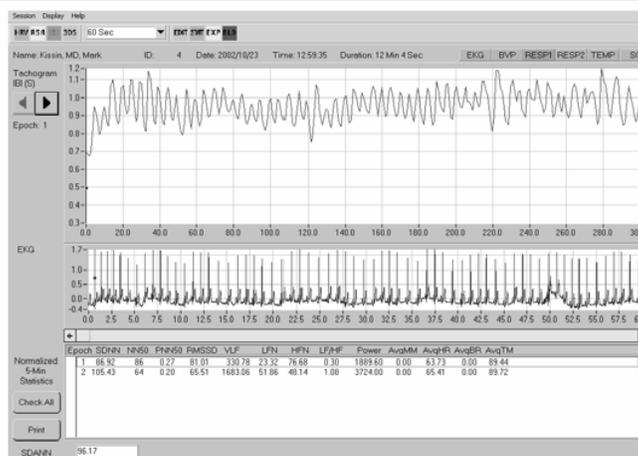


## Central Cholinergic Activation of the Vagus Nerve



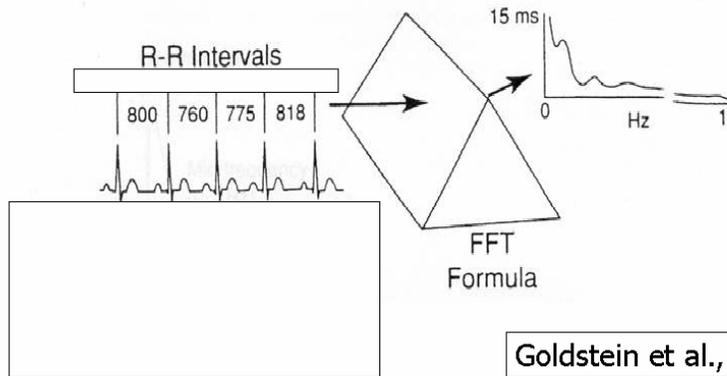
Pavlov et al., *Proc. Nat'l. Acad. Sci.* 103:5219-23, 2006

## Instantaneous Heart Rate: Tachogram

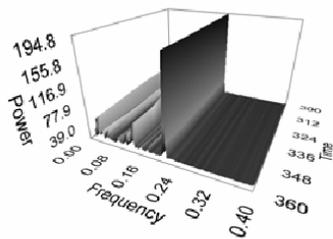


Goldstein et al., 2005

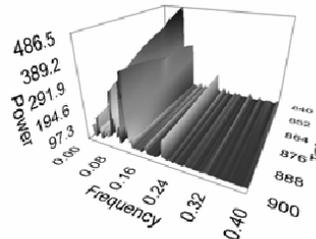
## Fast Fourier Transform of Tachogram



## Vagus Nerve Activity in Volunteers

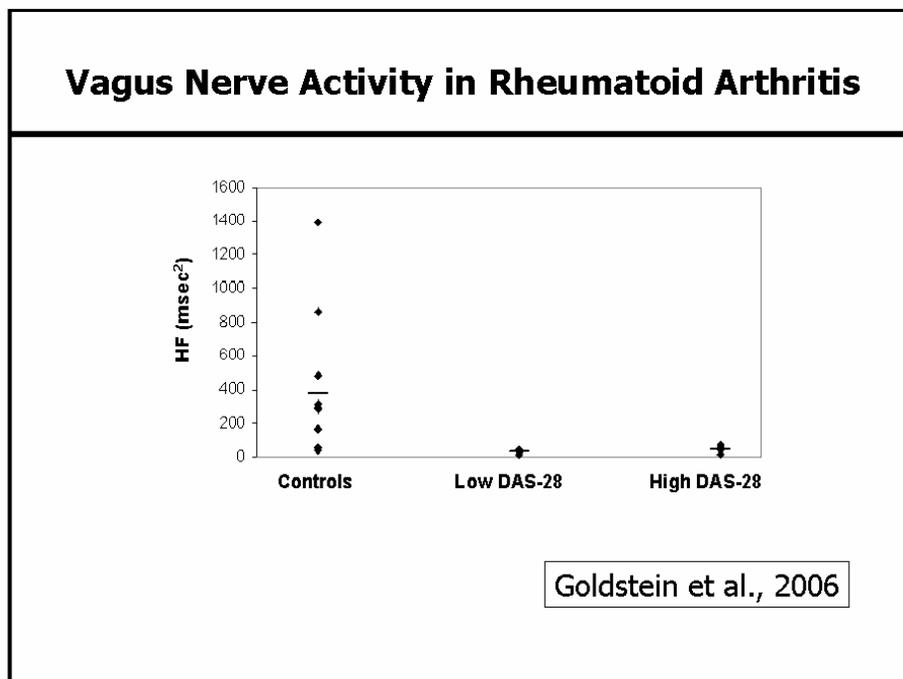
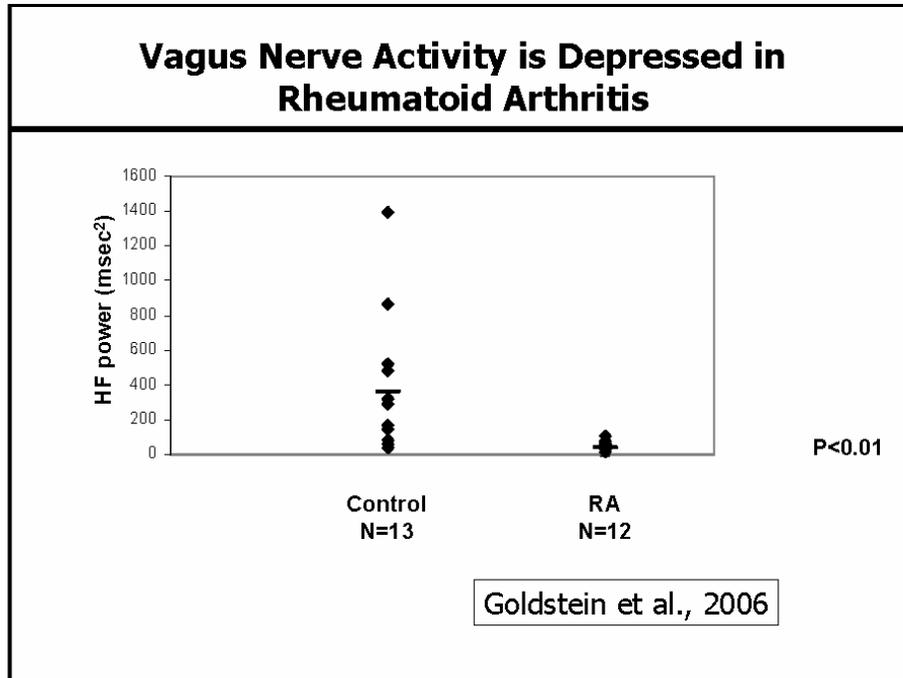


Vagus Predominate

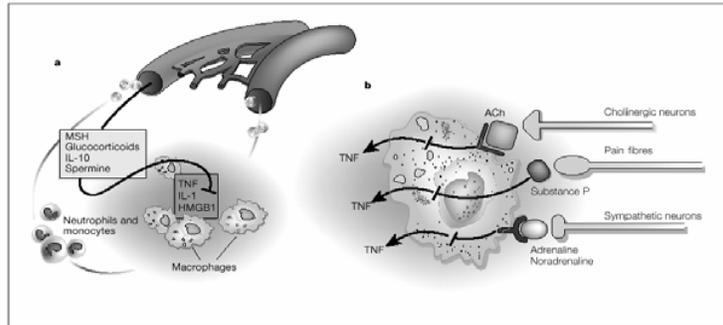


Sympathetic Predominate

Goldstein, Czura et al., 2003

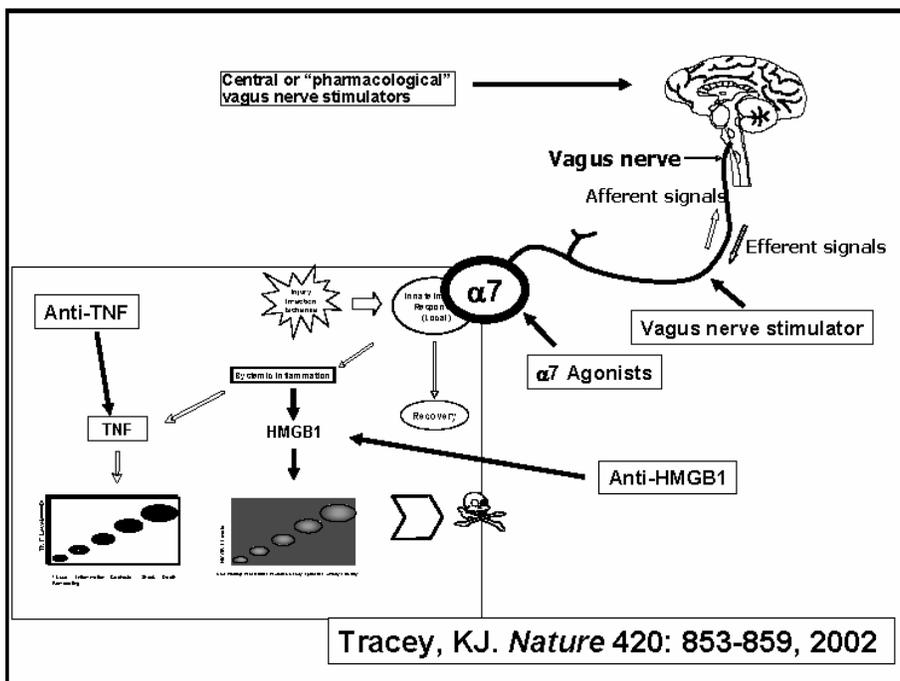


## Humoral vs. Neural Anti-inflammatory Pathways

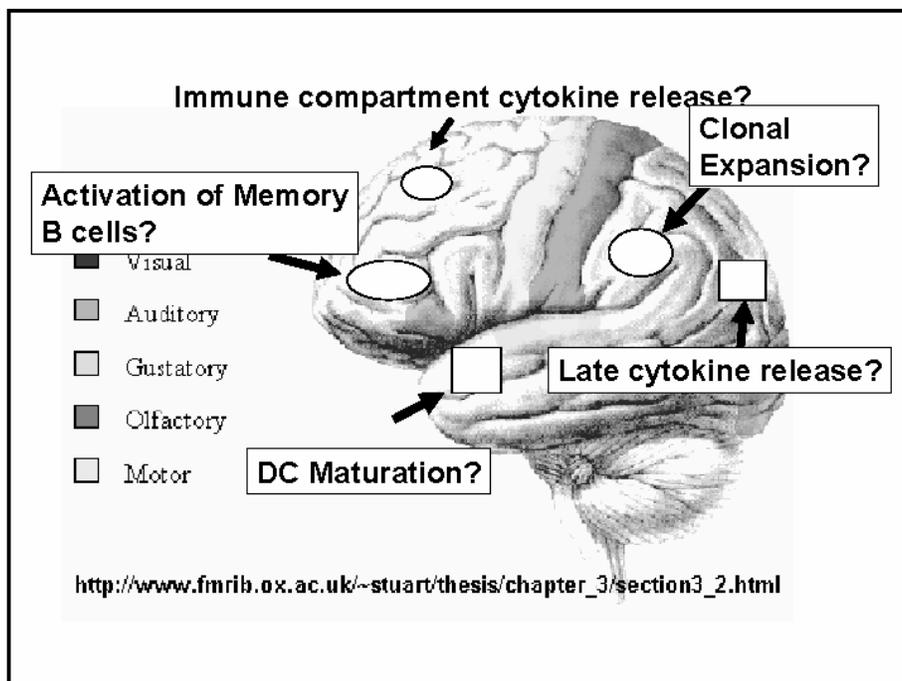
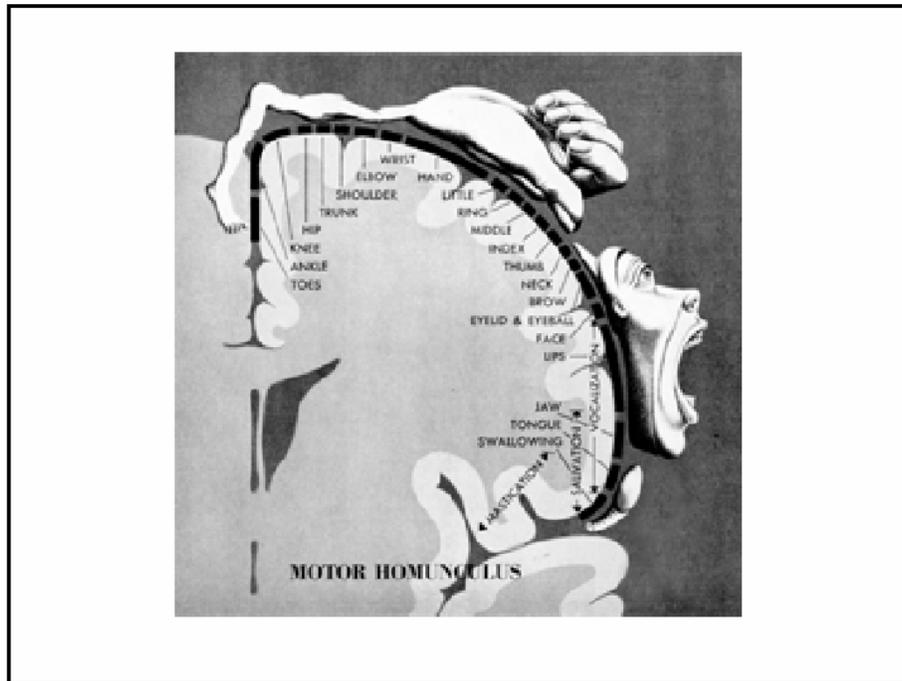


- |                         |             |
|-------------------------|-------------|
| •Concentration gradient | •Local      |
| •Slow                   | •Fast       |
| •Not-integrated         | •Integrated |

Tracey, KJ. *Nature* 420: 853-859, 2003



Tracey, KJ. *Nature* 420: 853-859, 2002



**Presentation 3 – Nancy Klimas**

# Immune abnormalities associated with CFS in the general population

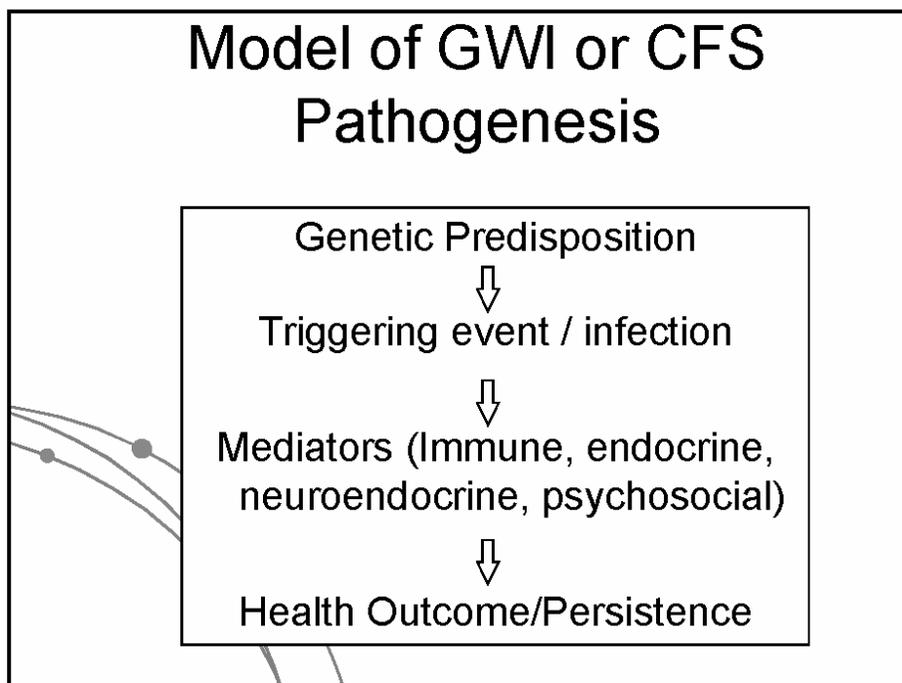
Nancy Klimas, MD

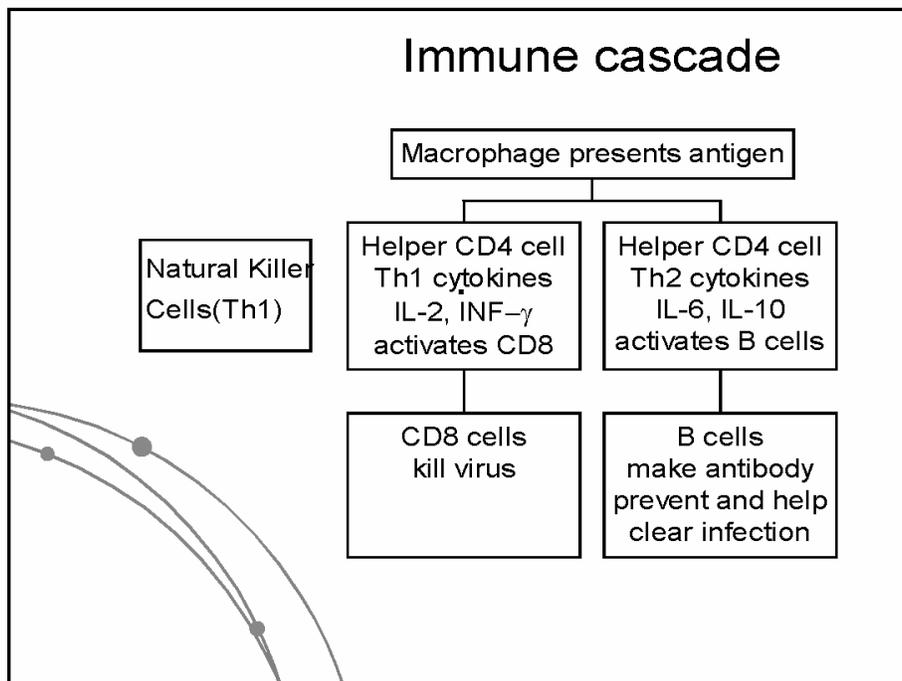
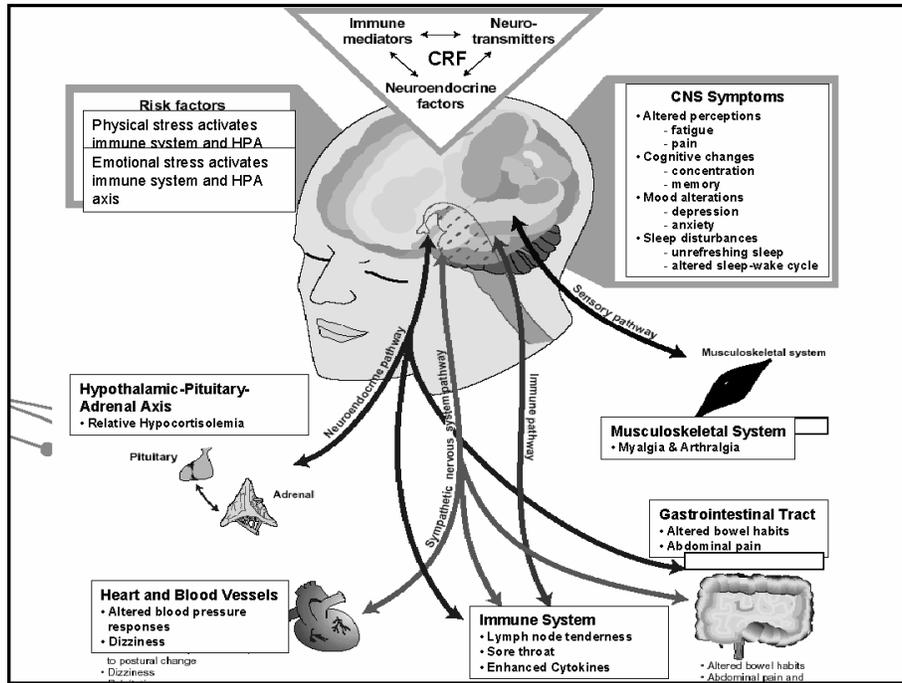
Miami VA Medical Center  
University of Miami Miller School of Medicine  
CFS and GWI Research Center

## GWV and CFS : Comparisons

- Both defined by symptoms which overlap
- Significant overlap in research findings
- Study of GW veterans showed a 16 fold increase risk of CFS, but no other increased risk over controls
- Issues surrounding the study of a multisymptom illness with a multisystem pathogenesis are the same

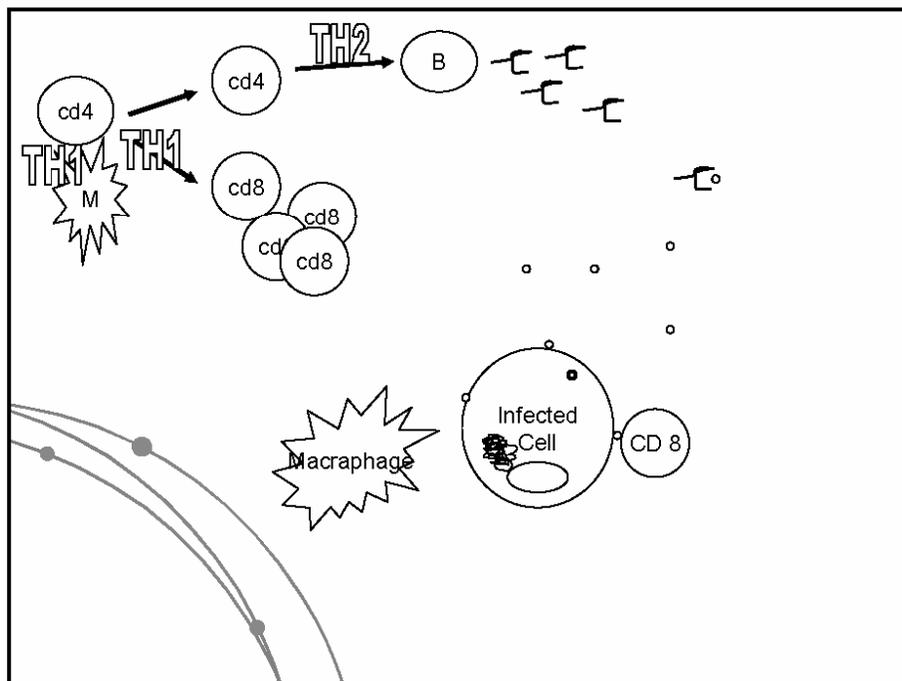
Gulf War Illness	Chronic Fatigue Syndrome***
Fatigue	Disabling fatigue
Depression	Exercise induced relapse
Arthralgia	Arthralgia
Myalgia	Myalgia
Sleep disturbance	Non restorative sleep
Cognitive dysfunction	Cognitive dysfunction
Headache	Headache
Diarrhea, intermittent	Sore throat
Wheezing, Cough, Chest pain, Shortness of breath*	Tender lymph nodes
Weight loss, low grade fever**	

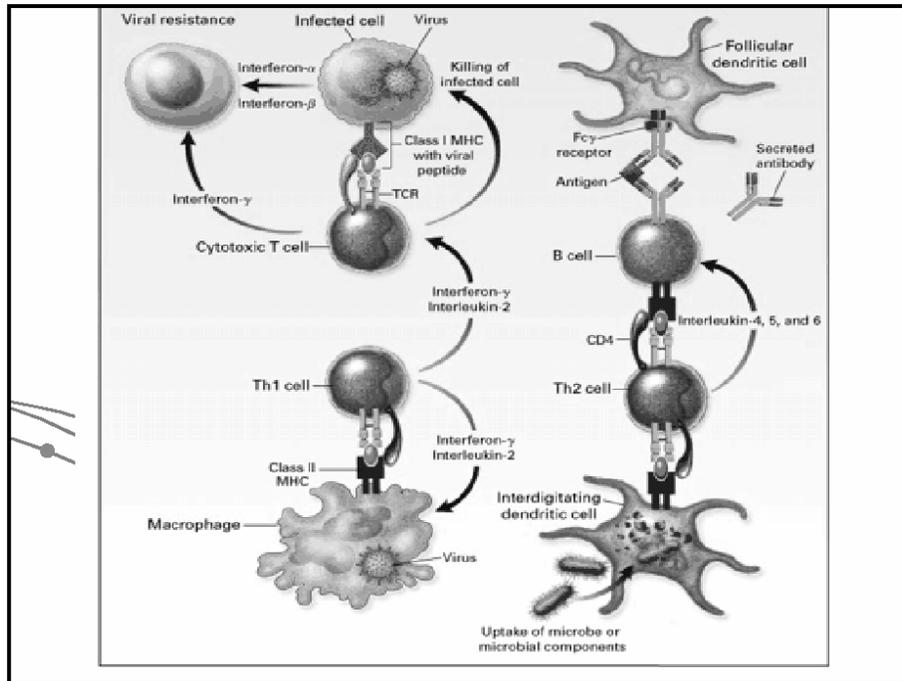




## Cytokines Patterns

- Type 1 - promote cell mediated immunity, antiviral responses. INF-g, IL-2, IL-12
- Type 2 - promote antibody production. IL-4, IL-5, IL-10
- Pro-inflammatory - mediate inflammatory responses. TNF-a, IL-1, IL-6



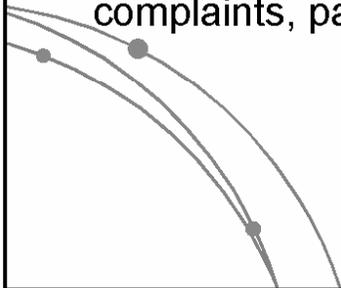


## Cytokines and the Immune System

- Lymphocytes and associated cells - function through a messenger system using cytokines.
- Cytokines deliver message from cell to cell, promoting cell growth, enhancing cell functions, turning off cell functions, and promoting cell death (apoptosis).

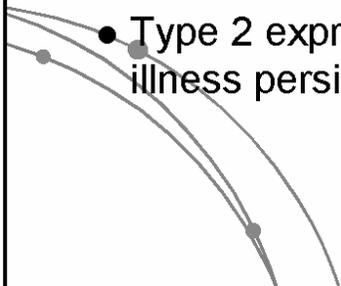
## Cytokines in CFS

- Th 2 imbalance
- Proinflammatory cytokine expression
- Activation of inflammatory cascade correlates with severity of cognitive complaints, pain



## Cytokine Changes Over Time

- Patterns of cytokine expression change with illness severity
- TNF- $\alpha$  receptor expression increases with flares of illness (Patarca et al, 1996)
- Type 2 expression increasingly evident as illness persists for years

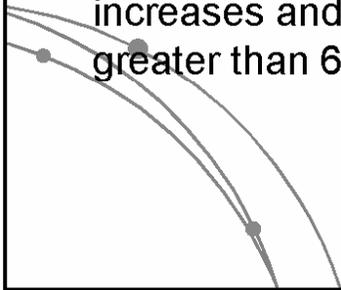


## Severity of Illness correlates with pro-inflammatory cytokine levels

- Hurricane Andrew study

Increase in cognitive difficulties directly correlated with IL1 increase

Post hurricane relapse resulted in cytokine increases and relapse that persisted greater than 6 months



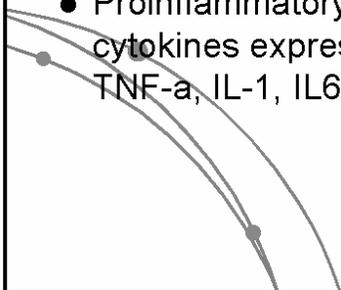
## Immune abnormalities in CFS

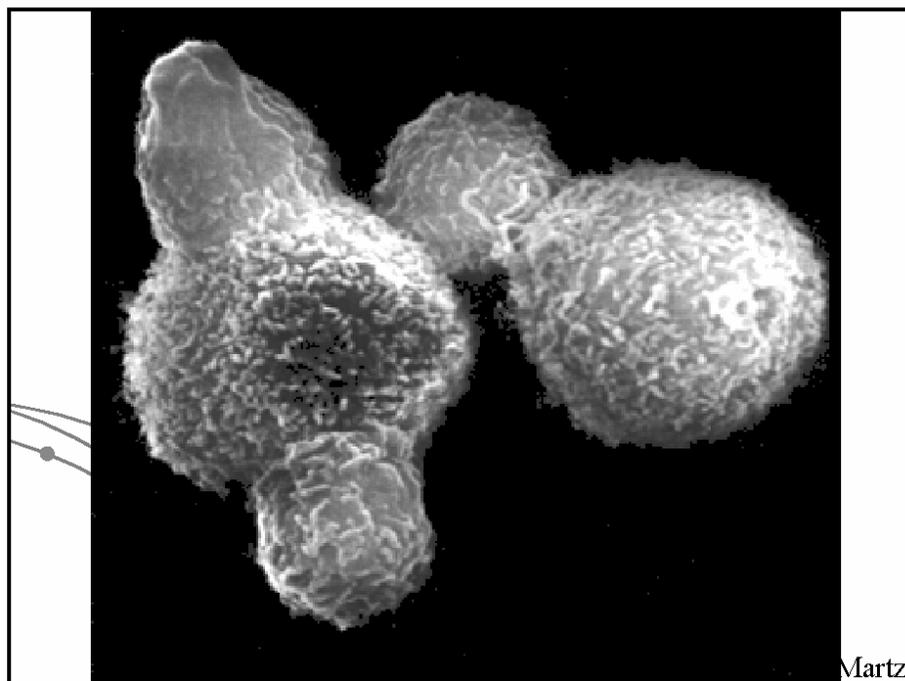
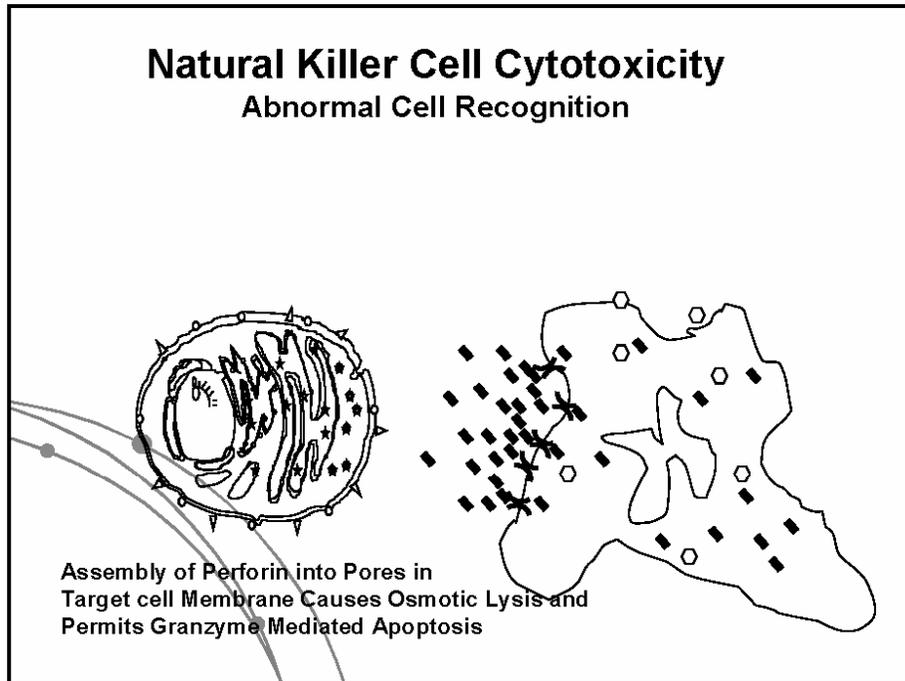
### Immune Activation

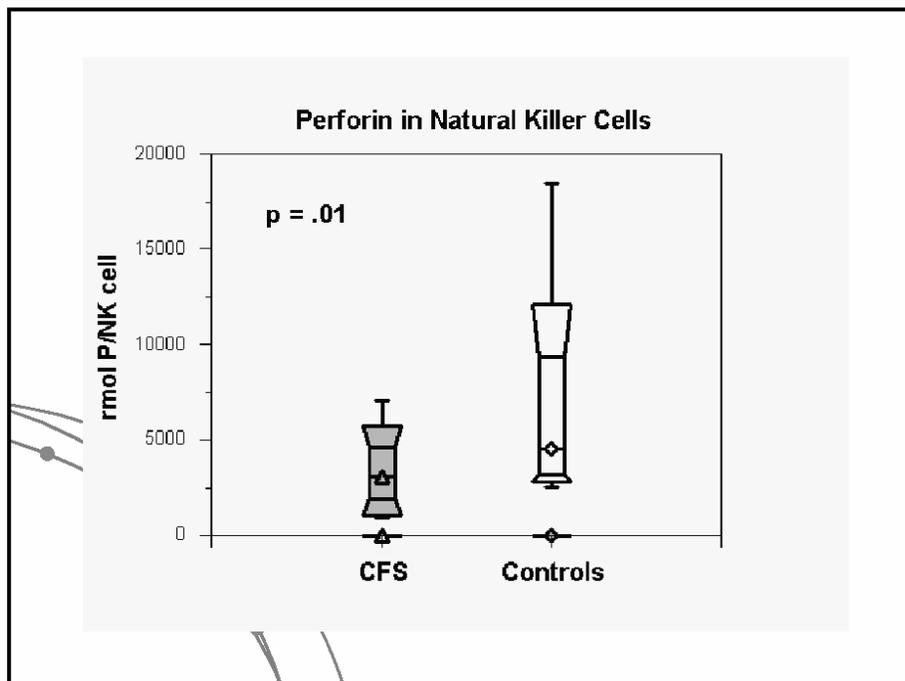
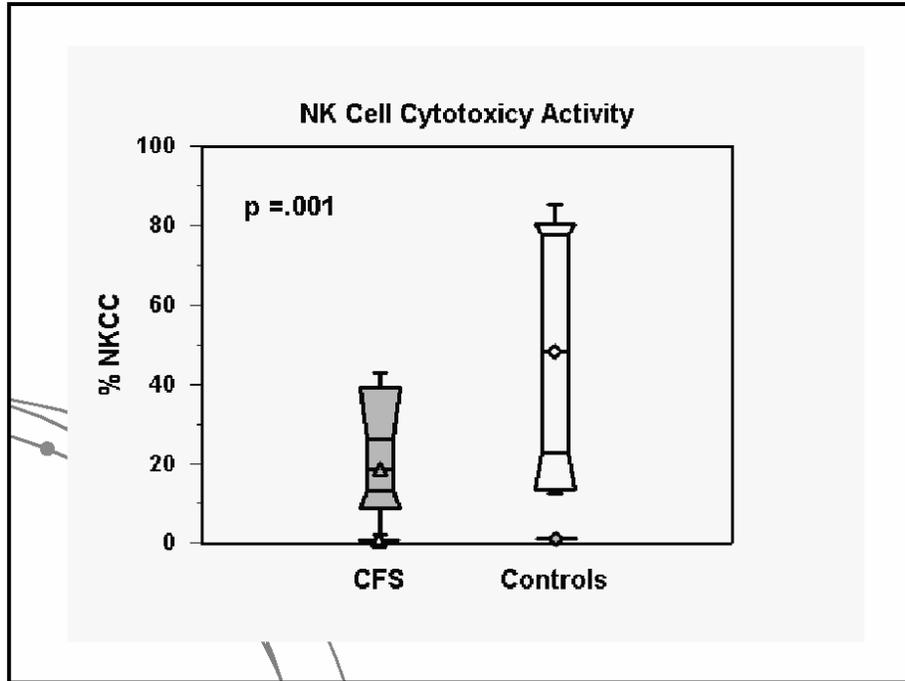
- DR, CD26 expression
- TH2 cytokine shift
- Proinflammatory cytokines expression  
TNF- $\alpha$ , IL-1, IL6

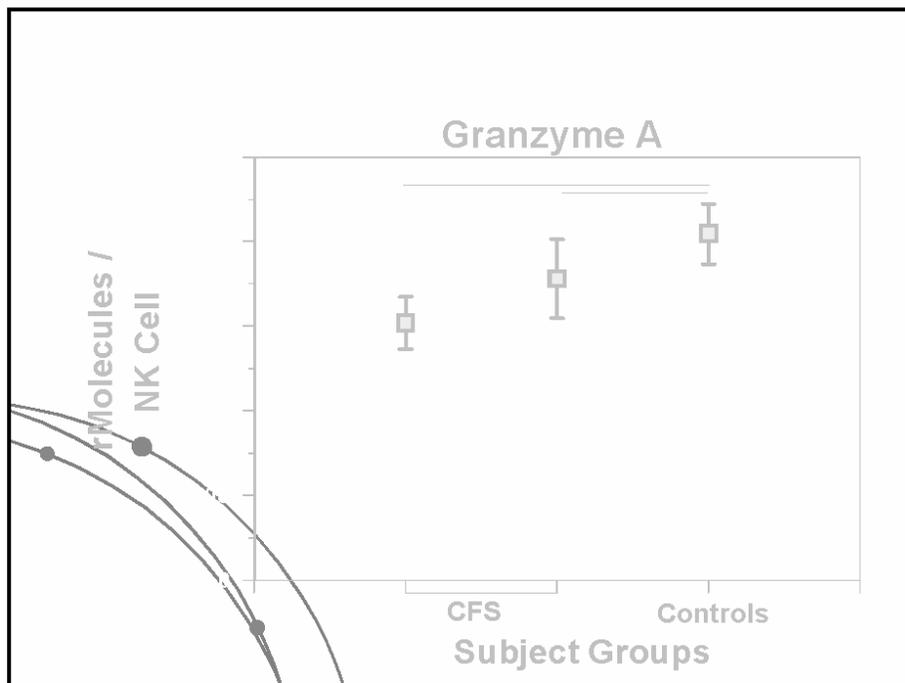
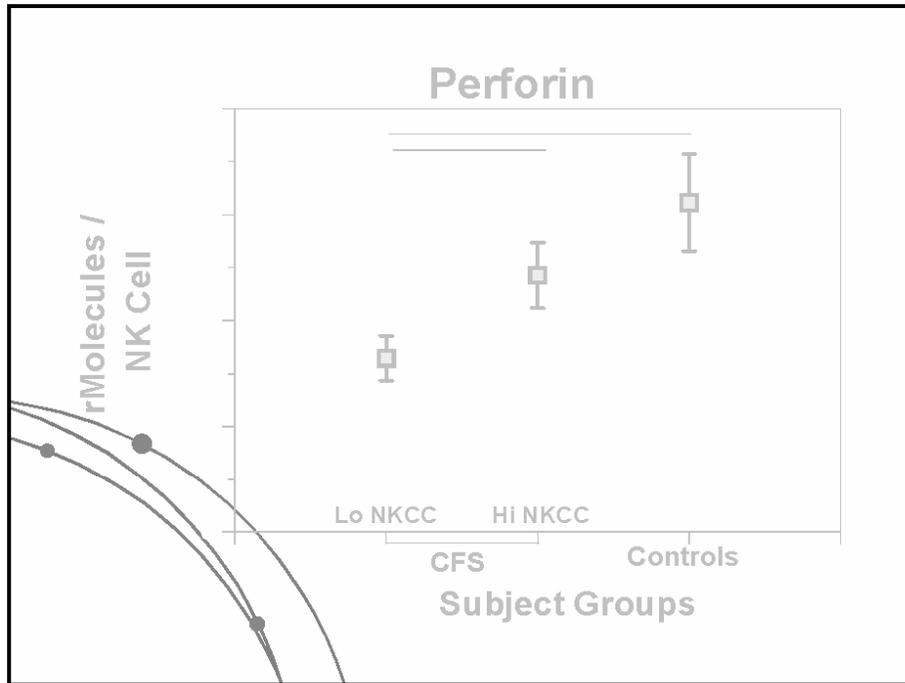
### Functional defects

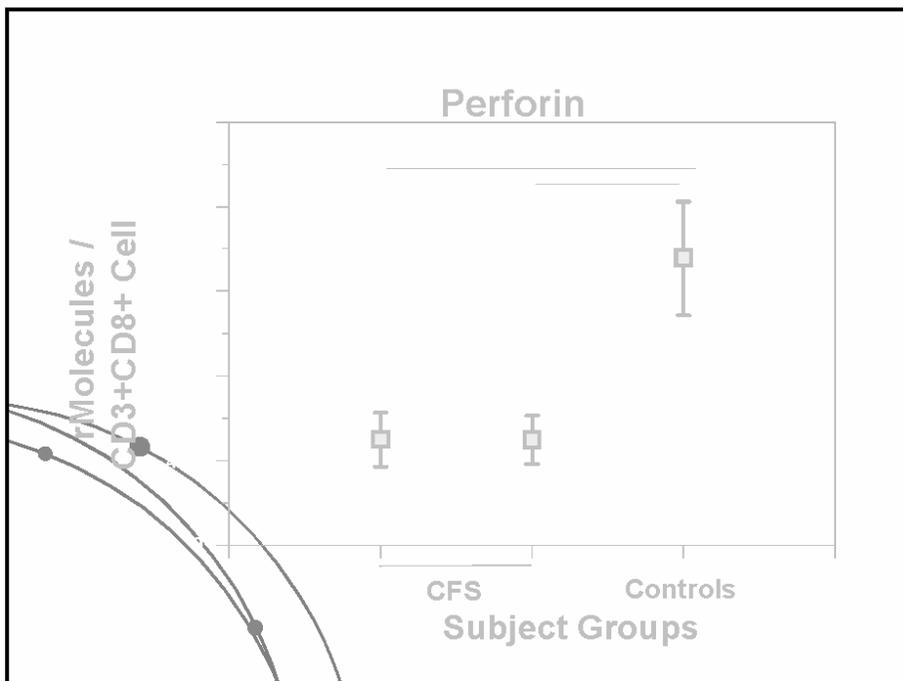
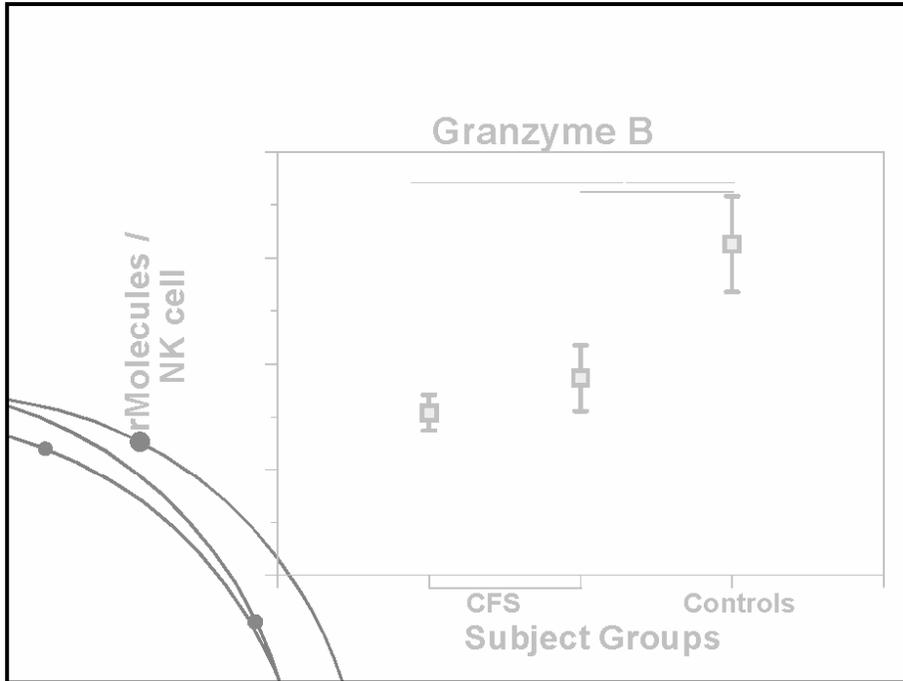
- NK Cell dysfunction
- CD8 abnormalities
- perforins, granzymes
- Macrophage abnormalities
- Antibody production

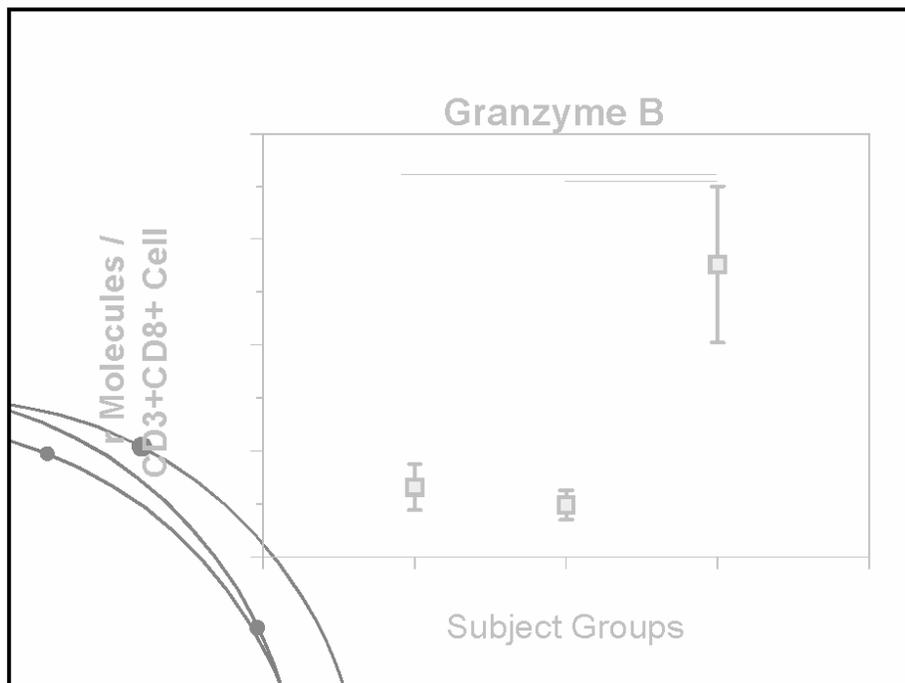
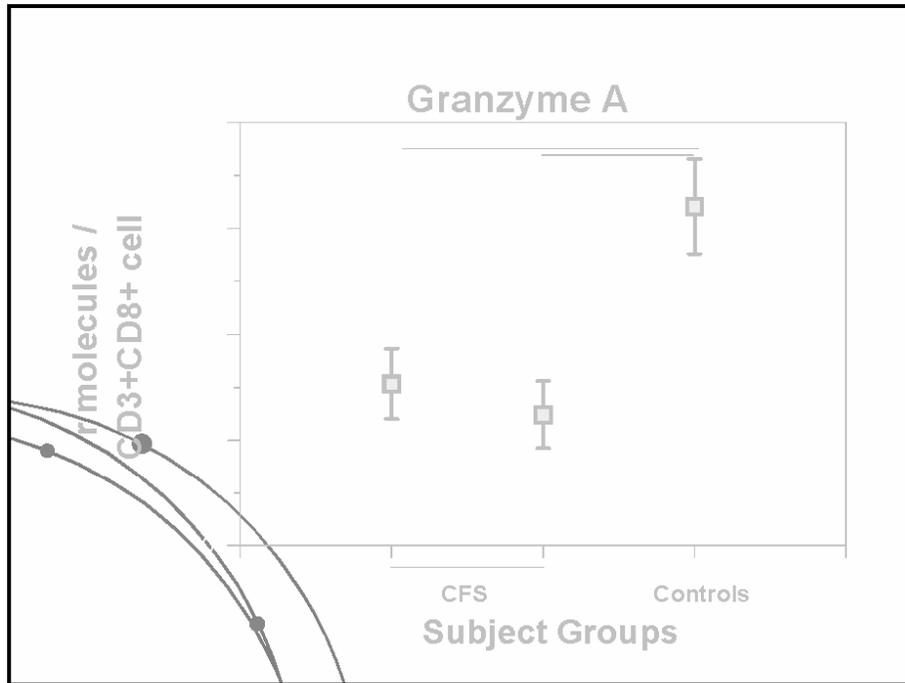


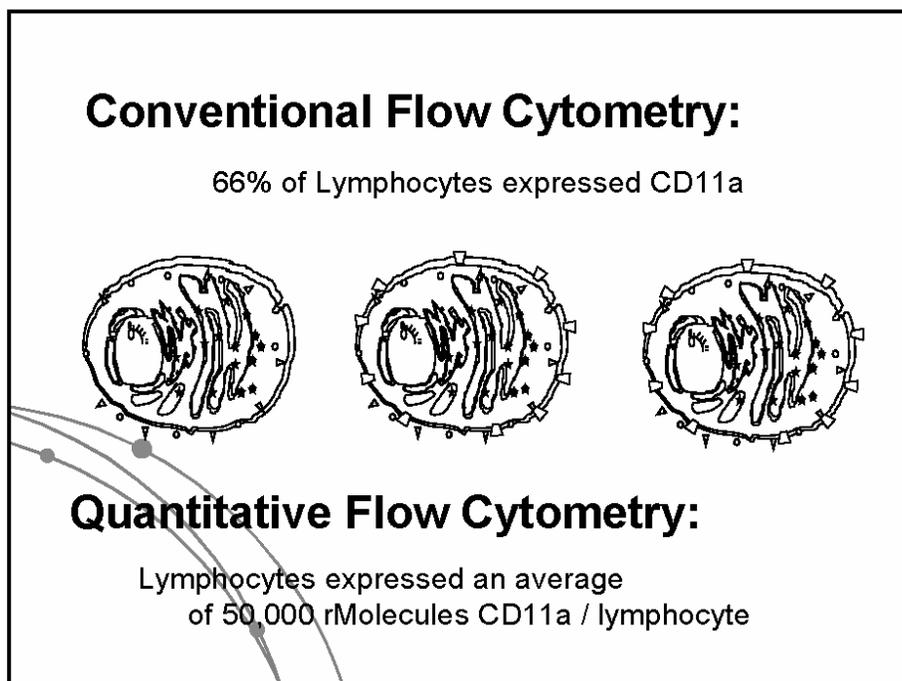
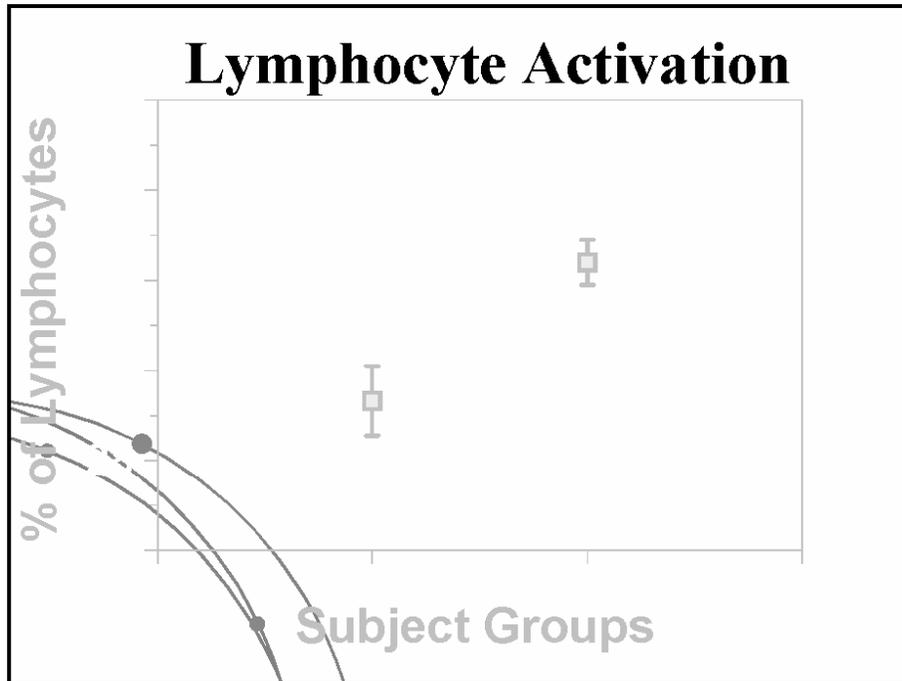


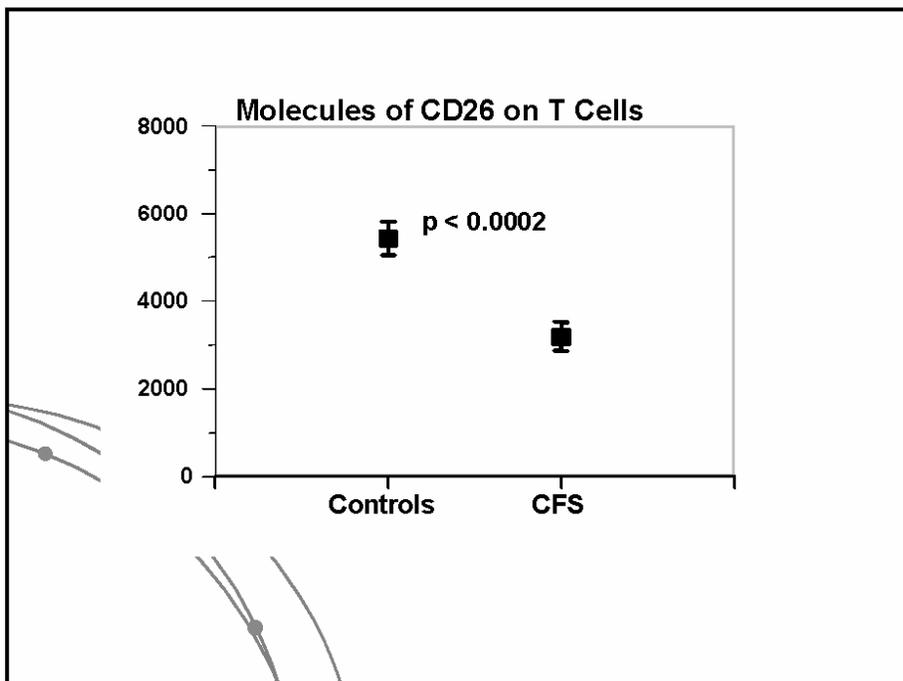
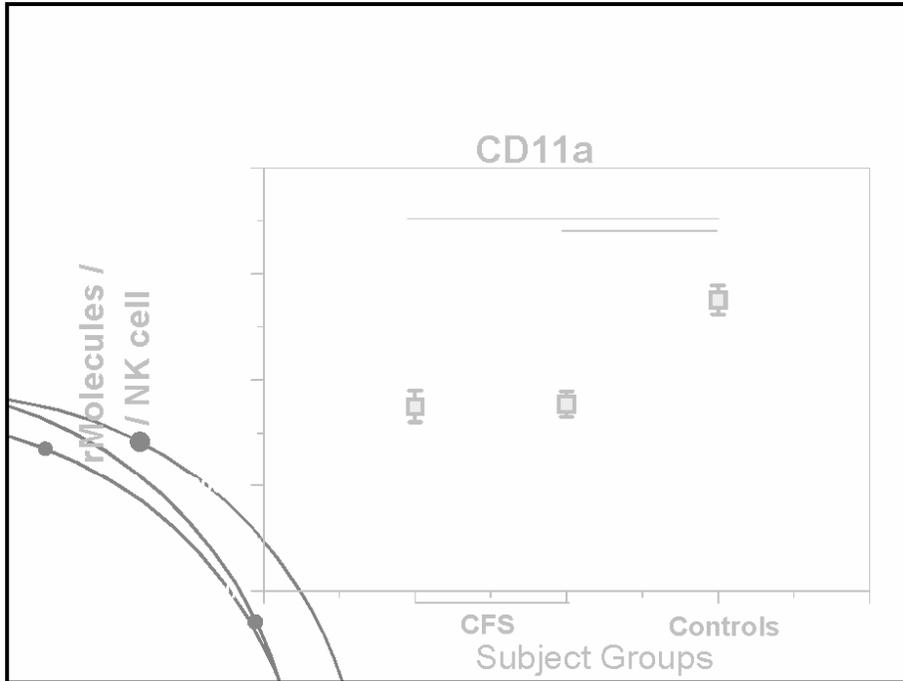


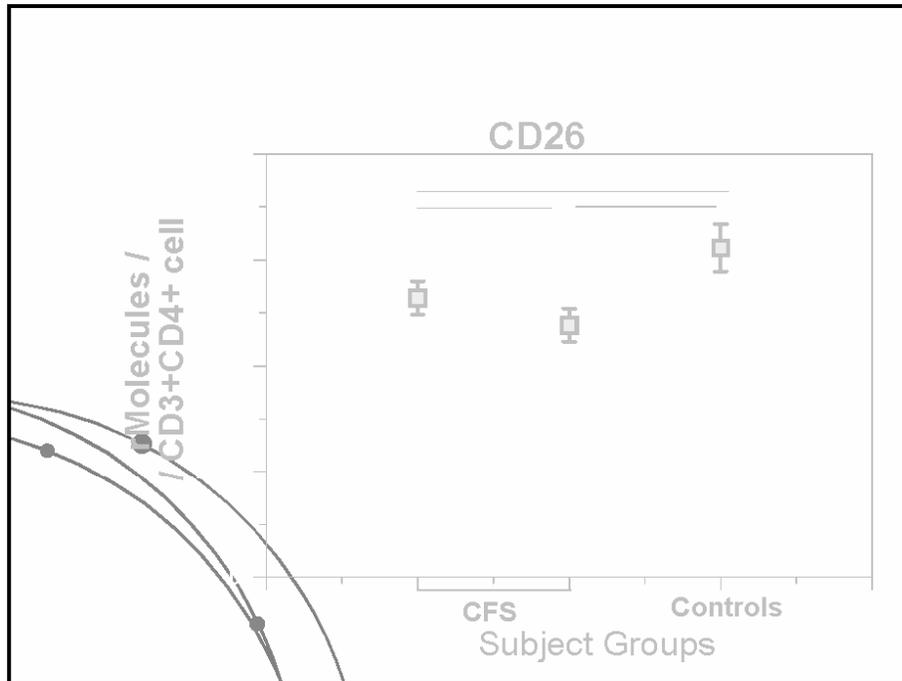












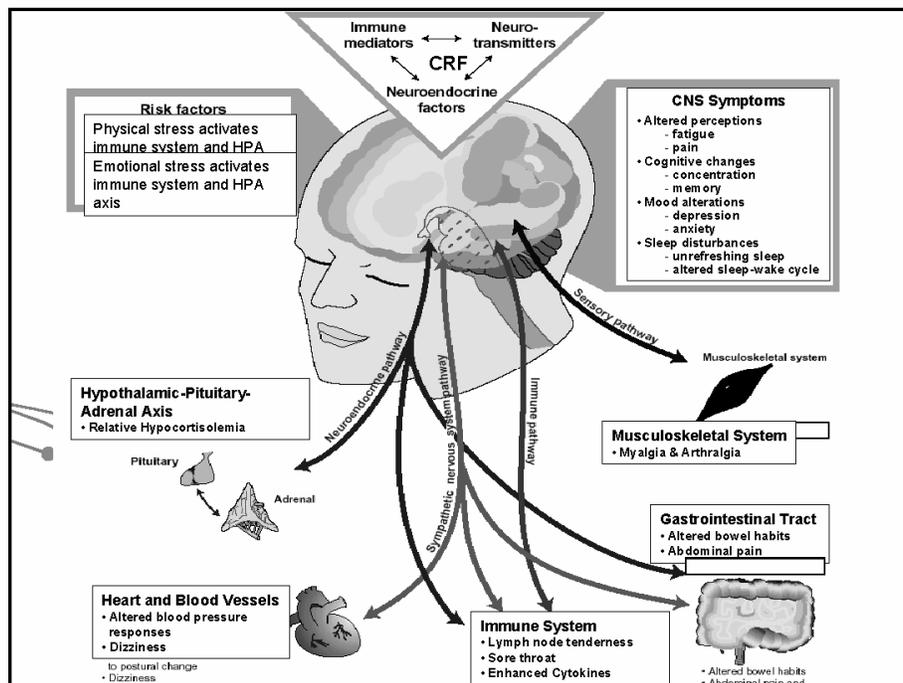
## Immune abnormalities in CFS

Immune Activation	Functional defects
<ul style="list-style-type: none"><li>● DR, CD26 expression</li><li>● TH2 cytokine shift</li><li>● Proinflammatory cytokines expression TNF-<math>\alpha</math>, IL-1, IL6</li></ul>	<ul style="list-style-type: none"><li>NK Cell dysfunction</li><li>CD8 abnormalities</li><li>↓ perforins, granzymes</li><li>Macrophage abnormalities</li><li>Antibody production</li><li>Cell receptor expression</li></ul>

# Gulf War Illness

- Immune Activation
- Higher T and B cell numbers
- Reduced NK, T and B cell function in vitro
- Elevations in immune complexes
- Autoantibody titers directed against MBP and striated or smooth muscle
- Elevated titers to EBV, HSV, HHV6
- Cytokine shift, IL10 over expression – increasing INF gamma/IL10 with increasing vaccine exposure
- 2 groups, TH1 or TH2 ; TH2 cytokine shift with pro-inflammatory cytokines TNF- $\alpha$ , correlating with cognitive impairment

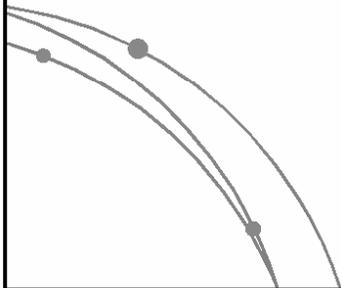
- **Volinn A** Environ Health Perspect. 2004 Jun;112(6):840-6
- **Suzuki A**. J Clin Immunol. 2004 Jan;24(1):66-73.
- **Ferguson and Cassaday** Behav Neuro 2002 10(2):93
- **Peakman** 2004 J Clin Immunol 24(1):60
- **Natelson** Neuroimmunomodulation 10(2):93



## Immune Mediator interactions

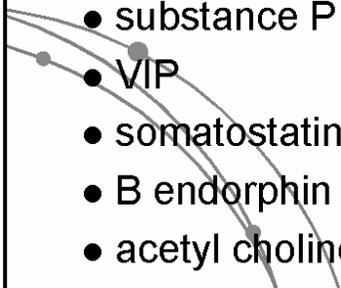
### Immune neuropeptide interactions

- Immune - Autonomic
- Immune - HPA
- Immune - Sleep



## Neuro immune connections: receptors in WBCs

- epinephrine, norepinephrine
- serotonin
- substance P (neurogenic inflammation)
- VIP
- somatostatin
- B endorphin
- acetyl choline



## Endocrine immune interactions

- Cortisol decreases inflammation through down regulation of immune activation, therefore low cortisol in CFS could play a role in chronic immune activation
- GH and thyroid impact on cell metabolism
- Autonomic impact on lymphocyte function
- models: stress, depression

## Stress Response and Immune Function

- Short term - “fight or flight”
- Long term - immune dysfunction, illness
- “stress diathesis model” - developmental implications , endocrine impact on brain vulnerability (Nemeroff, Plotsky)
- Recent proteomics data from the CDC group showing mutations in cortisol regulatory proteins

## Depression and immune function and the HPA axis

- Decreases T cell function, NK cell function
- Blunted GH, TRF
- Increased CRF, ACTH, cortisol (as opposed to Demitrack and others finding in CFS)
- Enlarged adrenal mass, pituitary (as opposed to Dinan's findings in CFS)
- When a CFS patient develops depression, what are the neuro-endocrine, immune implications?

## Sleep

- Circadian Sleep - Wake neuroendocrine and immune functions in CFS (Modolfsky)
- altered diurnal patterns in cortisol, prolactin
- altered diurnal patterns of NK cell function
- alpha wave intrusion on sleep EEG
- studies associating pro-inflammatory cytokines directly with sleep abnormalities

## Conclusion

- Immune abnormalities include chronic immune activation and cellular functional defects
- CFS as a model for illness caused by neuro - endocrine - immune interactions
- Result - Health outcome, termed CFS - cognitive, fatigue, sleep, pain
- Significant implications to GWI research

Presentation 4 – Lea Steele

**Gulf War Exposures and Dysregulation of  
CNS Inflammatory Processes:**

**Research Previously Considered**

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**Lea Steele, PhD.**  
**August 14, 2006**

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

**Research Previously Considered**

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- **LRRI: low-level sarin effects on cholinergic receptors, cytokines, immune measures, glucocorticoids**
- **Duke: sarin, exposure combinations: AChE levels, gene expression, ROS, GFAP, neuronal death, etc**
- **New Mexico: inhaled DU associated with glial activation; enhanced with nasal inflammation**
- **EPA: Particulates associated with microglial activation, ROS, proinfl cytokines, neuronal damage**  
*(Kuwaiti sand, oil fires, tent heaters, diesel)*
- **Vaccines: Rook hypothesis, immune activation by adjuvants?**

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

Presentation 5 – Marina Morris

# Low Level Chemical Toxicity Study of Autonomic Neural Balance

Mariana Morris, Ph.D.  
Pharmacology &  
Toxicology  
Boonshoft School of  
Medicine  
Wright State University  
Dayton, Ohio  
mariana.morris@wright.edu



## Research Advisory Committee on Gulf War Veterans' Illnesses August 14, 2006

DAYTON DAILY NEWS  
FRIDAY  
SEPTEMBER 24, 1999

### VA center joins study on Gulf War afflictions

Volunteers will be followed through various therapies.

By James Rasmach

War veterans are seeking help and negative behavioral therapy, which involves techniques for relaxing the symptoms of the syndrome. The program is a voluntary one and the other study will be...

### Ailments may be linked to nerve gas antidote

250,000 U.S. troops received the drug during the Gulf War

RELATED STORY  
Cincinnati VA Center leading treatment program. BA

### VA tests remedies for Gulf illness

Dayton, Cincinnati centers each have trial programs under way

By KEVIN LAMB  
Dayton Daily News

CINCINNATI — Gulf War veterans experiencing the joint pain, muscle aches, fatigue, concentration difficulties and rashes that have been termed Gulf War syndrome are invited to join the Cincinnati VA Medical Center's demonstration treatment program for veterans with these symptoms.

Cincinnati is one of five U.S. sites conducting the one-year treatment trial, which will become a model for further programs if it is effective.

The individualized treatment includes physical therapy, therapeutic exercise, access to a support group and treatment for physical symptoms or underlying mental disorders such as depression or post-traumatic stress syndrome. For information, call Carolyn Homan toll-free at (888) 855-3321 or directly at (513) 861-3100, extension 4255.

The Cincinnati trial is different from two other trials for Gulf War syndrome for which the Dayton VA Center is among 38 participating centers.

One trial is to determine whether exercise alone or in combination with psychological cognitive behavioral therapy can diminish the severity of symptoms, and the other will test whether an antibiotic can effectively treat the symptoms. Call 288-6011, Ext. 1212, for information on the Dayton trials.



NORTH CAROLINA VETERAN Brian Martin suffers from ailments he attributes to toxins to which he was exposed during the Persian Gulf War. He cannot be ruled out as a cause of Gulf War syndrome.

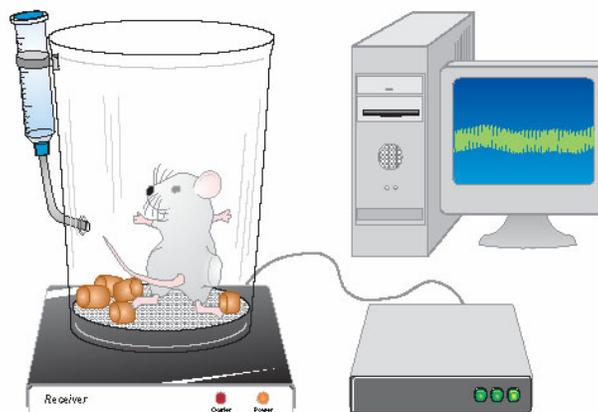
Beatrice Alexandra Golomb of Rand Corp., who headed the review, told a Pentagon news conference she speculated that "PB" might be wrong.

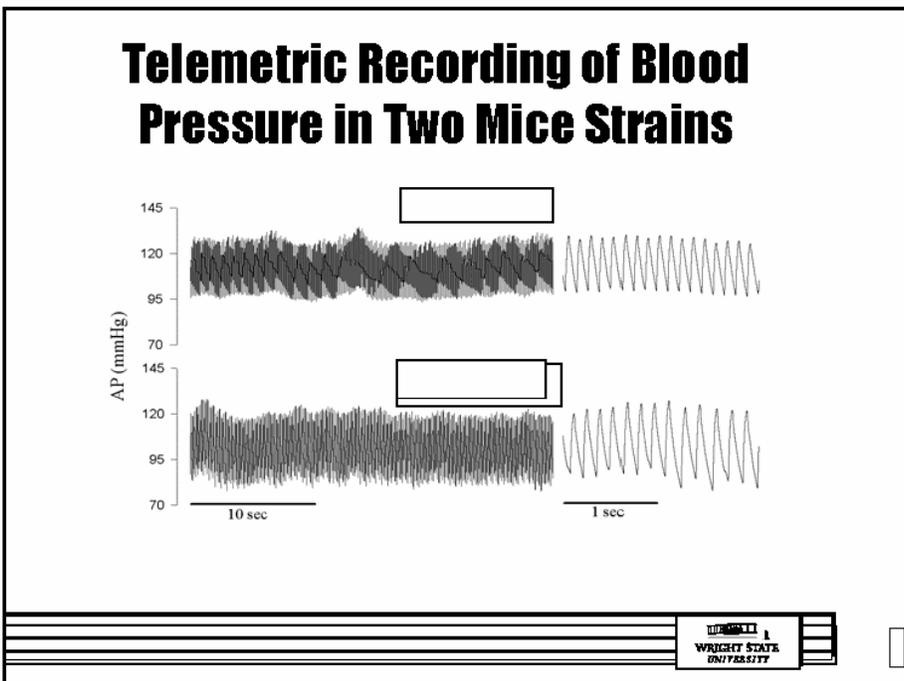


## Experimental Goals

- ◆ Phase 1: Stress/Chemical Interactions
  - *How does stress alter the body's ability to resist chemical challenges?*
  - *Studies of pyridostigmine/stress interactions: focus on autonomic neural function*

## Studies of Autonomic Balance Integrative Cardiovascular Laboratory

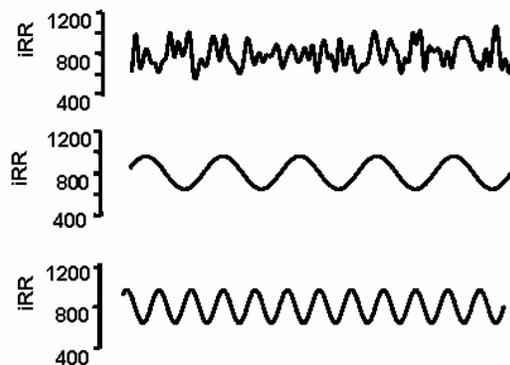




## What is Spectral Analysis ?

- ◆ A statistical method for characterization of parameters of variability. Variation over time is considered variance. Power spectra of variability are composed of two oscillatory components; low frequency (LF: 0.1-1 Hz) and high frequency (HF: 1- 5.0 Hz).
- ◆ The components are associated with autonomic balance. For HR and BP variability, LF is related to sympathetic modulation, whereas, for PI variability the HF is related to vagal modulation of the sinus node.

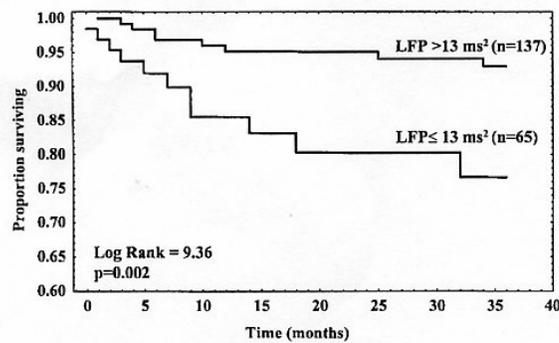
## Signal Analysis

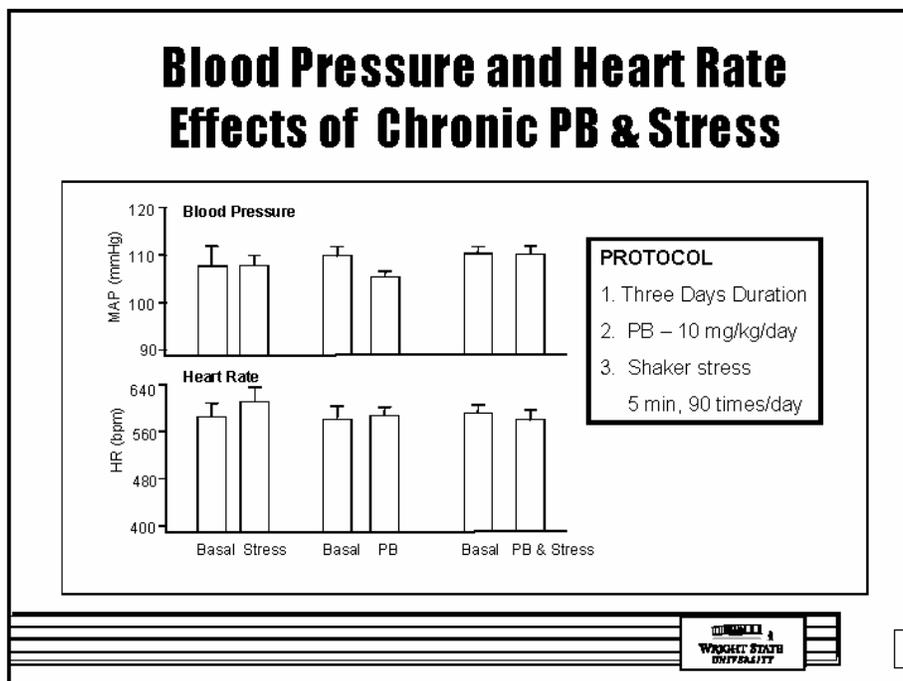
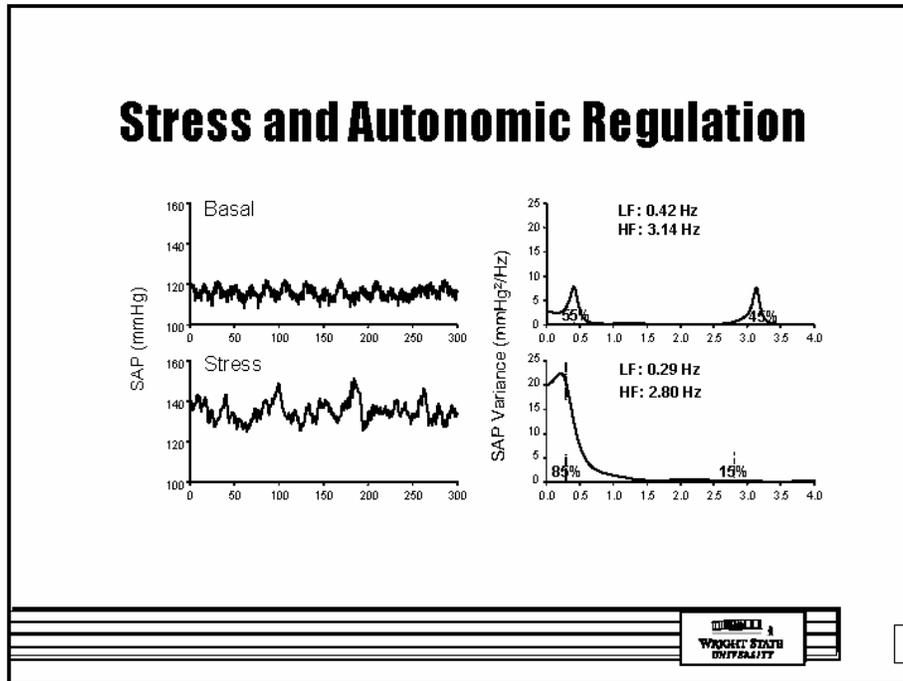


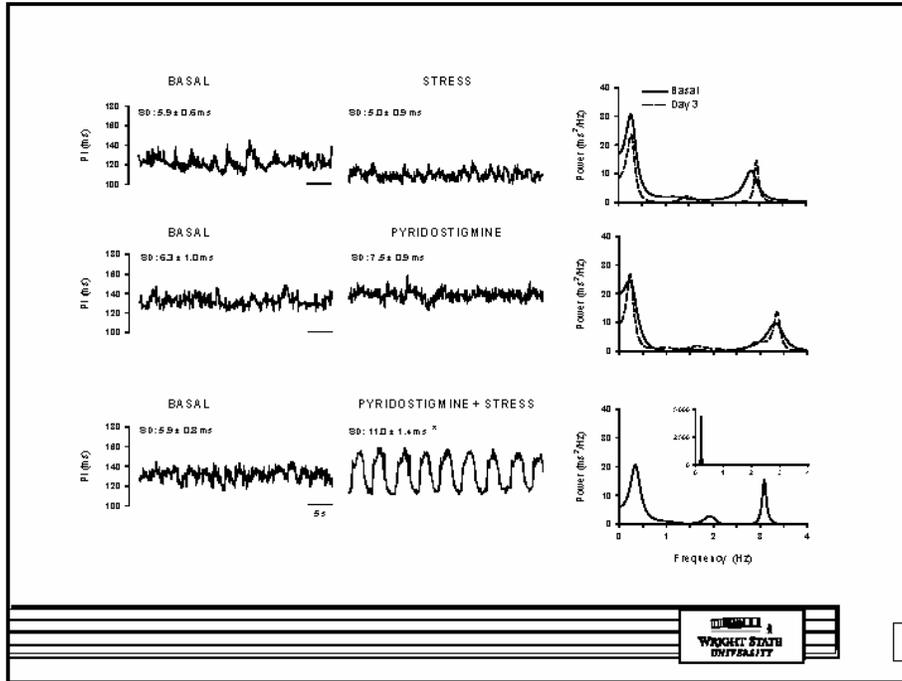
## Clinical Relevance of Heart Rate Variability (HRV)

- ◆ HRV variability in chronic heart failure: Target for therapy? Sueta, 2003
- ◆ Decreased HRV variability is associated with increased mortality after acute myocardial infarction. Kleiger et al., 1987
- ◆ HRV strongly predicts cardiac death in heart failure patients. LaRovere et al., 2003

## Reduced Heart Rate Variability Associated with Sudden Cardiac Death







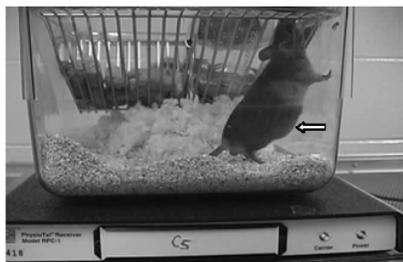
## Use of AChE Inhibitors in the Clinic

- ◆ Cholinergic stimulation with pyridostigmine reduces ventricular arrhythmias and enhances heart rate variability in heart failure. Behling et al., 2003
- ◆ Cholinergic stimulation with pyridostigmine reduces the QTc interval in coronary artery disease. Castro et al., 2003

## Experimental Goals

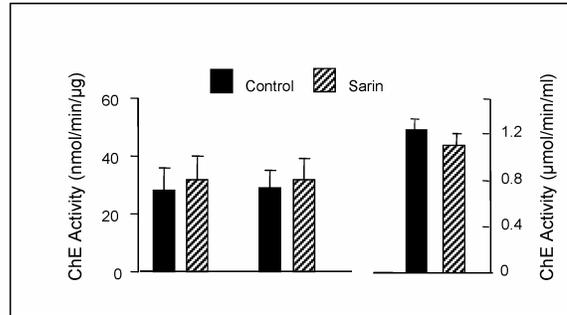
- ◆ Phase 2: Low Level Sarin Exposure
  - *Genomic and Proteomic Expression*
  - *Autonomic Neural Function*
  - *Neurobehavioral and Cholinergic Function*

## Autonomic Cardiovascular Effects of Low Dose Sarin

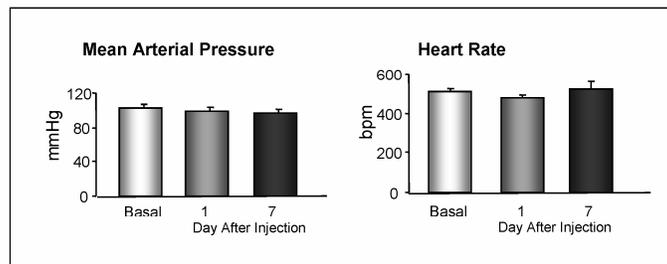


- ◆ Radiotelemetry (5000Hz)
- ◆ Sarin sc (8  $\mu\text{g}/\text{kg}$ )
- ◆ Spectral analysis - autoregressive method
- ◆ Blood and brain AChE

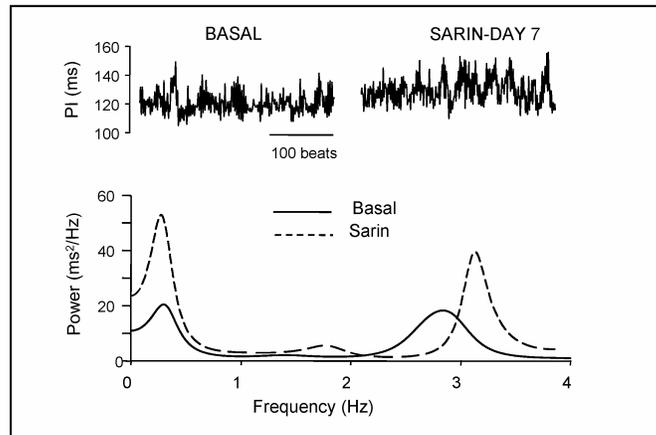
## Low Dose Sarin Brain and Blood Cholinesterase



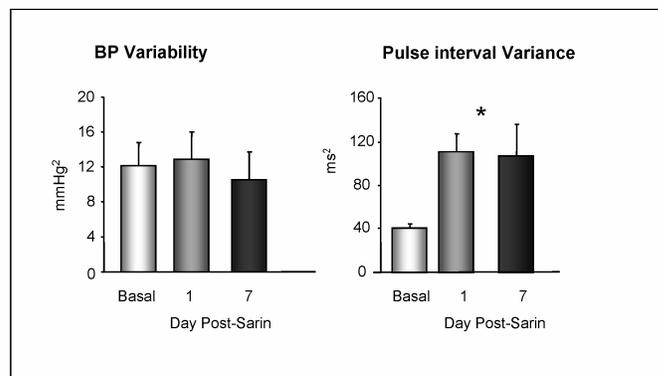
## Low Dose Sarin Blood Pressure and Heart Rate



## Low Dose Sarin: Acute Increase in Heart Rate Variance



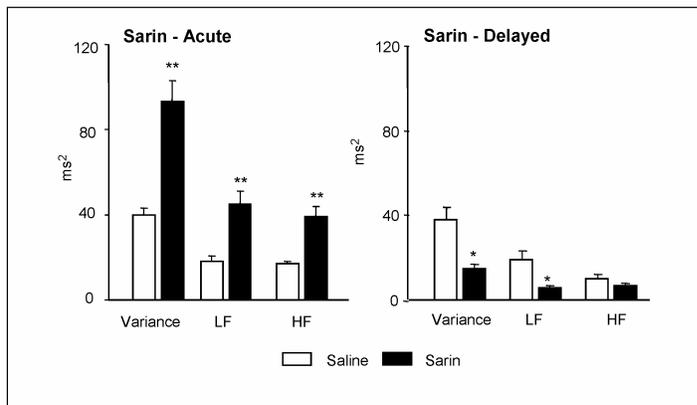
## Acute Changes in Blood Pressure and Heart Rate Variance



## Serendipity: The gift of finding valuable things not sought for

- ◆ Action: Your graduate student leaves the sarin treated mice in the animal facility for an extended vacation.
- ◆ Outcome: Cardiovascular monitoring shows that sarin produced delayed changes in heart rate variance, associated with cardiac dysfunction.

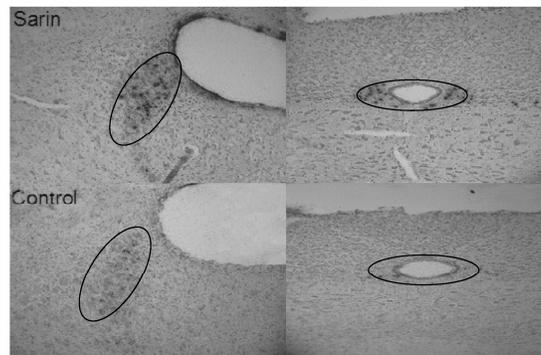
## Delayed Effect of Sarin on PI Variance and Frequency Domains



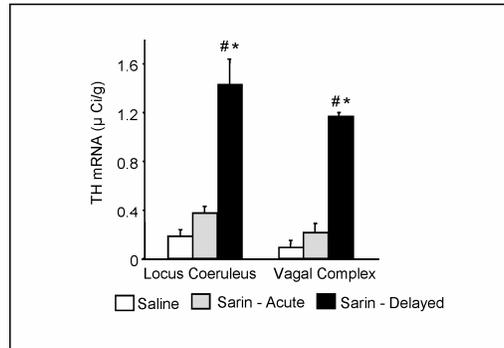
## Sarin on Blood Pressure Variance

Treatment	Variance (mmHg <sup>2</sup> )	Low Freq (mmHg <sup>2</sup> )	High Freq (mmHg <sup>2</sup> )
Saline	13 ± 2	9 ± 2	3 ± 0.3
Sarin Acute	13 ± 3	9 ± 3	3 ± 1
Sarin Delayed	14 ± 2	10 ± 3	3 ± 1

## Effect of Low Dose Sarin on Brainstem Catecholamine Systems

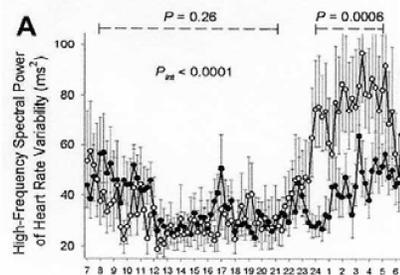


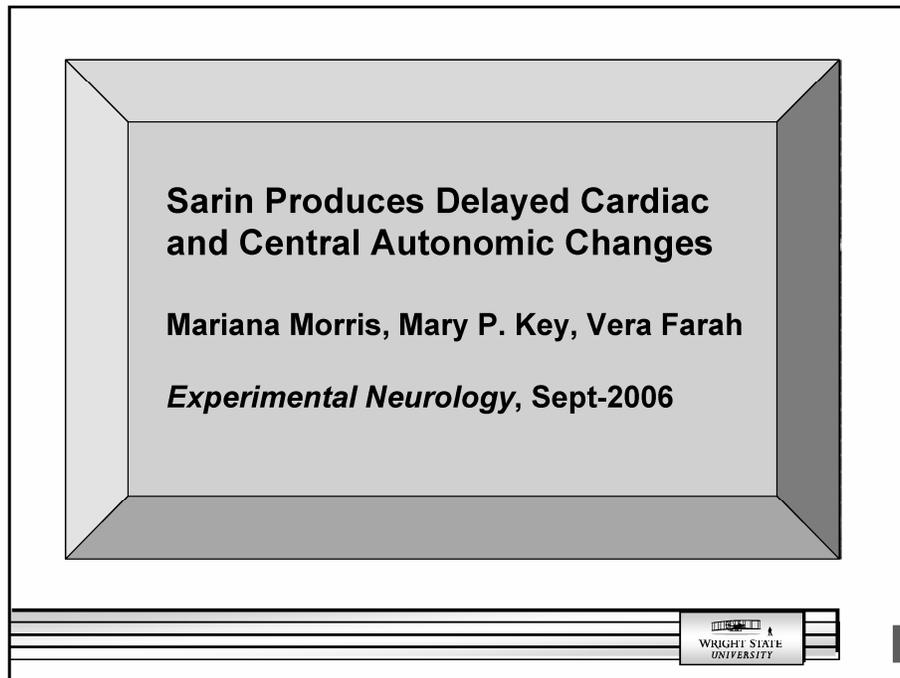
## Low Dose Sarin Produces Delayed Changes in Brainstem Amine Function



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

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



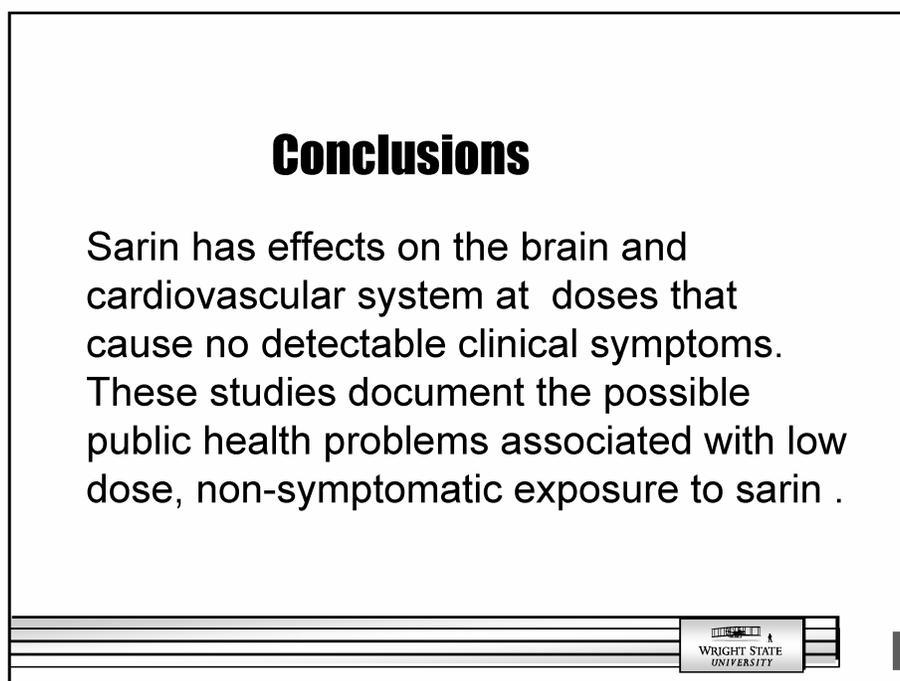


**Sarin Produces Delayed Cardiac  
and Central Autonomic Changes**

**Mariana Morris, Mary P. Key, Vera Farah**

*Experimental Neurology, Sept-2006*

WRIGHT STATE  
UNIVERSITY



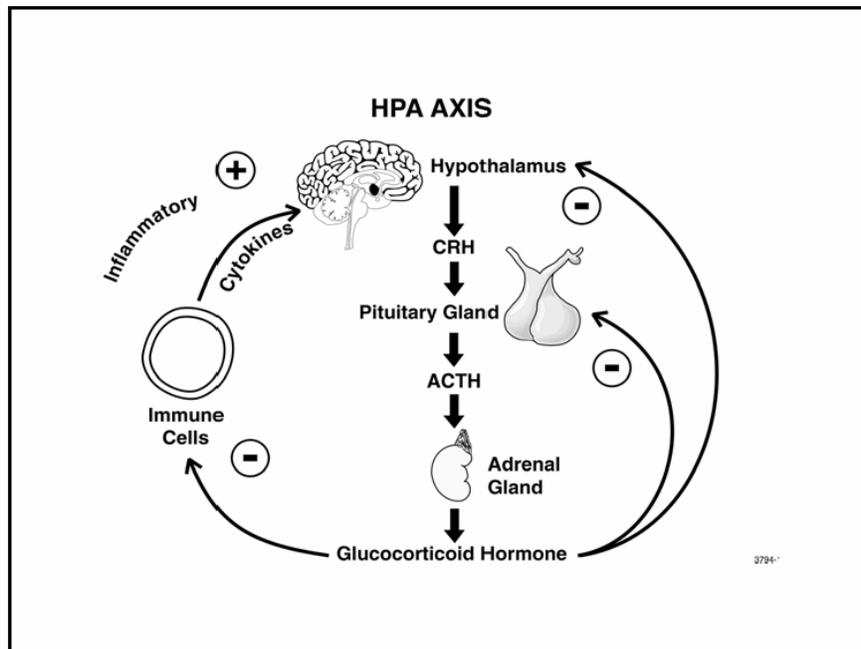
**Conclusions**

Sarin has effects on the brain and cardiovascular system at doses that cause no detectable clinical symptoms. These studies document the possible public health problems associated with low dose, non-symptomatic exposure to sarin .

WRIGHT STATE  
UNIVERSITY

### Presentation 6 – Mohan Sopori

- Brain and the immune system communicate bidirectionally.
- Many neuroactive substances have the potential to affect the immune system through the CNS.
- Two established pathways by which the brain affects the immune system:
  1. Activation of the Hypothalamus-pituitary-adrenal (HPA) axis.
  2. Through sympathetic cholinergic innervations.

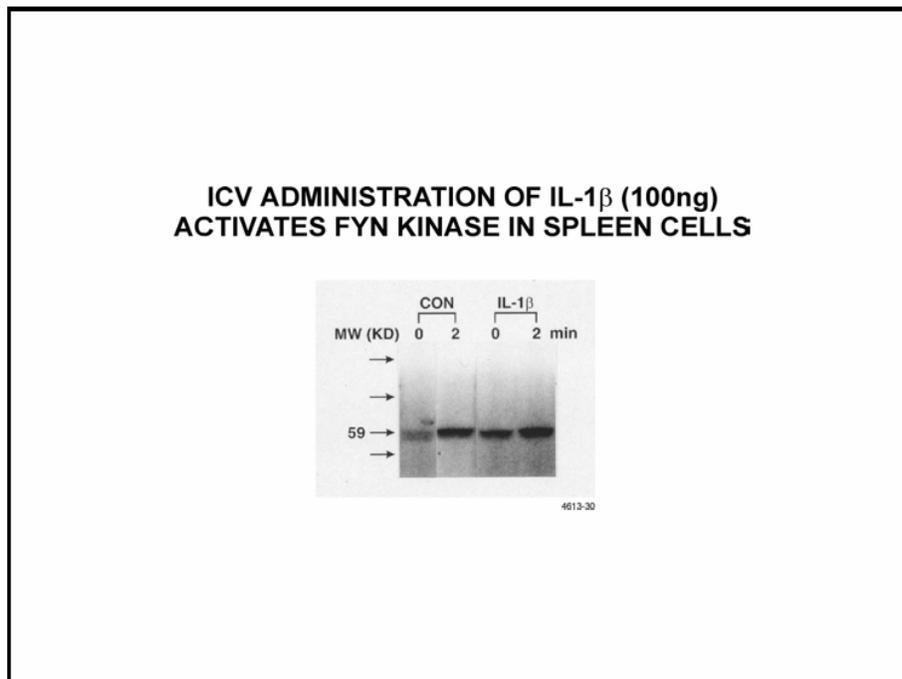
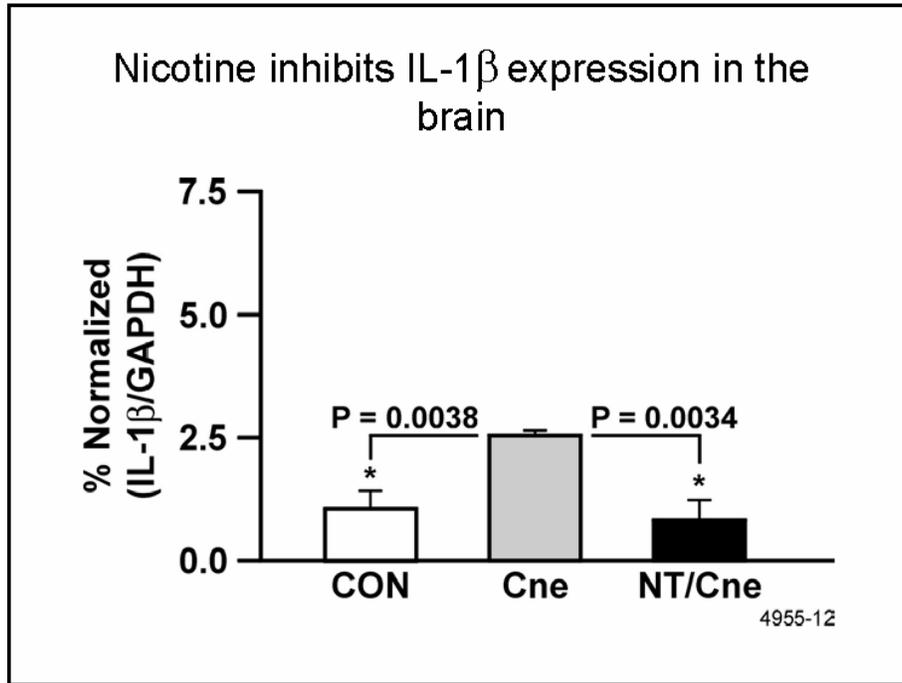


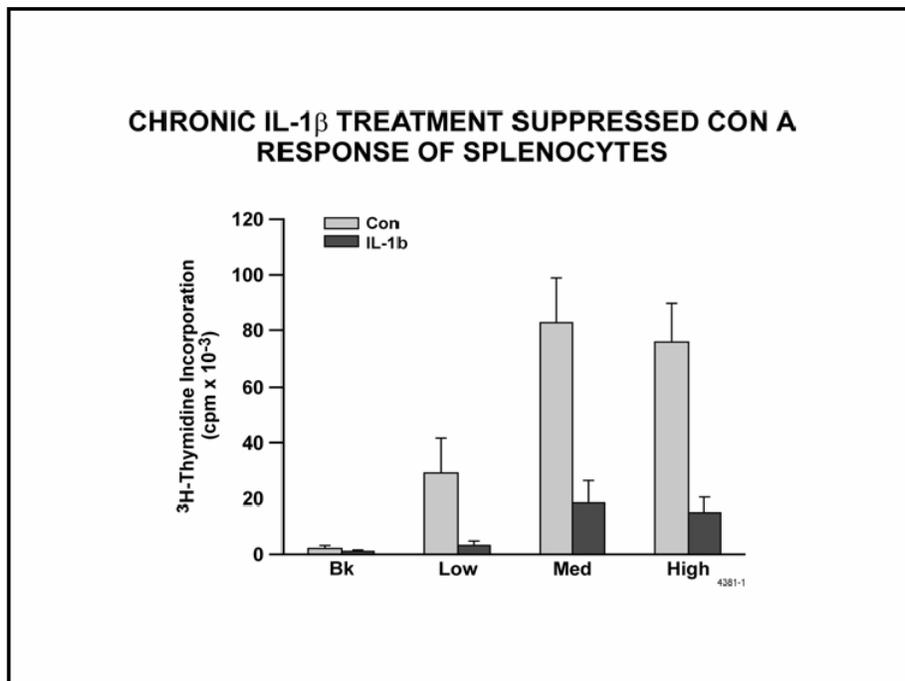
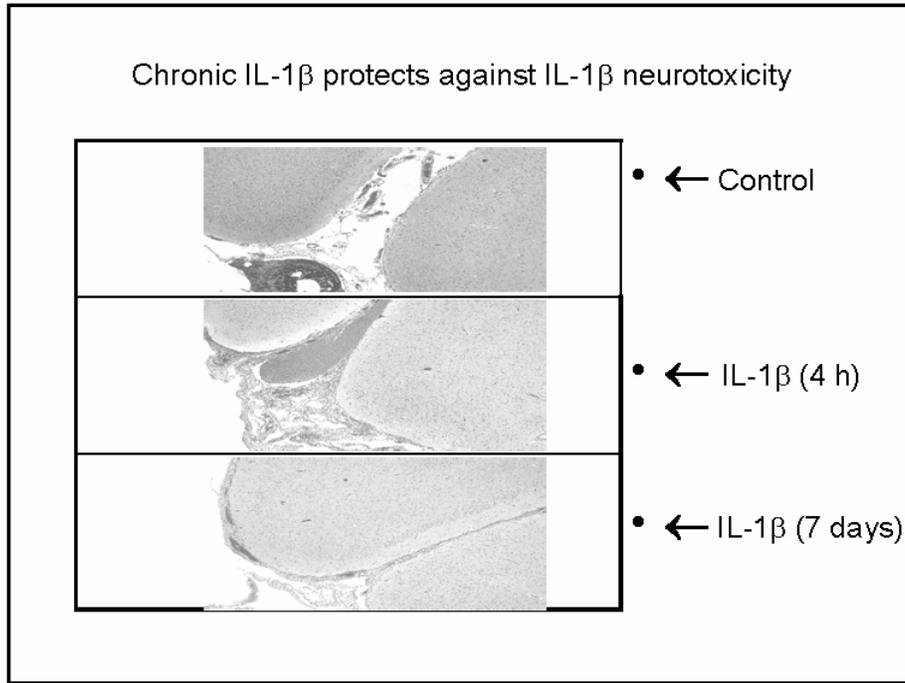
## Cytokine Effects

- Cause sickness behavior (e.g., malaise, listlessness, fever, pain, loss of interest in social activities) ....Keith Kelly, Suzzane Broussad et al.
- Correlation between acute viral infection (e.g., EBV, Q fever) and the levels of IL-1 $\beta$  and IL-6 in serum and produced by PBMC in culture (Ute Vollmer-Conna et al.).
- There is lack of tight correlation between serum and brain cytokine levels (LPS-tolerance, Sulie Chang).
- How does a cytokine signal reach the brain? Vagal afferents sense peritoneal cytokines, but not IV or subcutaneous cytokines.

## Cytokine Effects

- Pyrogenic effects of cytokines are critical for survival of cold-blooded animals from infections (M. Kluger).
- Use of antibodies against proinflammatory cytokines has adverse effects on human sepsis patients (C. Dinarello).
- Ischemic brain injury is more severe in TNF- $\alpha$  and IL-6 KO animals.
- Chronic nicotine treatment blunts inflammation, but increases viral, bacterial, and viral load (Razani-Boroujerdi et al.; Tom Klein et al.).
- Tolerance: sublethal exposures to endotoxin or TNF- $\alpha$  protects against subsequent lethal infections (Hymie Anisman).



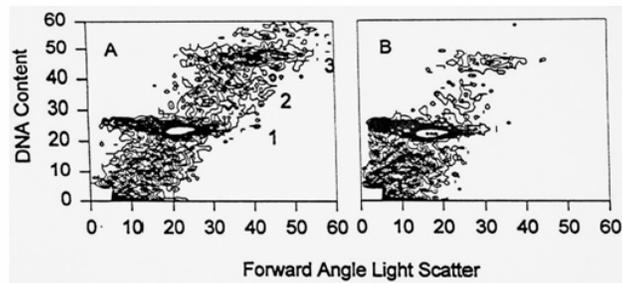


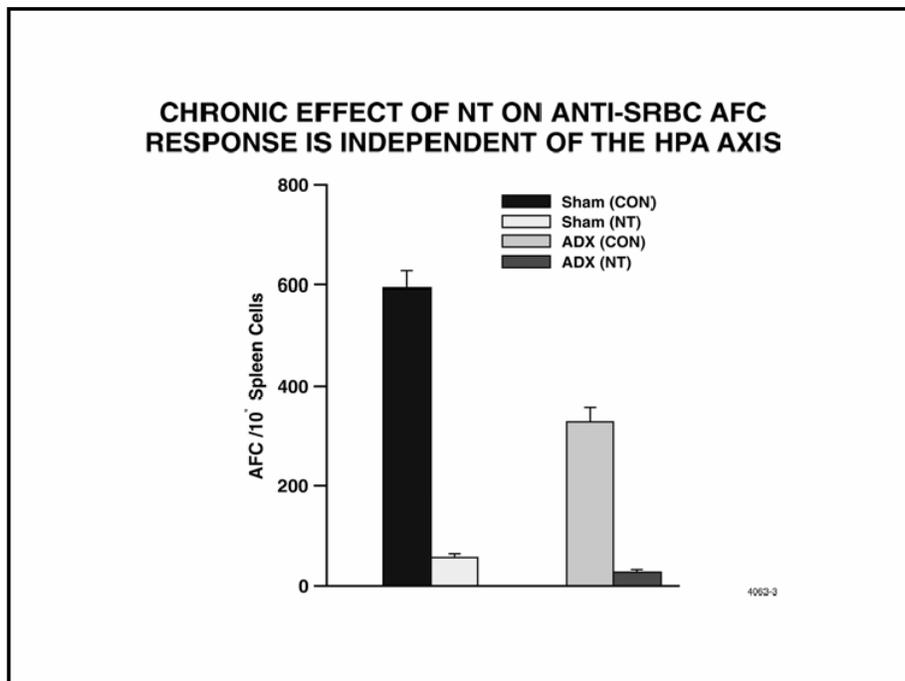
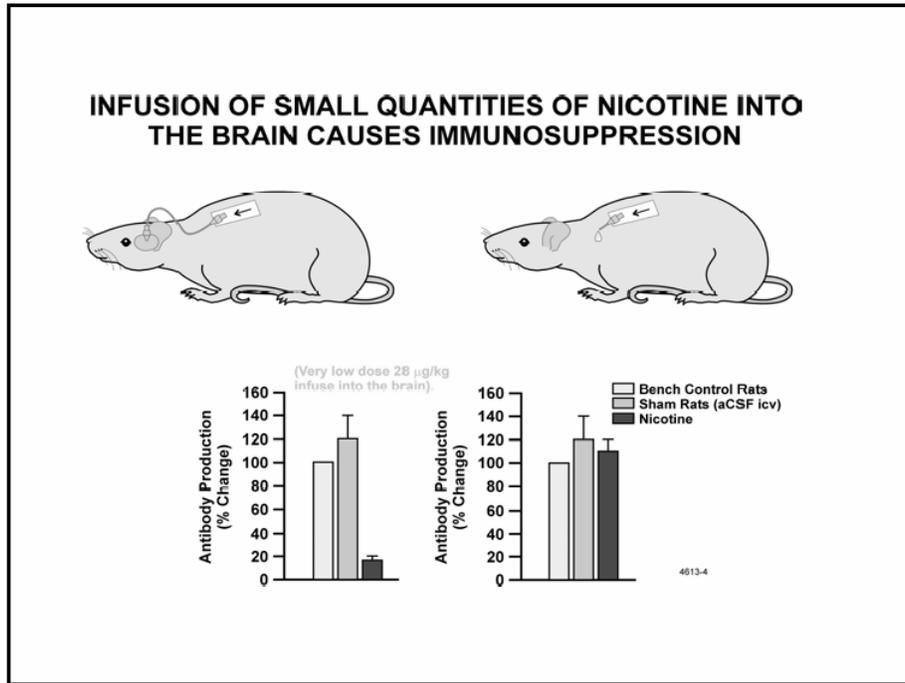
## Cholinergic compounds and neuroimmune interaction

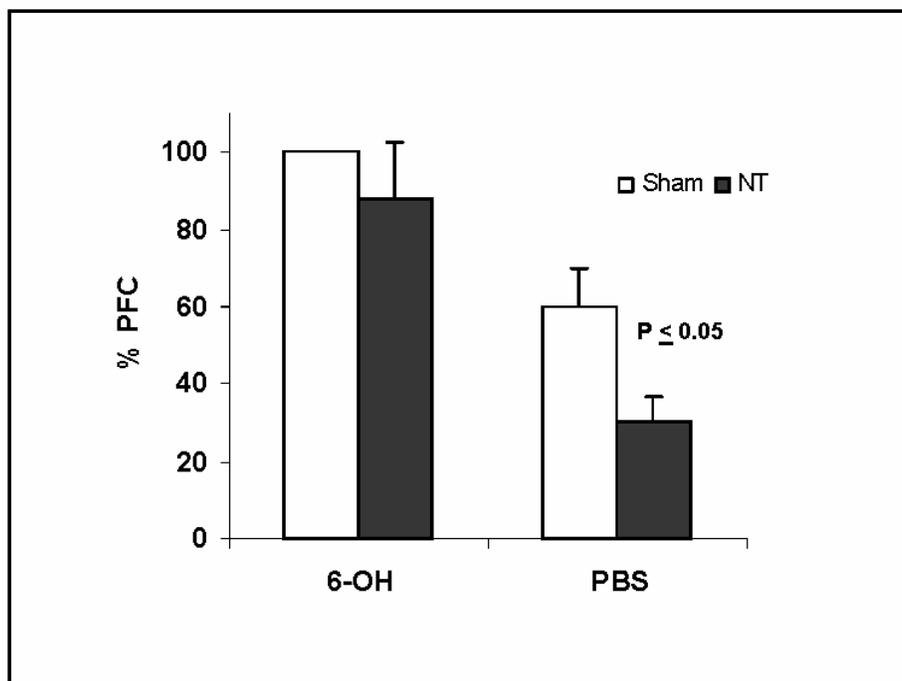
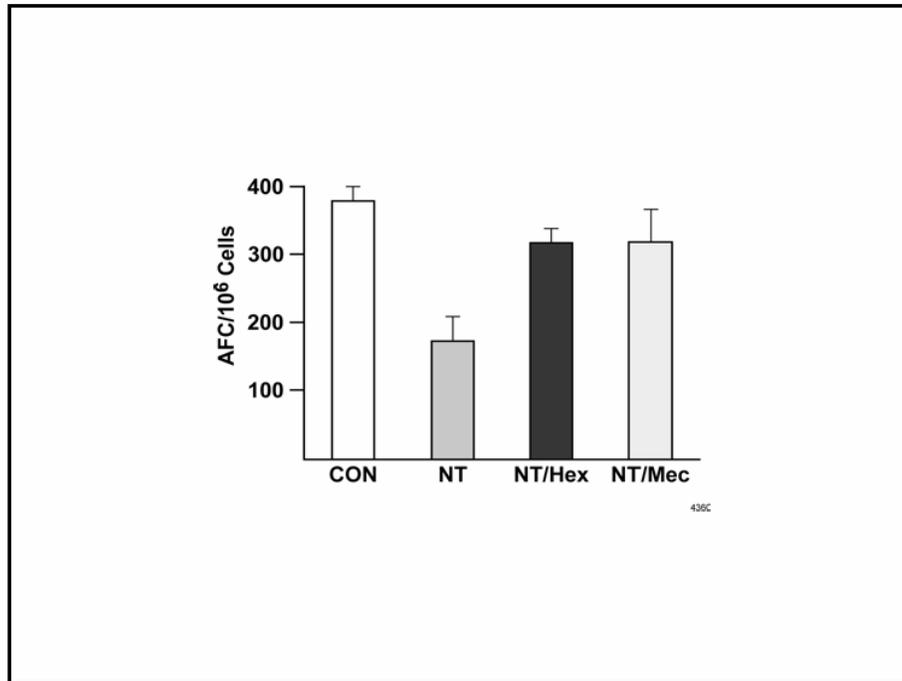
- Lymphocytes contain both muscarinic and nAChRs.
- Sarin - a powerful acetylcholine esterase inhibitor, in high doses causes cholinergic shock -respiratory failure and death.
- Nicotine –a neuroactive substance. It is an established immunosuppressive and anti-inflammatory.

- Lymphoid tissues have dense sympathetic innervations.
- Leukocytes, including lymphocytes and dendritic cells also have beta-2-adrenergic receptors .
- The sympathetic neurotransmitter norepinephrine (NE) binds beta-2-adrenergic and suppresses immune and inflammatory responses.

## EFFECTS of NICOTINE







### TCR-Mediated Signaling for Ca<sup>2+</sup> response

**ANTIGEN**

↓

**Protein Tyrosine kinases  
(PTK)**

↓

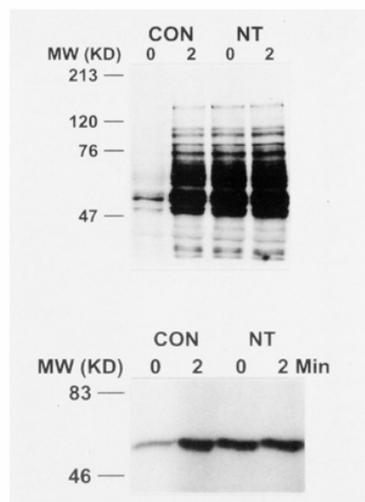
**IP3 (PLC- $\gamma$ 1)**

↓

**Calcium from stores**

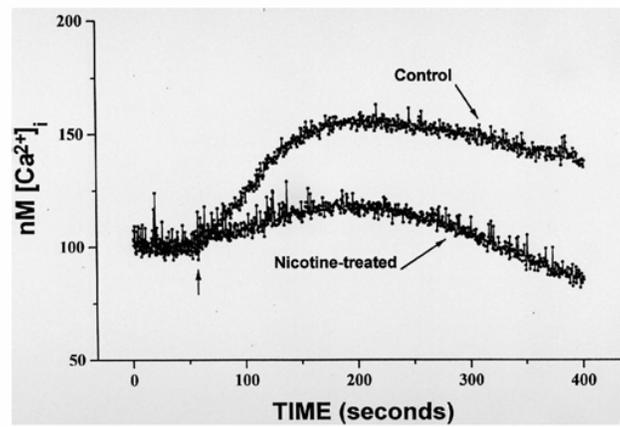
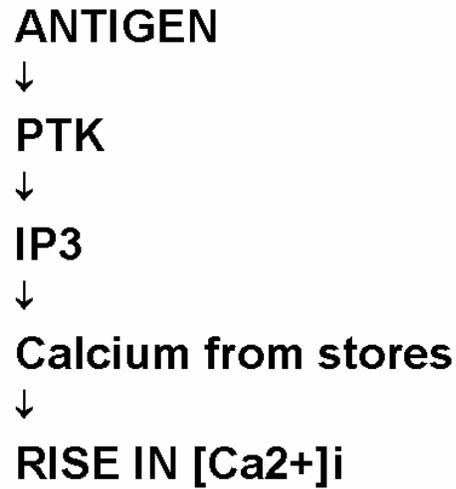
↓

**RISE IN [Ca<sup>2+</sup>]<sub>i</sub>**



4613-32

### TCR-Mediated Signaling for Ca<sup>2+</sup> response



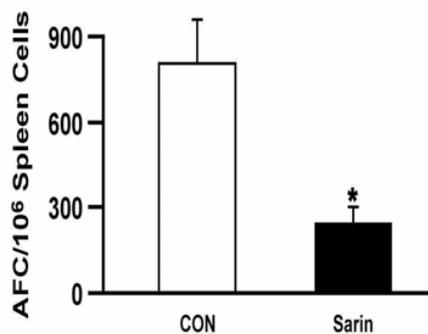
4613-2B

## Health effects of low-doses sarin

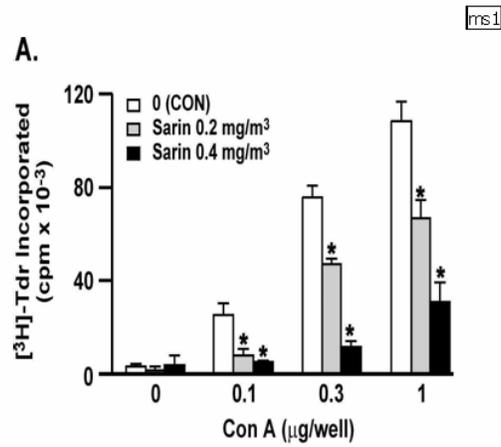
- The possibility that some GW-I veterans were exposed to sarin, engendered the possibility that some symptoms of the GWS might be related to low-dose exposure to sarin.
- The health effects of low-dose sarin are not known. Some survivors of the 1994 subway sarin attack in Japan have exhibited chronic neurological problems.
- Some of the survivors died from opportunistic infections, such as Legionella, indicating sublethal doses of sarin might have long-term effects on the immune system.

DOES SARIN AFFECT THE IMMUNE SYSTEM?

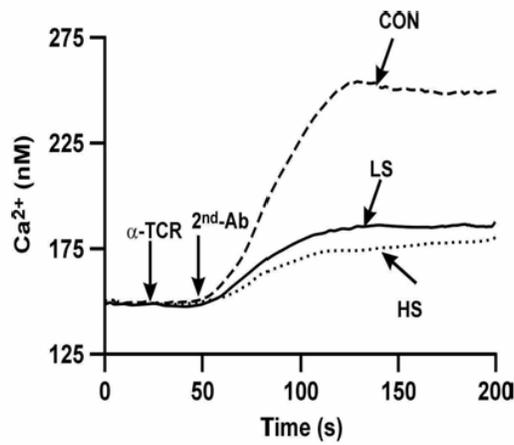
## Sarin inhibits antibody response



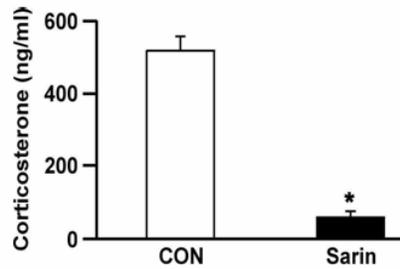
### Sarin suppresses T cell mitogenesis



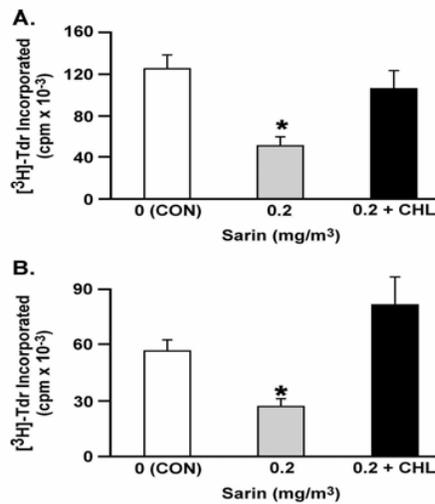
### Sarin impairs T cell signaling (calcium response)

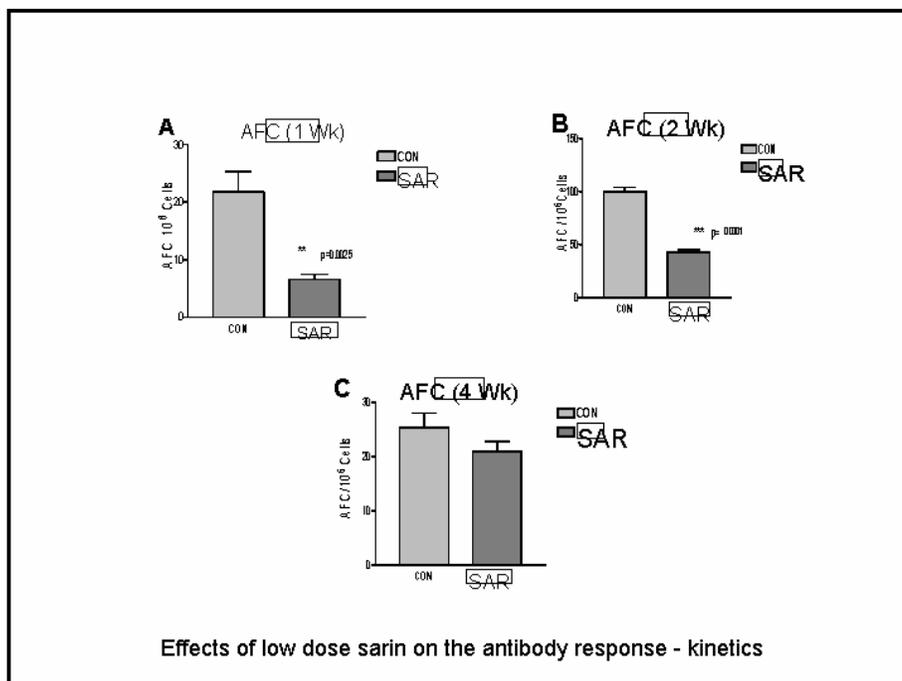
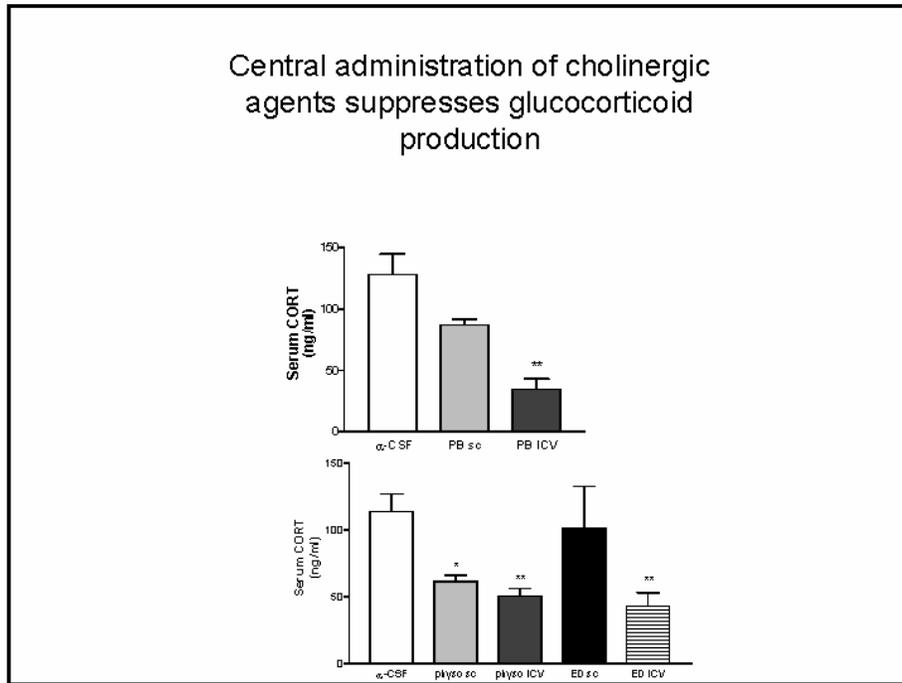


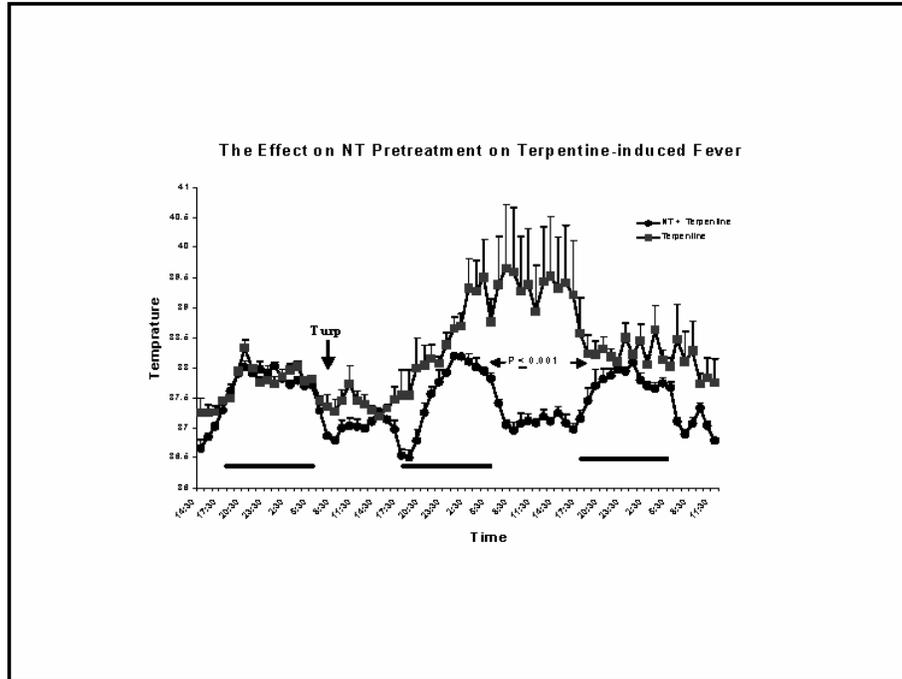
### Sarin suppresses corticosterone production



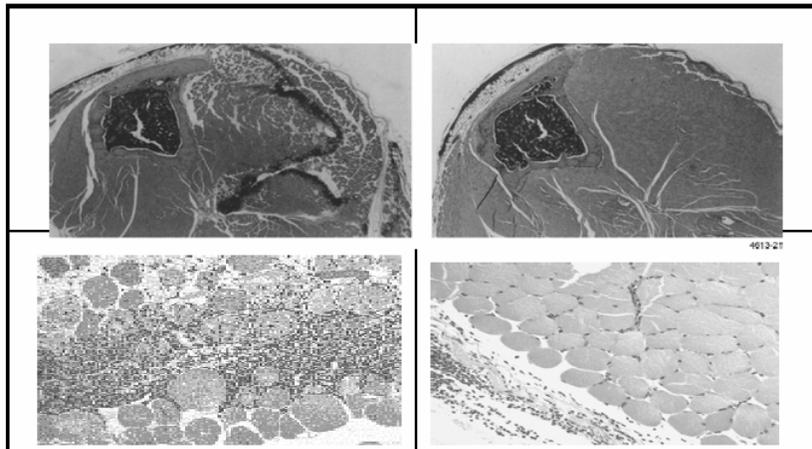
### Chlorisondamine blocks sarin-induced immunosuppression (A: Con A; B: anti- $\alpha\beta$ TCR)

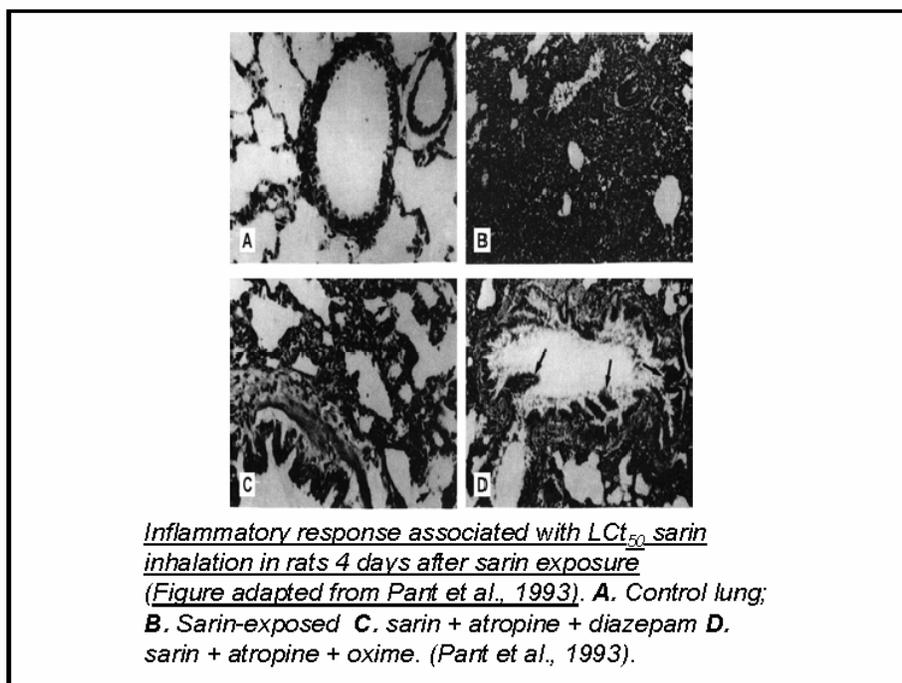
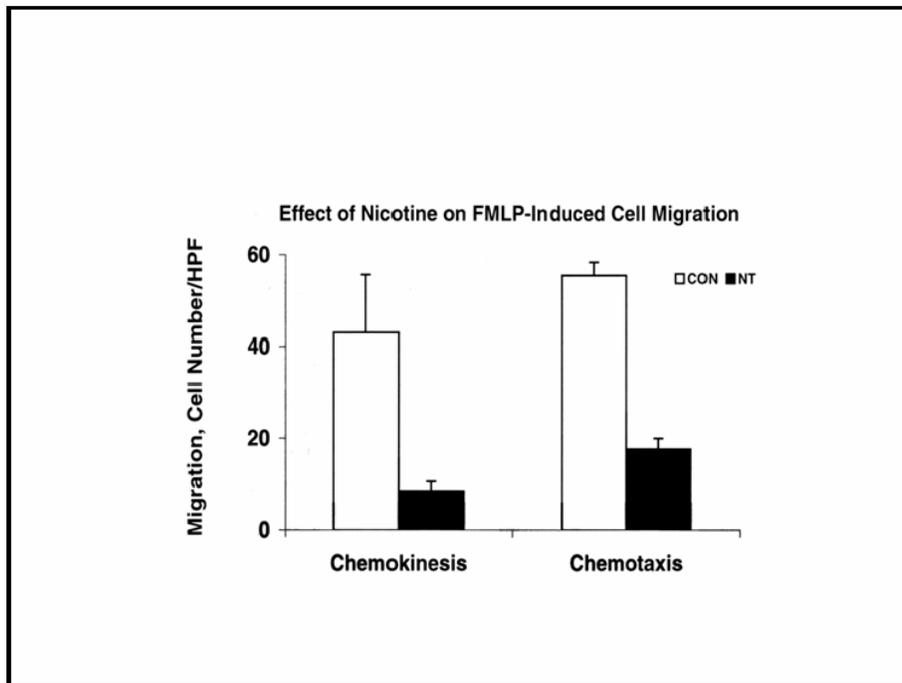


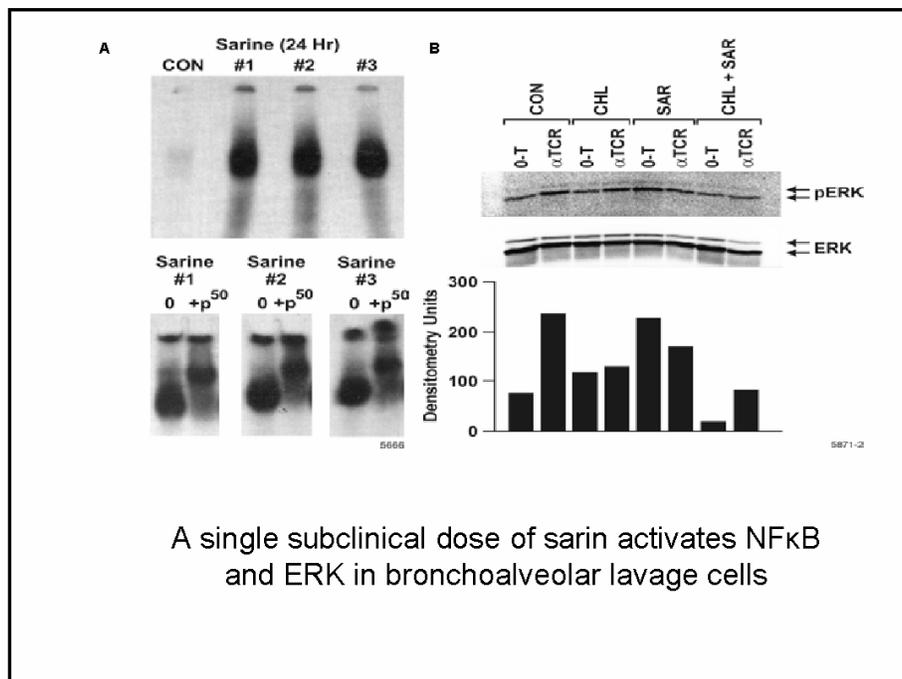
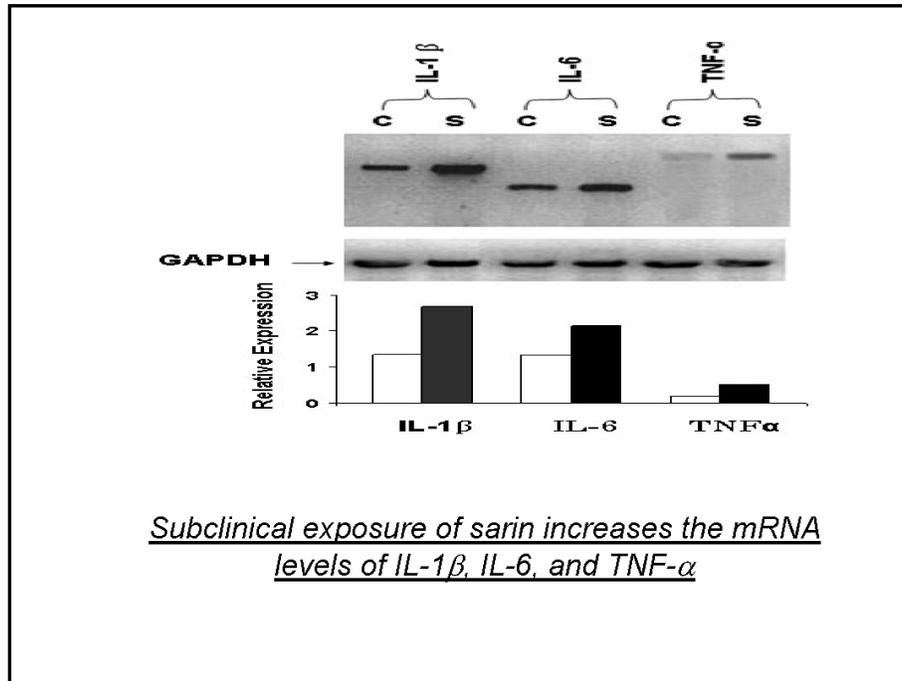


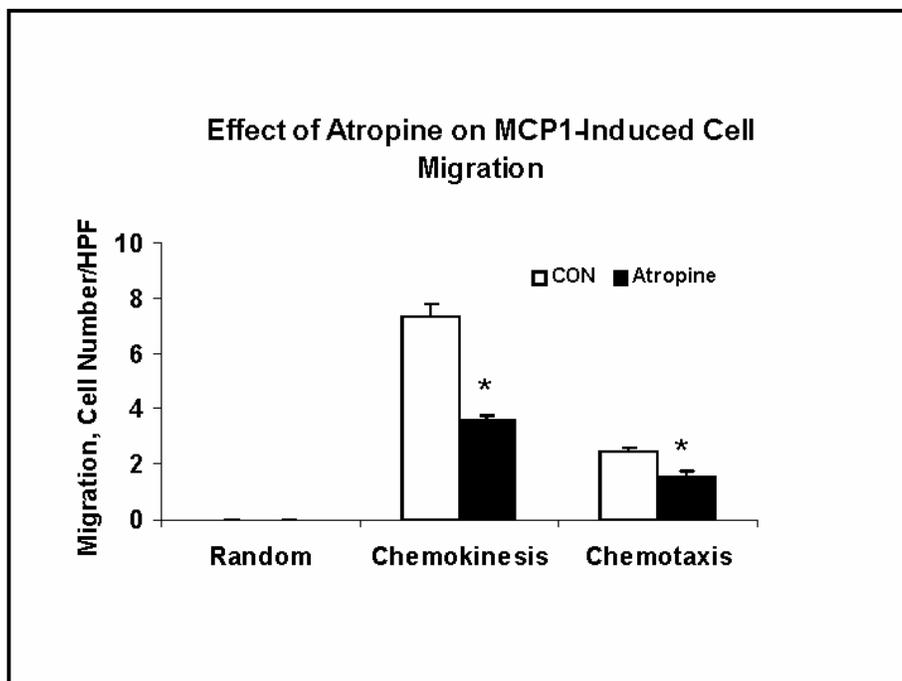
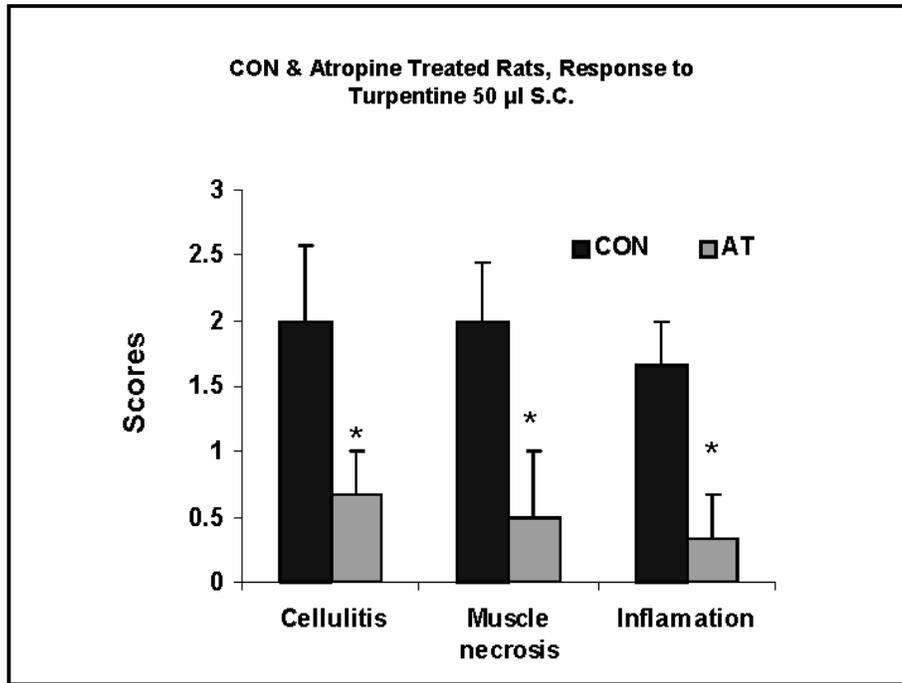


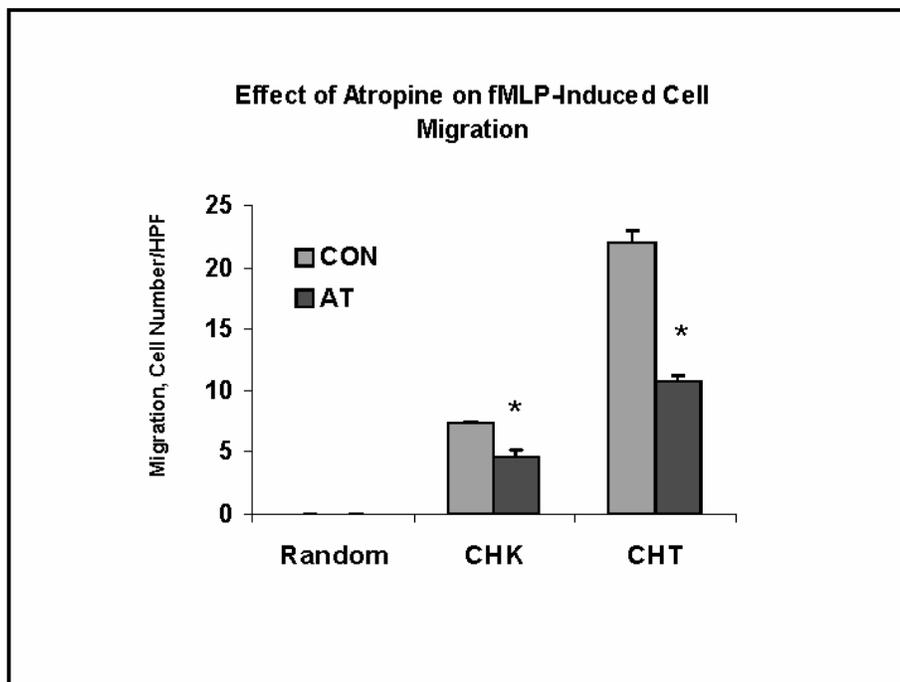
**Nicotine inhibits the migration of leukocytes to the site of inflammation**











## CONCLUSIONS

1. Both sarin and nicotine inhibit T cell activation.
2. While nicotine has no significant effects on CORT levels, sarin and other cholinesterase inhibitors decrease serum CORT levels.
3. Nicotine is anti-inflammatory, while sarin is proinflammatory. Nicotine inhibits the expression of IL-1 $\beta$  in the brain, while sarin induces the expression of proinflammatory cytokines in the lung and the brain (activation of the NF $\kappa$ B/ERK pathway).
4. While activation of nAChRs suppresses, activation of muscarinic receptors stimulate the immune/inflammatory responses.

- Seddi Razani-Boroujerdi
- Roma Kalra
- Ray Langley
- Juan Carlos Philippides
- Shashi Singh
- Neerad Mishra
- Fletcher Hahn
- Julie Hutt
- RO1 DA017003
- R01 DA04208-15
- RO1DA04208-7S
- W81XWH-04-C-0071

**Presentation 7 – Jau-Shyong Hong**

**Role of Inflammation in the Pathogenesis of  
Neurodegenerative Diseases**  
*Models, Mechanisms, and Therapeutic Interventions*

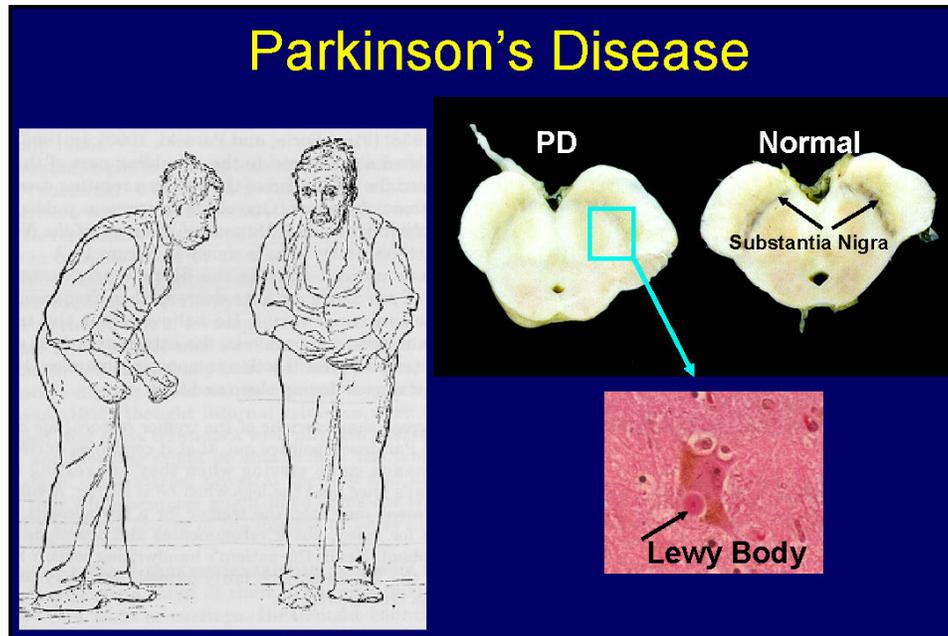
J.S. Hong, PhD  
Neuropharmacology Section  
Laboratory of Pharmacology and Chemistry  
NIEHS / NIH  
RAC 2006-08-14



**TIME**

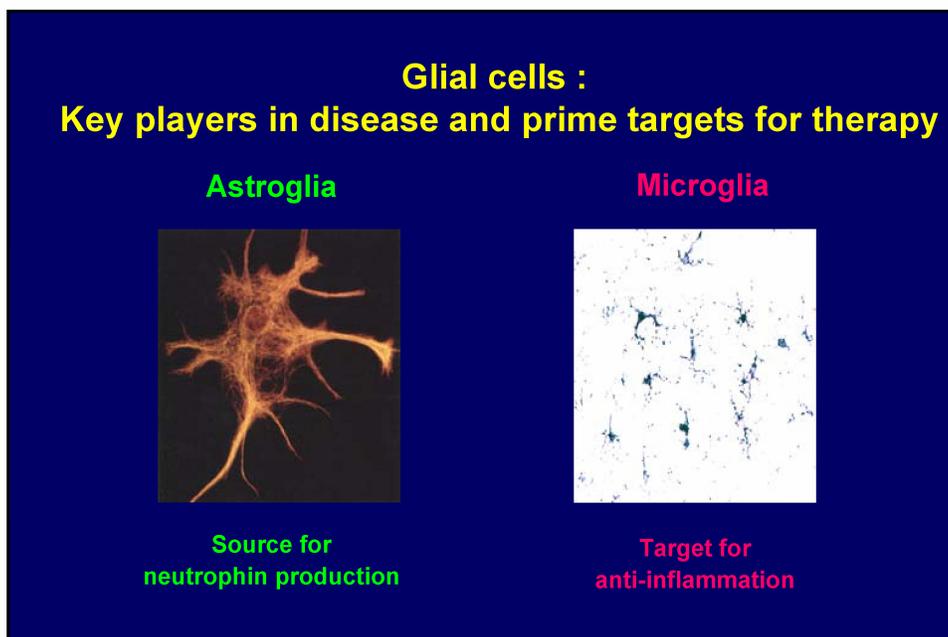
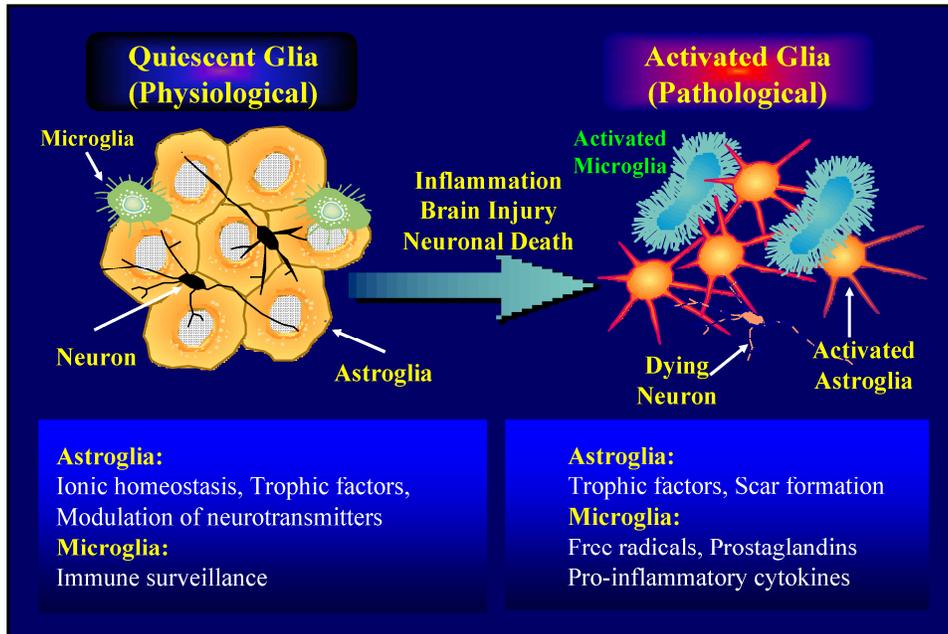
**THE SECRET KILLER**

■ The surprising link between **INFLAMMATION** and HEART ATTACKS, CANCER, ALZHEIMER'S and other diseases  
■ What you can do to fight it



## Parkinson's Disease (PD)

1. PD is an age-related, **progressive (8-10 years)**, and **self-propelling** neurological disease with a **selective** loss of dopaminergic neurons in the substantia nigra.
2. Less than **5%** of PD patients show genetic mutations ( $\alpha$ -synuclein, Parkins). In contrast, **95%** of PD cases are sporadic. Twin studies suggest that **environmental factors** are closely associated with PD.
3. Among the environmental toxins, the following have been considered important **risk factors**:
  - (a) **Infectious agents: Bacteria (LPS), Virus (HIV)**
  - (b) **Pesticides & Herbicides: Rotenone, Paraquat**



## Research Aims

1. Creation of progressive and inflammation-mediated rodent Parkinson's disease **models**
2. Elucidation of **mechanisms** of inflammation-mediated degeneration: role of microglia
3. Development of novel anti-inflammatory **therapy** for Parkinson's disease

## AIM 1

### Creation of Progressive and Inflammation-mediated Rodent Parkinson's Disease Models

#### Rationale:

The existing models **did not** address the role of **inflammation** in the pathogenesis, nor reflect the **delayed** and **progressive** nature of PD.

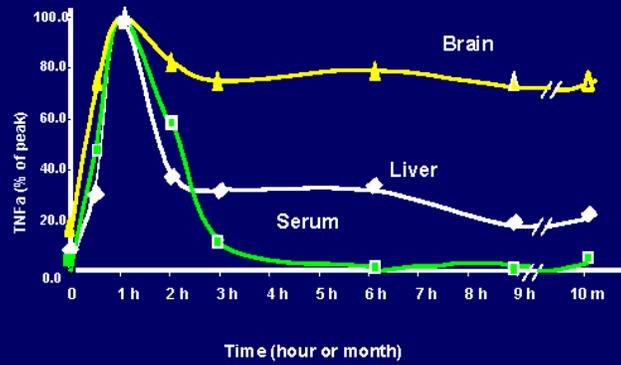
#### a) *In vivo* model:

1. *Intra-nigral infusion of lipopolysaccharide (LPS, an inflammagen from bacteria)*
2. *Systemic injection of LPS*

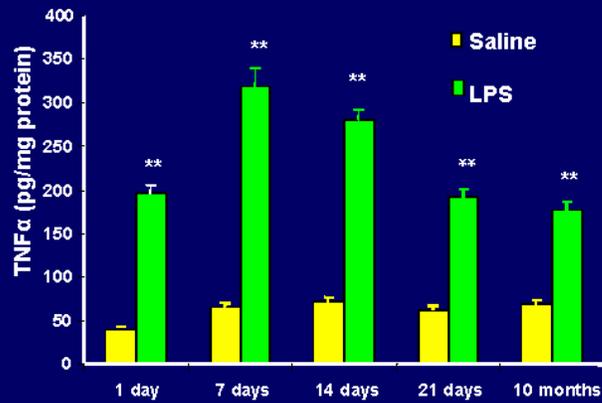
#### b) *In vitro* model: (Primary cell cultures)

*Bin Liu, MD, Ph.D, Huiming Gao, MD, Ph.D, Liya Qin, Ph.D.*

### Tissue TNF $\alpha$ levels after a single intra-peritoneal injection of LPS (5mg/kg)

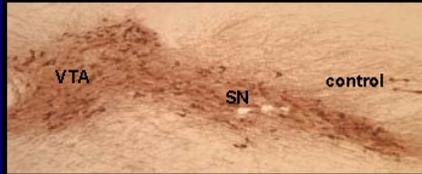


### Brain TNF $\alpha$ levels after a single intra-peritoneal injection of LPS (5mg/kg)

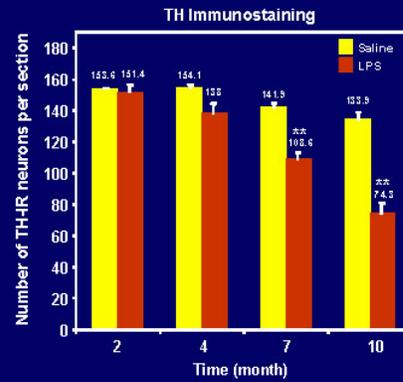
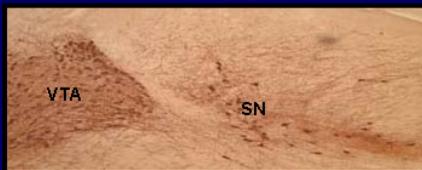


### Progressive loss of dopaminergic neurons in substantia nigra after a single injection of LPS (5 mg/kg. ip)

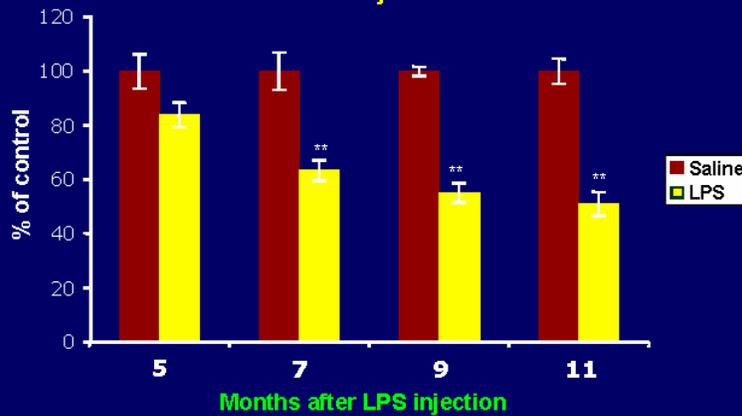
Dopaminergic neurons, control, (10 month)



10 months after LPS (5mg/kg, ip)



### Time-related decrease in rotarod activity after LPS injection



## AIM 2

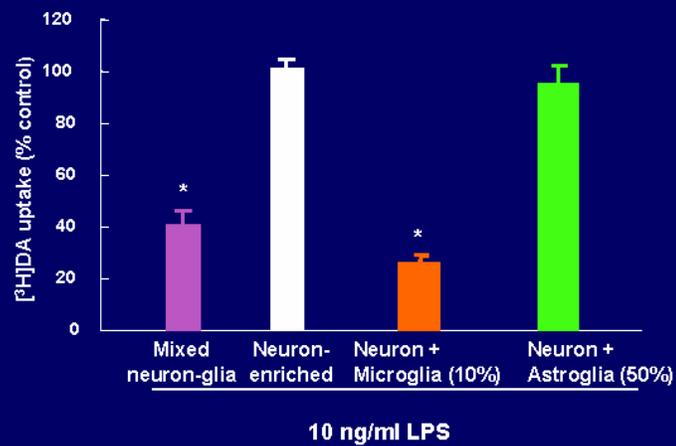
### Elucidation of Mechanism of Inflammation-mediated Dopaminergic Neurotoxicity: Role of Microglia

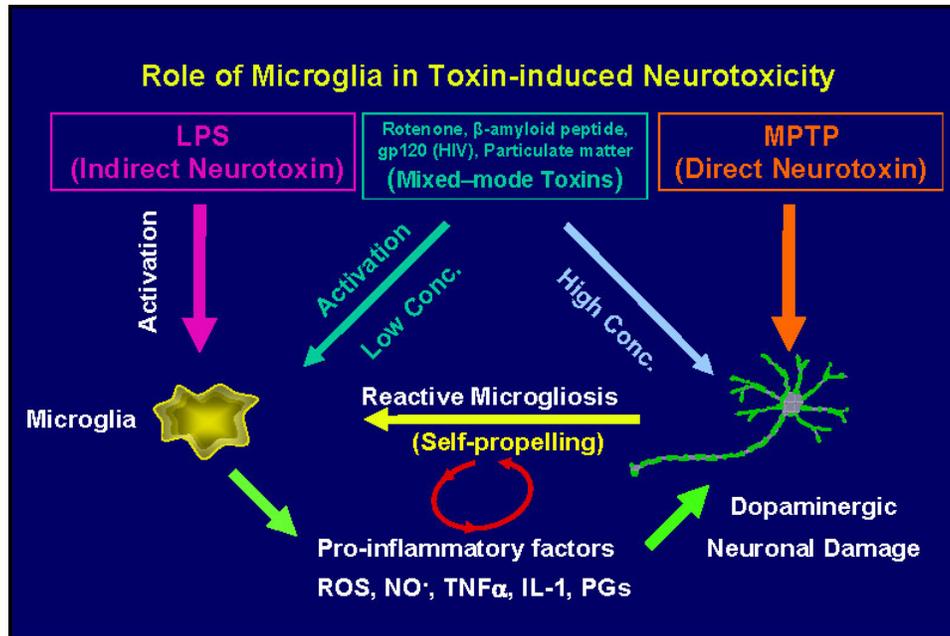
#### *Rationale:*

The role of **inflammation** and the **cell type** responsible in the pathogenesis of PD was **not** clearly defined .

*Liya Qin, Ph.D. Wei Zhang, MD, Ph.D, Yuxin Liu, Ph.D.*

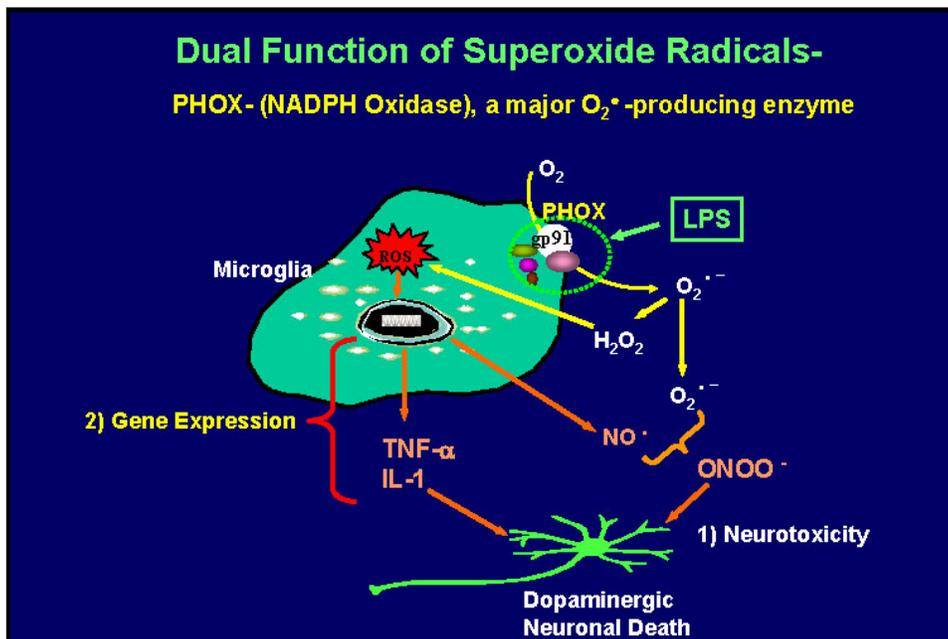
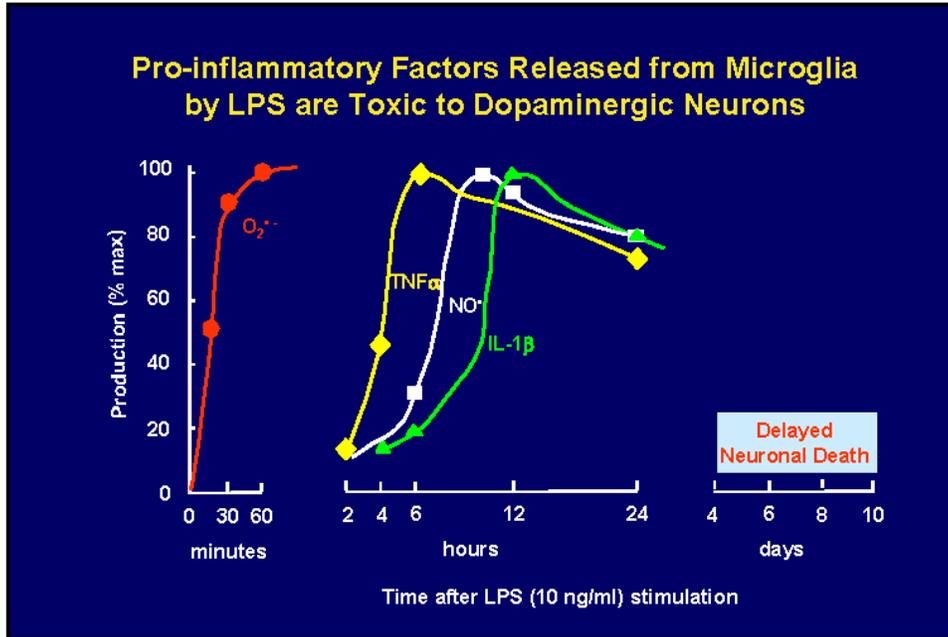
### LPS-elicited Dopaminergic Neurotoxicity is Microglia Dependent

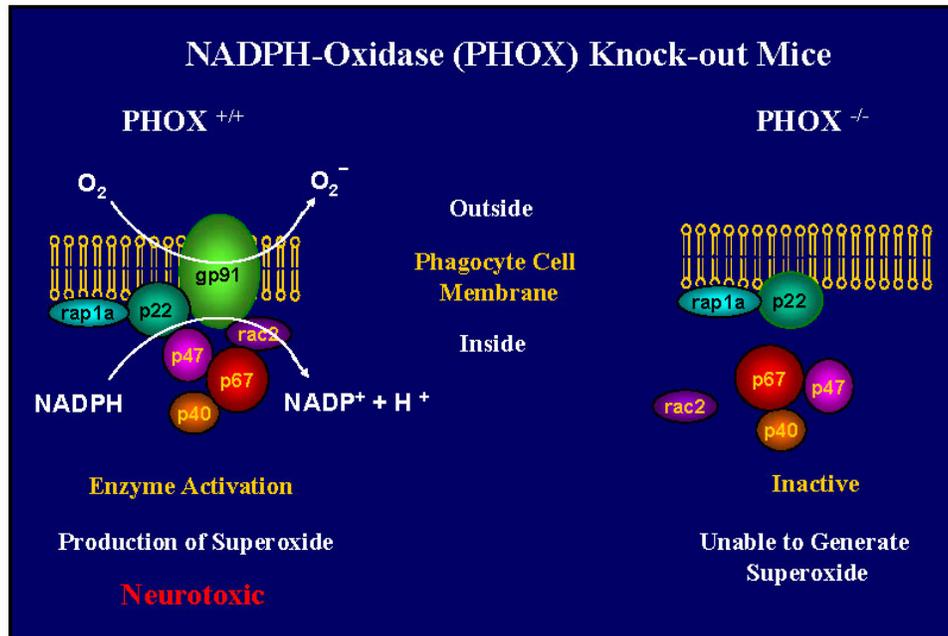




## Mixed-mode Toxins

- **Infectious agents:**  
Bacteria, Fungus, Virus (gp-120, HIV coat protein)
- **Pesticides:**  
Rotenone, Paraquat
- **Heavy metals:**  
Manganese, Cadmium etc.
- **Air pollutants:**  
Particulate matter, Diesel engine exhaust, Nano-particles



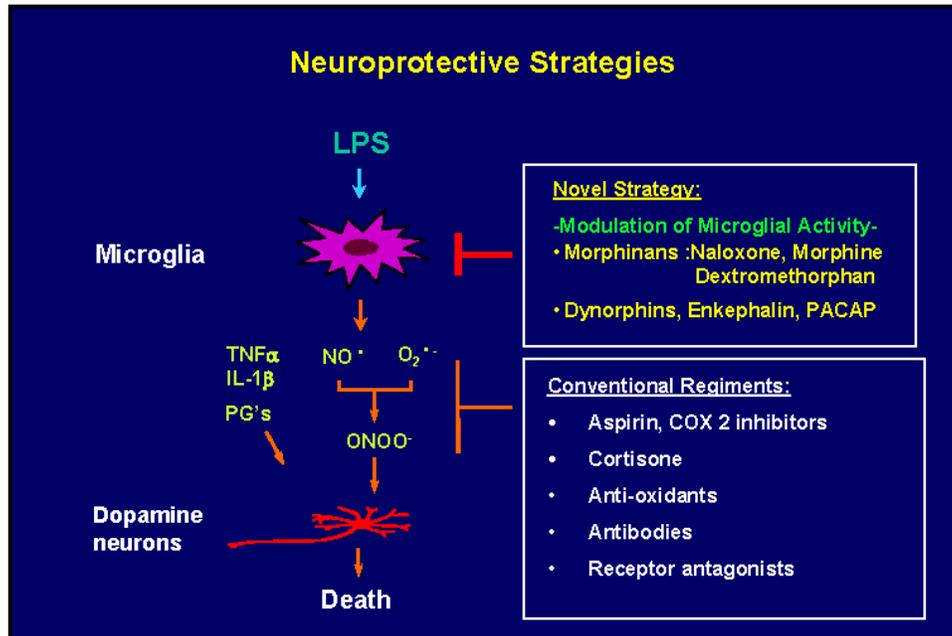


### AIM 3

## Development of Novel Anti-inflammatory Therapy for Parkinson's Disease

### Rationale:

1. Current therapy (L-DOPA) **does not slow the progression of PD.**
2. Clinical trials show that anti-inflammatory therapy of PD is effective. However, due to their low potency and safety issues, current anti-inflammatory drugs are not suitable for **long-term therapy.**
3. **More potent and safer** anti-inflammatory drugs are urgently needed.

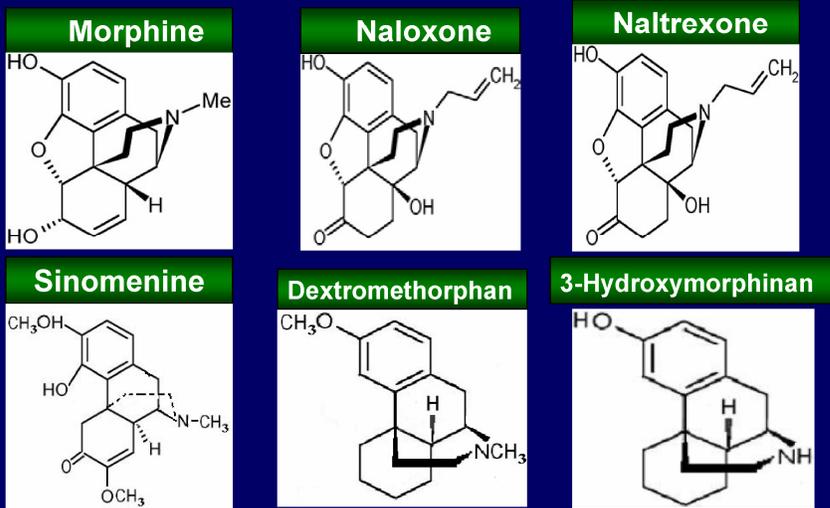


## Neuroprotective and anti-inflammatory effects of morphinans (naloxone, dextromethorphan):

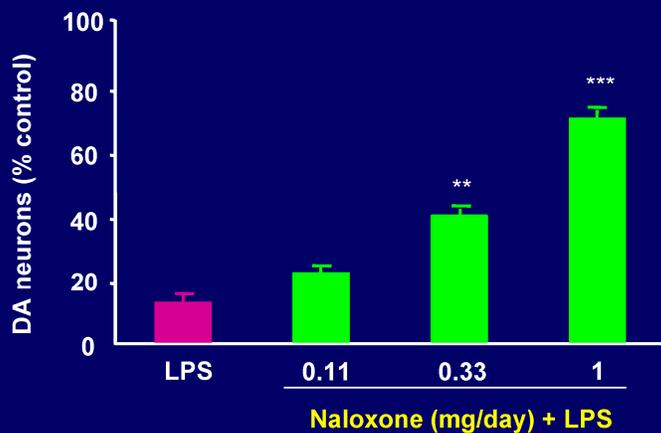
Implication for the therapy of Parkinson's disease.

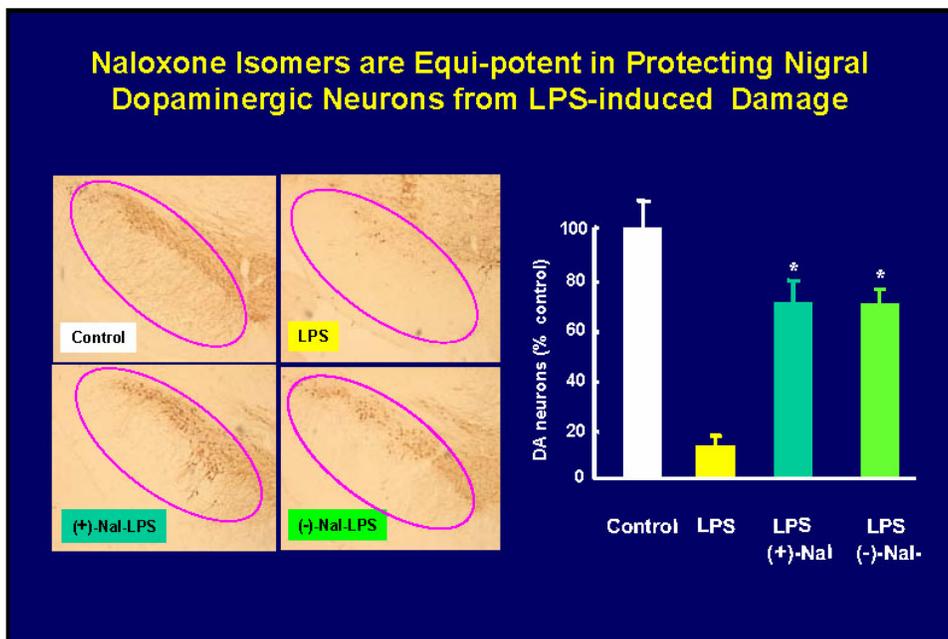
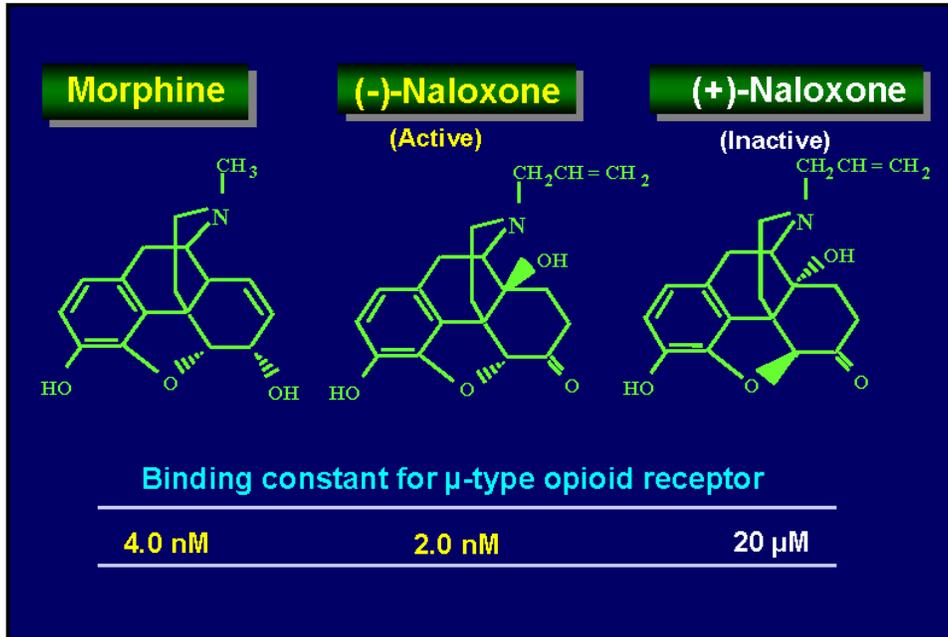
*Wei Zhang, MD, Ph.D.*  
NIEHS/NIH

**Morphinans which are anti-inflammatory and neuroprotective**

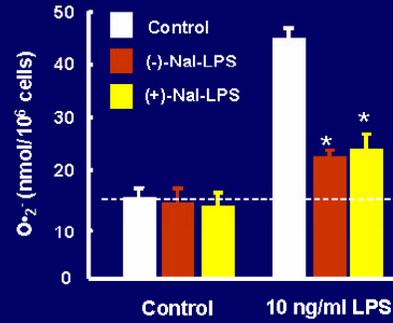
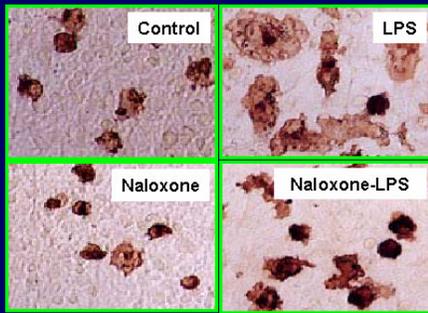


**Naloxone Reduces LPS-induced Loss of Nigral Dopaminergic Neurons (*in vivo* study)**

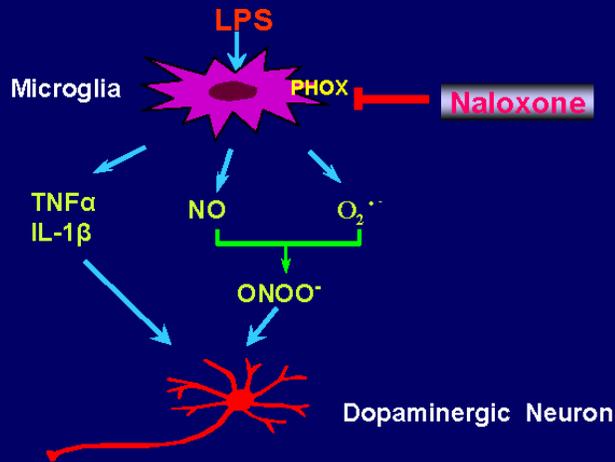




### Naloxone Inhibits LPS-induced Activation of Microglia and Production of Superoxide



### Microglial PHOX is the Site of Action for Naloxone-elicited Neuroprotective Effect



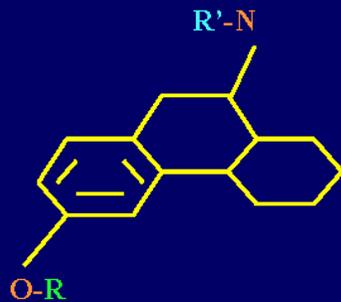
### What is LDN (Low Dose Naltrexone)

1. **Low Dose Naltrexone (LDN)** is a treatment for MS and other neurological diseases. This method was devised and developed by **Dr. Bernard Bihari**, a neuro-physician in New York.
2. It has been reported that naltrexone (**3 mg per day**, in contrast to 150 mg per day a day) is beneficial for patients who suffer from cancer, AIDS and neurological disorders, such as MS, PD.
3. **How Naltrexone Works:** The benefits are due to the temporary **inhibition of brain opioid receptors and production of endorphins**. This results in the reduction of painful symptoms and an increase sense of well-being.

### Low-dose Naltrexone and Pain (Pain Therapeutic Inc.)

1. **Inhibition of pain by opioid painkillers is achieved by inhibiting nerve cells that have opioid receptors**
2. **Opioid painkillers also activate an excitatory signaling pathway linked to opioid receptors, thereby stimulating the transmission of pain.**
3. **Tolerance and physical dependence can be prevented by co-administration of **ultra-low-dose naltrexone**, an opioid antagonist. We believe ultra-low-dose naltrexone blocks the excitatory pathway, but not the inhibitory pathway, on opioid receptors.**
4. **The inhibition of excitatory signals enhances analgesia and attenuates tolerance, physical dependence and addiction.**

### Structure of 3-hydroxy-morphinan (3-HM): a metabolite of DM



	R	R'
DM	CH <sub>3</sub>	CH <sub>3</sub>
3-HM	H	H

### Potential Beneficial Effect of Morphinans

#### Opioid-related?

- Alcohol abuse
- Compulsive eating disorder
- Opiate addiction
- Smoking

#### Non-opioid and/or inflammation-related

##### CNS

- Alzheimer's dis.
- Brain Ischemia.
- Parkinson's dis.
- MS
- Spinal injury

##### Peripheral

- Asthma
- Arthritis
- Arteriosclerosis
- Cancer
- Diabetes
- Heart attack
- Hepatitis
- Inflammatory pain
- Irritable bowl dis.
- Lupus
- Sepsis



**Presentation 8 – Tomás Guilarte**



*Peripheral Benzodiazepine  
Receptor Imaging of central  
nervous system  
inflammation and injury*

**Tomás R. Guilarte, PhD**  
**Neurotoxicology & Molecular Imaging Laboratory**  
**Department of Environmental Health Sciences**  
**Johns Hopkins Bloomberg School of Public Health**

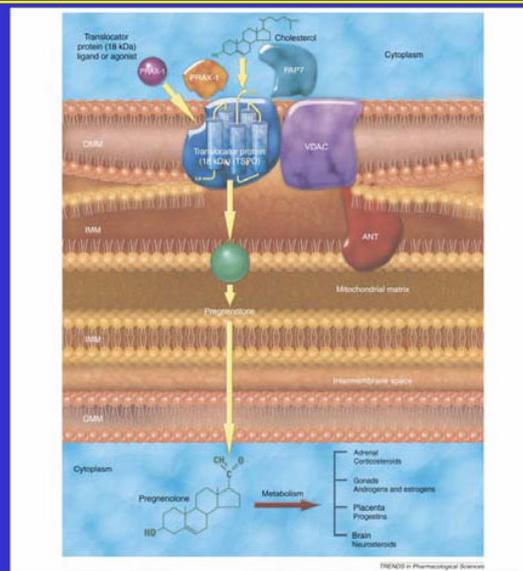
**Reactive gliosis as an Index of Brain  
Injury & Inflammation**

- Reactive gliosis is the hallmark response of the nervous system to injury & inflammation.
- Microglia are the immune-competent cells of the brain and they proliferate and migrate to sites of injury and inflammation.
- Astrocytes are also activated and hypertrophy.
- The ability to track cell-specific responses allows the assessment of brain pathology *in vitro* and *in vivo*.

## Peripheral Benzodiazepine Receptor

- Peripheral Benzodiazepine Receptor exclusively localized in glial cells.
- Different from the “central” type benzodiazepine receptors:
  - 1) Pharmacology
  - 2) Subcellular distribution (mitochondria)
  - 3) Function (*steroidogenesis*, cell growth & differentiation)

### Schematic of the Peripheral Benzodiazepine Receptor



From: Papadopoulos V, Baraldi M, Guilarte TR et al., Trends Pharmacol. Sci. 27: 402-409, 2006

## Peripheral Benzodiazepine Receptor

- Very low expression in the brain neuropil with high expression in the ependymal cells of the ventricles and in the choroid plexus.
- Availability of pharmacologically selective, high affinity (nM) radioligand (isoquinoline, PK11195. Now other ligands are available).
- PK11195 can be labeled with positron emission tomography ( $^{11}\text{C}$  and  $^{18}\text{F}$ ) and single photon emission computed tomography ( $^{123}\text{I}$  and  $^{125}\text{I}$ ) radioisotopes.

## Peripheral Benzodiazepine Receptor

- PBR is the rate limiting step in neurosteroid synthesis. It controls the transport of cholesterol into mitochondria for the synthesis of pregnenolone. This has been demonstrated in glial cells.
- Emerging evidence suggests that activation of PBR may have neuroprotective effects [Ferzaz et al., JPET 301: 1067, 2002; Veiga et al., J. Neurosci. Res. 80: 129, 2005]
- The PBR has been validated as a marker of neuronal injury & inflammation, but its activation may provide an avenue for neuroprotection and recovery from brain injury.

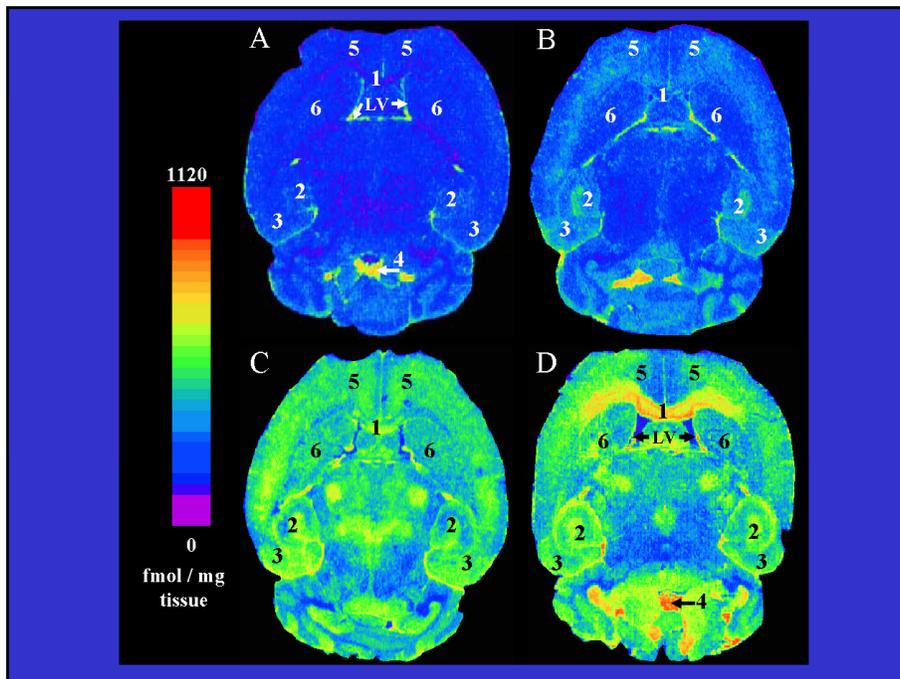
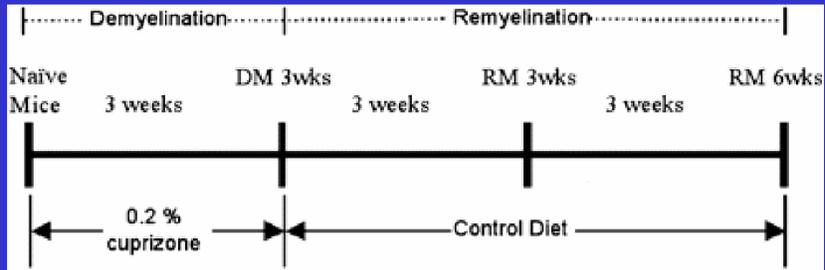
## Animal models-PBR validation

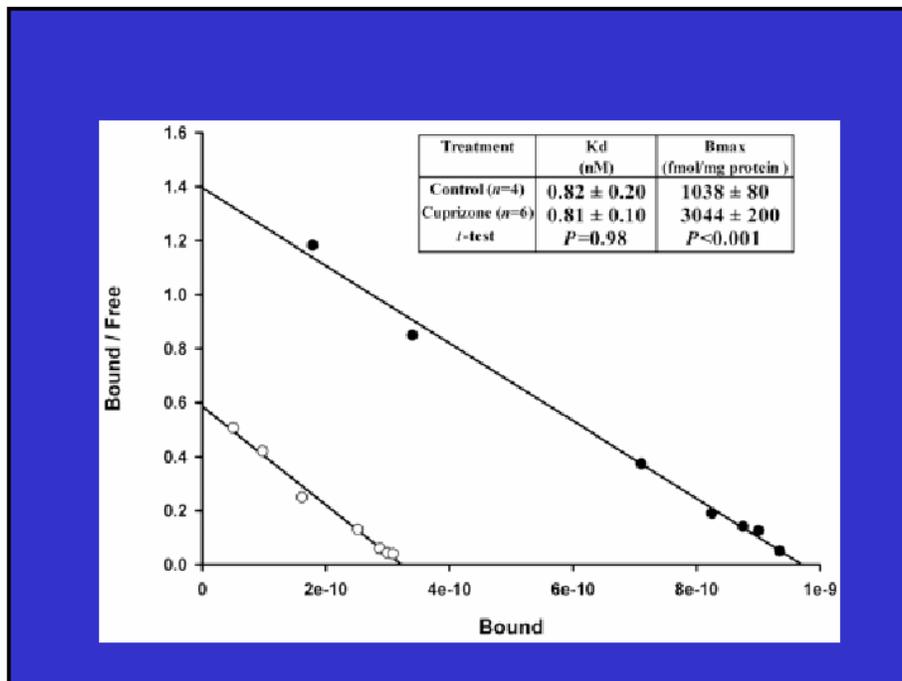
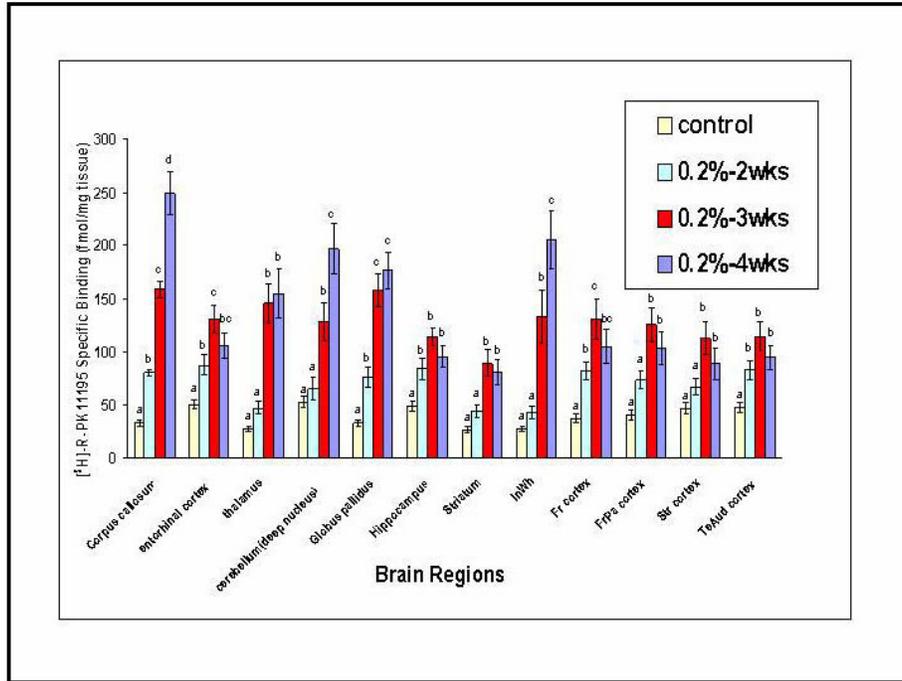
- Trimethyltin (TMT) - Hippocampus (Rat)
- Domoic Acid - Hippocampus (Rat)
- Kainic Acid - Hippocampus (Rat)
- MPTP - Basal ganglia (Mouse)
- Methamphetamine - Basal ganglia (Rat)
- Cuprizone - Demyelination (Mouse)
- Facial Nerve Axotomy (Rat)
- Transient Global Forebrain Ischemia (Rat)
- Traumatic Brain Injury (Rat)
- Simian Immunodeficiency Virus Encephalitis (Rhesus)

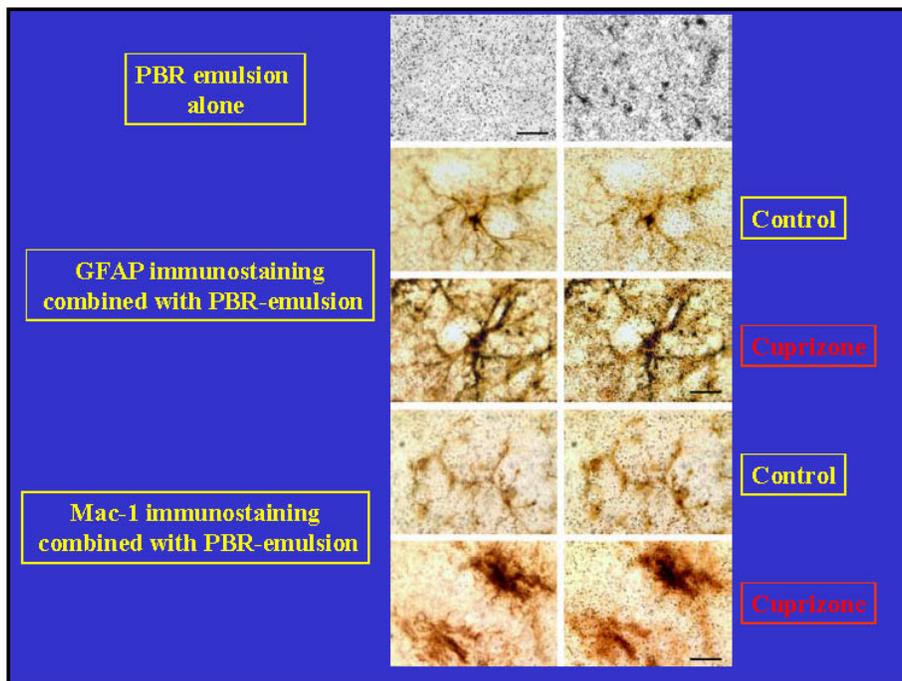
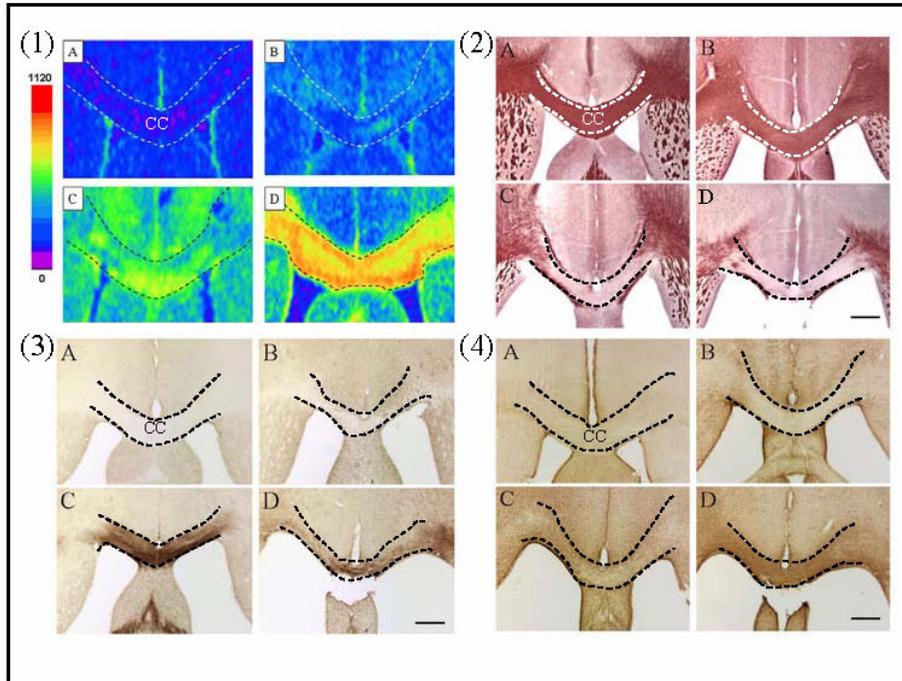
## Animal Model of Demyelination Cuprizone

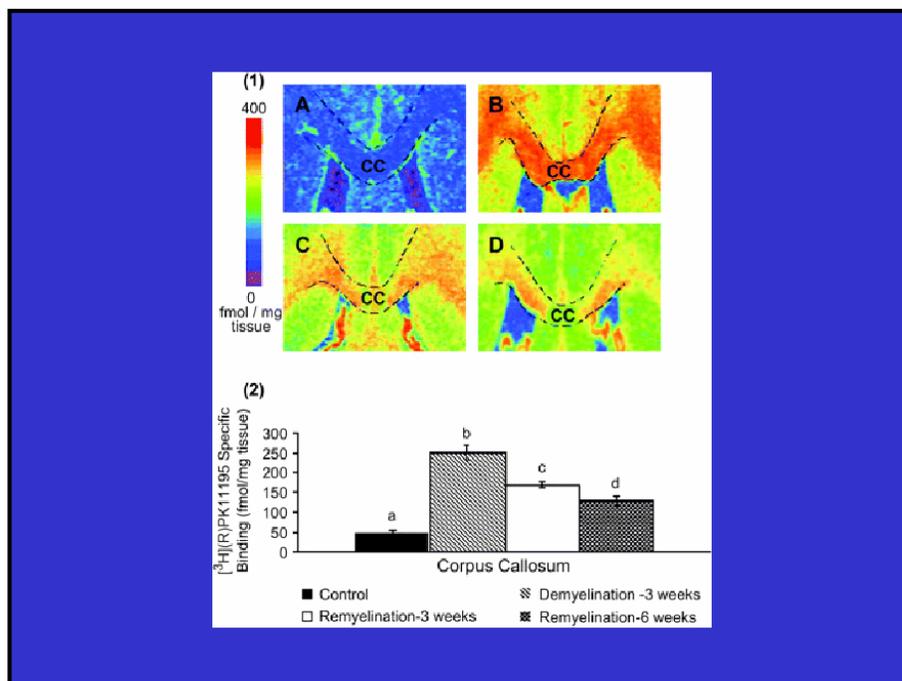
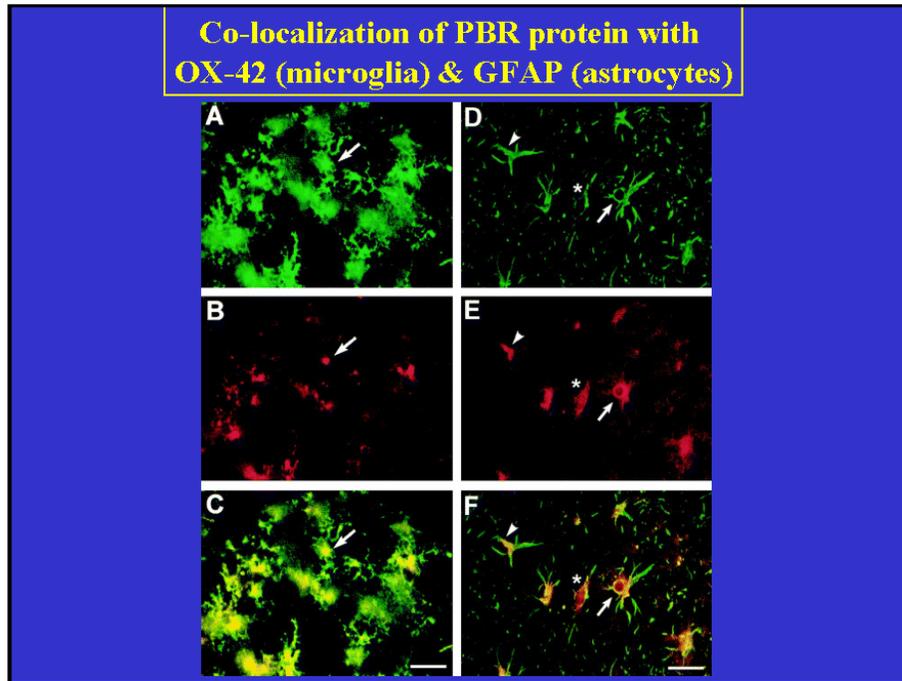
Chen et al., *Brain* 127: 1379-1392, 2004

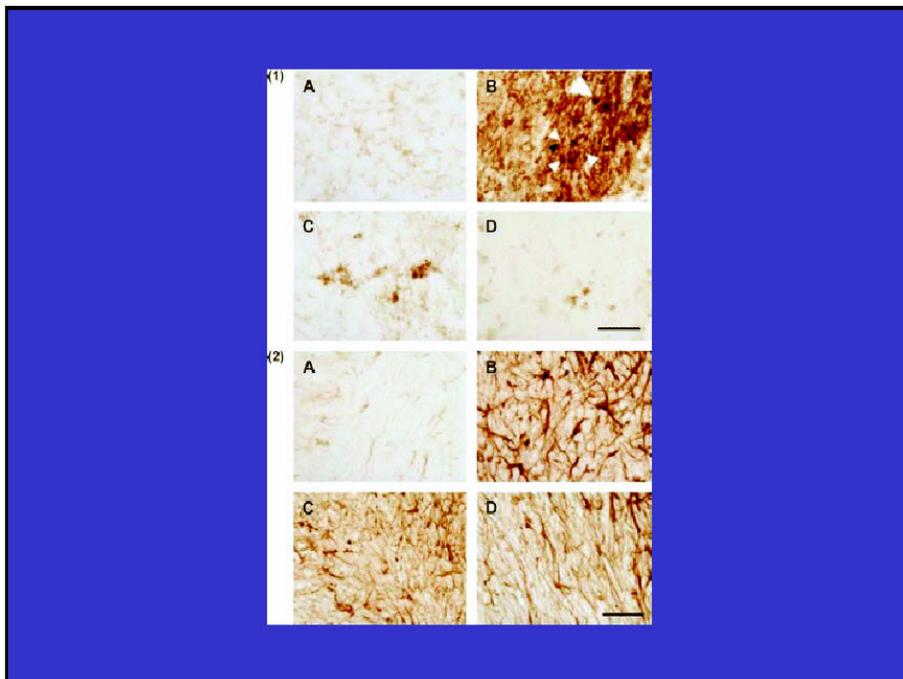
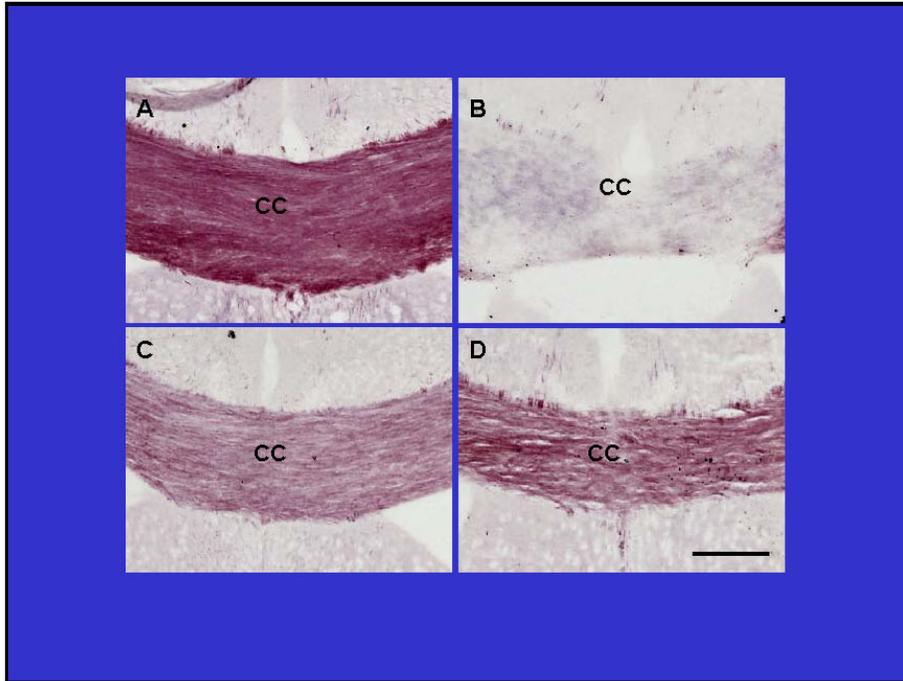
### Experimental design for Cuprizone-demyelination/remyelination studies



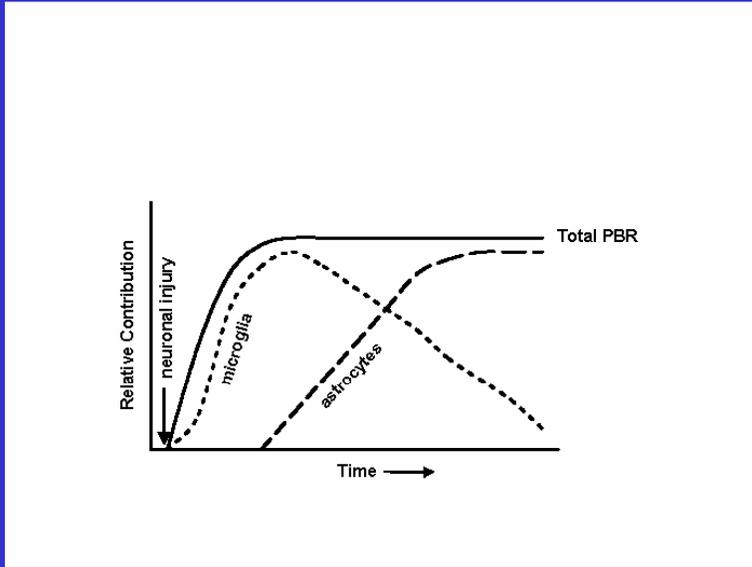




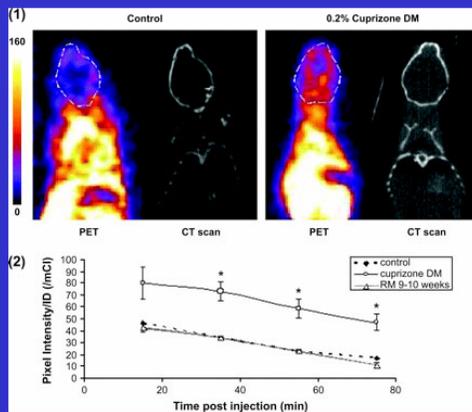




### Cellular sources of PBR response in the brain following injury

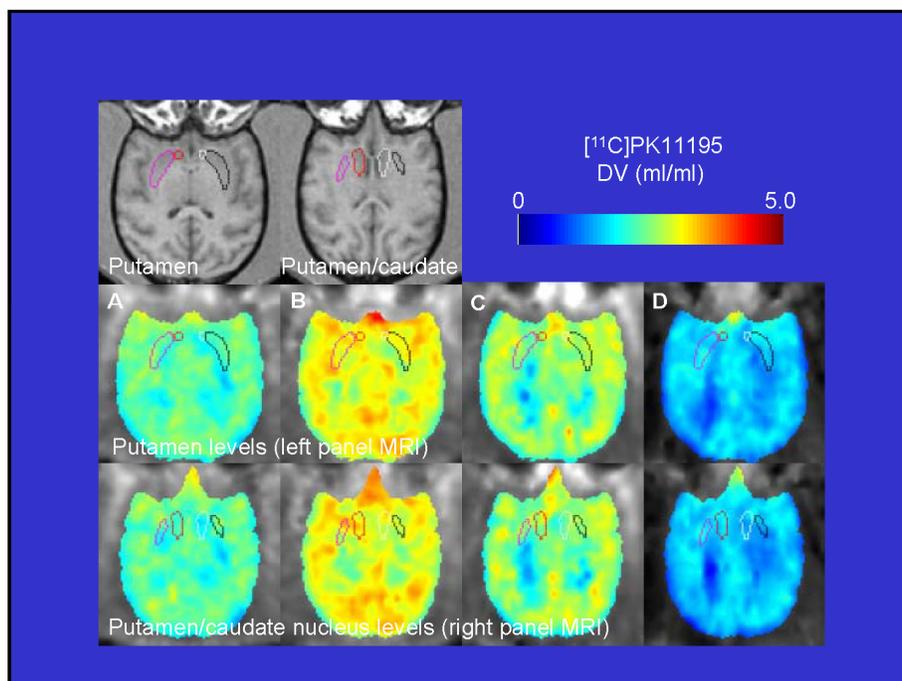
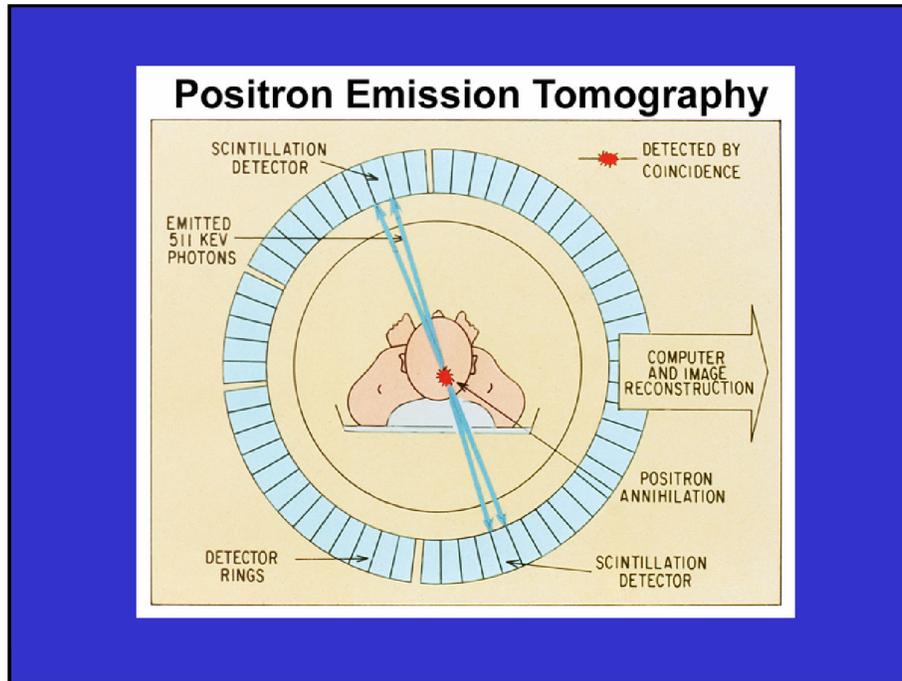


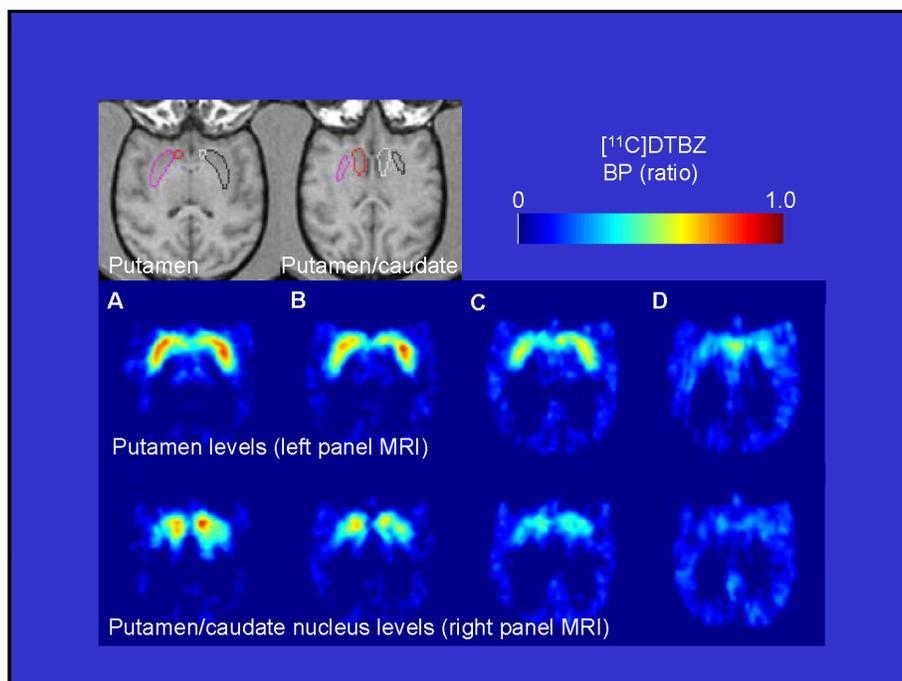
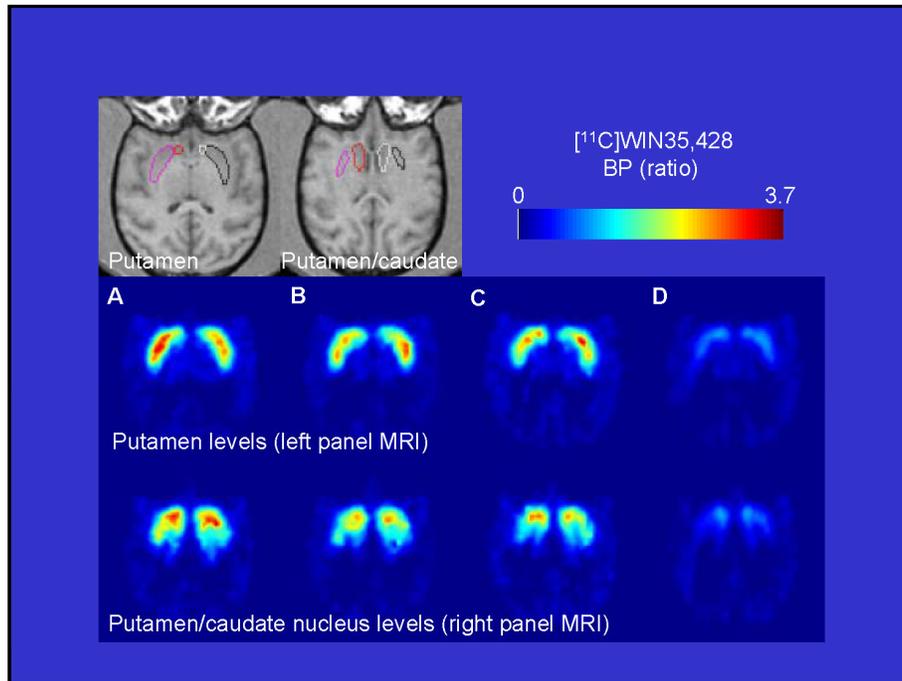
### Small animal PET

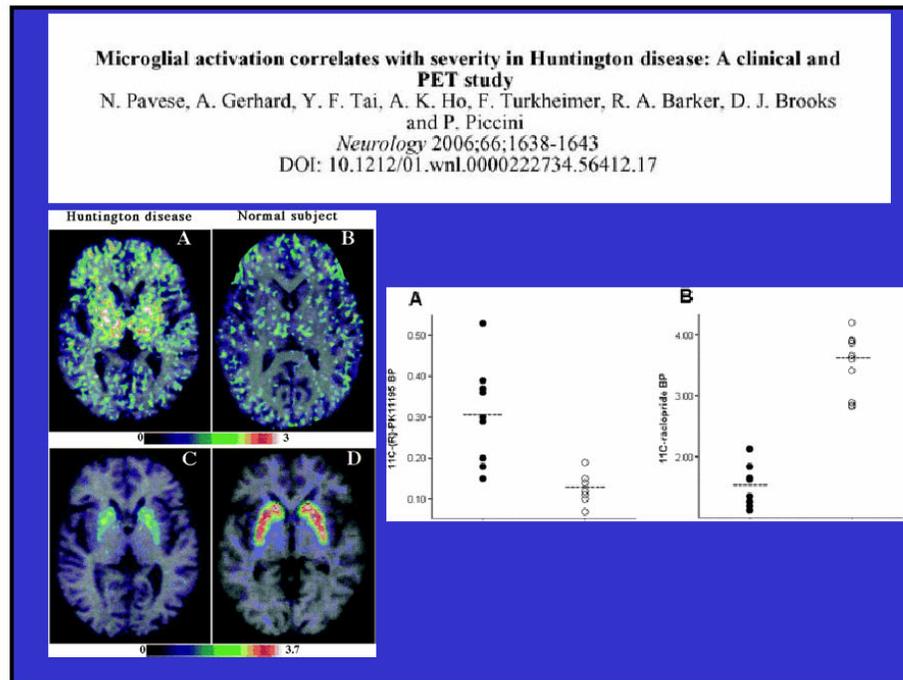












### Advantages of the Peripheral Benzodiazepine Receptor as a Biomarker of Neuronal Injury & Inflammation

- 1) Able to detect primary and secondary sites of brain injury with no *a priori* knowledge.
- 2) Highly sensitive to brain injury from frank neuronal loss to subtle damage to nerve terminals. It is able to detect areas of brain damage prior to commonly used histological methods.
- 3) Because it uses the principle of receptor autoradiography, it is quantitative.

### **Advantages of the Peripheral Benzodiazepine Receptor as a Biomarker of Neuronal Injury**

4) Availability of selective and high affinity iodinated radioligands allows a high throughput for *in vitro* screening. Further, because of recent advances in small animal imaging instrumentation it can be used in rodent models of CNS disease.

### **Ongoing Studies using PBR-PET**

- Application of PBR imaging and quantification in rodent and non-human primate brain in models of neurodegeneration.
- Application of PBR imaging in human neurodegenerative & inflammatory disease.

## Concluding Remarks

- The PBR is a sensitive and early indicator of brain injury and inflammation.
- It can be used to detect primary and secondary sites of injury from physical, chemical, viral or other types of brain insults.
- *In vivo* application to animal and/or human studies is now feasible and it may be useful in the early detection of brain disease, help in our understand of disease progression and to monitor the effectiveness of therapeutic interventions.

## Acknowledgements

- Dr. Anthony Kuhlmann
- Dr. Ming-Kai Chen
- Dr. Jim O'Callaghan
- Dr. Kwamena Baidoo
- Dr. Joseph Mankowski
- Dr. Martin Pomper
- Dr. Dean Wong
- Funding: National Institute of Environmental Health Sciences & Michael J. Fox Foundation for Parkinson's Research

**Presentation 9 – Nancy Klimas**

## **GWV – The Miami Experience**

- Our Center is comparing GWV, CFS and deployed healthy GW veterans in a longitudinal study.
- The initial assessment is an extensive evaluation and blood and saliva collections pre-post an exercise challenge; the subjects are then followed over time to assess the trajectory and variability of the illness as it relates to biomarkers.

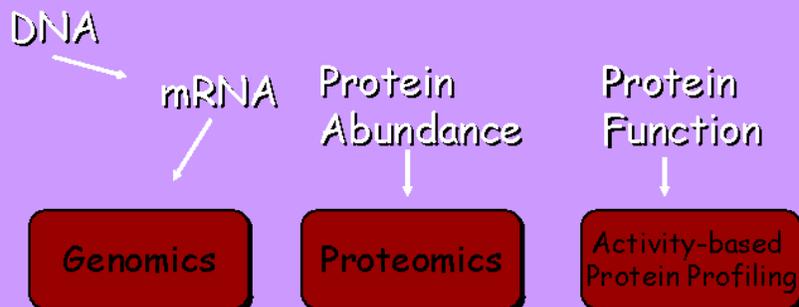
## **GWV and CFS comparative studies – The Miami experience**

Nancy Klimas MD  
Miami VA Medical Center  
Miller School of Medicine  
University of Miami

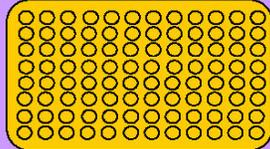
## GWV - The Miami Experience

- Samples are then processed for use in the following:
- Genomics – 20,000 gene array pre post exercise; (proteomics to follow)
- Immune regulation – functional studies, cytokine
- Neuroendocrine studies
- Neuropeptide Y

## Molecular Epidemiology Laboratory Strategy



## Microarray Technology

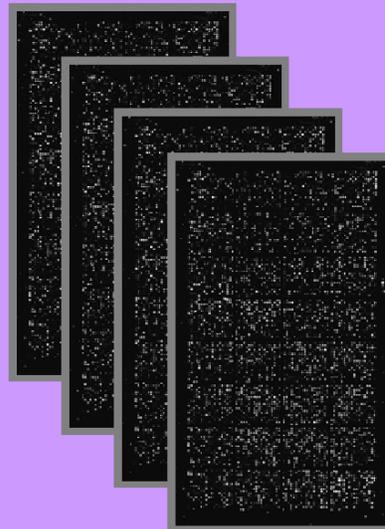
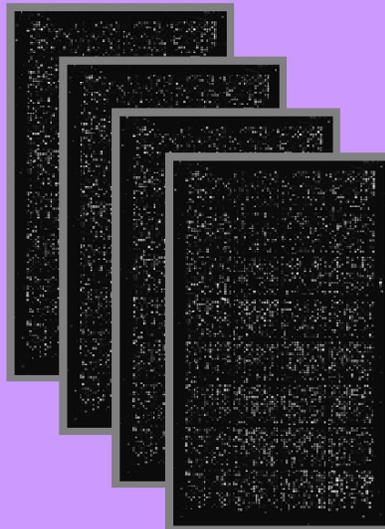


- A tool to measure the expression (mRNA) of genes
- An ordered array of spots (that represent genes) on a glass microscope slide

## Atlanta Case Control Study Gene Expression

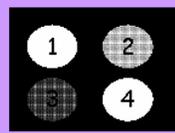
CFS Case Arrays

CFS Control Arrays

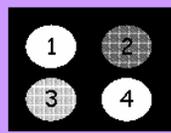


## How do we use microarray data?

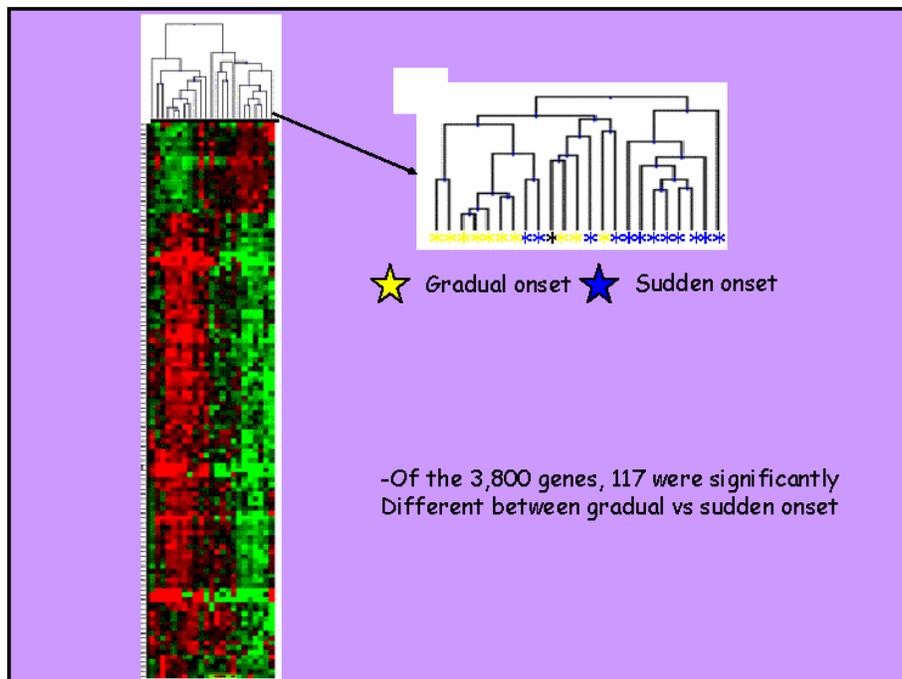
- Compare the intensity of one spot (CFS) to the intensity of the corresponding spot (control)



vs



	CFS	Cont	Ratio
1	100	100	1
2	60	30	2
3	20	80	4
4	100	100	1



## Patterns of gene activation and protein expression

- Gene expression patterns break population into two groups, one with increases in gene expression involved in immune activation, another with lower levels of gene expression in areas reflecting metabolism

Whistler et al BMC Physiology 2005 (5)5 e- journal

## CFS subtyping by gene expression

- “Molecular” evidence of a difference between suddenly & those with gradual illness onset
- Different profiles imply different pathophysiology
- Several immune, endocrine and metabolic genes and pathways involved
- Differentially expressed genes in RNA processing and metabolic pathways account for the differences between CFS and controls
- All gene ontology categories are required to distinguish someone with CFS from someone without (can’t just use one)
- There are hundreds of gene ontologies – CDC studies narrowed CFS classification to 26

## What Did the Genes Teach Us?

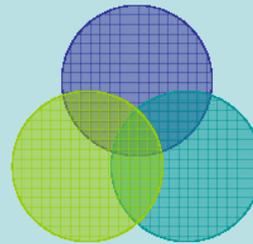
- CDC CFS study: Of 20,000 genes studied the activity of 26 genes did accurately predict which of six categories of chronic fatigue a patient had on the basis of symptoms and other clinical tests.
- Most of these genes are involved in immune system regulation, the HPA axis, and mitochondrial function.
- Studies of hormones and immune factors confirm these predictions
- Kerr's group: 35 genes in 25 pts/25 controls suggesting T cell activation, and perturbations of neuronal and mitochondrial function.

## What Did the Genes Teach Us in CFS?

- In other analyses, involving 50 genes that some people inherit with seemingly minor "misspellings," five of the 500 genetic glitches that were tracked repeatedly correlated with an apparent susceptibility to chronic fatigue.
- Those five include genes that affect levels of serotonin and glutamate
- Additional studies of cortisol regulation pathway genes reveal mutations in regulatory pathway

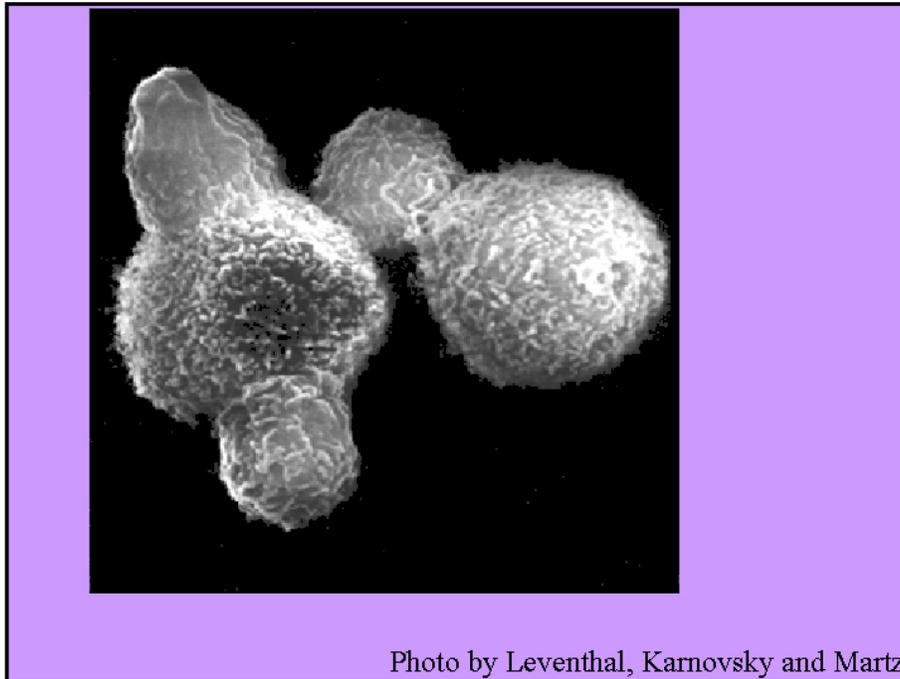
## CFS is a Complex Illness

- CFS Illness represents alterations in complex systems of homeostasis
- Not a result of a single mutation or single environmental factor
- Arise from a combined action of many genes, environmental factors and risk-conferring behavior



## GWVI - Miami

- Recruiting is underway
- Interim data available on immune studies pre post exercise challenge
- Gene array data is being performed at the CDC for comparability to their data set



## GWV

- Impact of acute exercise on circulating populations of cells
- Selective shift in compartment of cell subpopulations

## Conclusion

- 1. Gene array work and suggested proteomics offers a unique opportunity to better understand the complex underpinnings of multi symptom illness.
- 2. The peripheral blood compartment is uniquely suited to study neuro-immune regulation at the genomic level.
- 3. CFS and GWI share many commonalities, with what seems to be overlapping mechanistic studies of immune dysfunction.

**Presentation 10 – Mariana Morris**

## **Gulf War-related Research at Wright State University**

Mariana Morris, Ph.D.  
Professor and Chair  
Department of Pharmacology and Toxicology



### **The Mission:**

To understand the basis of toxicity from environmental, chemical and life-style interactions and to use this to study and develop countermeasures



## Research Advisory Committee on Gulf War Veterans' Illnesses

### August 15, 2006

**VA tests remedies for Gulf illness**

Dayton, Cincinnati centers each have trial programs under way

By KEVIN LAMB  
*Dayton Daily News*

CINCINNATI — Gulf War veterans experiencing the joint pain, muscle aches, fatigue, concentration difficulties and rashes that have been termed Gulf War syndrome are invited to join the Cincinnati VA Medical Center's demonstration treatment program for veterans with these symptoms. Cincinnati is one of five U.S. sites conducting the one-year treatment trial, which will become a model for further programs if it is effective.

The individualized treatment includes physical therapy, therapeutic exercise, access to a support group and treatment for physical symptoms or underlying mental disorders such as depression or post-traumatic stress syndrome. For information, call Carolyn Homan toll-free at (888) 865-3321 or directly at (513) 861-3189, extension 4265.

The Cincinnati trial is different from two other trials for Gulf War syndrome for which the Dayton VA Center is among 30 participating centers.

One trial is to determine whether exercise alone or in combination with psychological cognitive behavioral therapy can diminish the severity of symptoms, and the other will test whether an antibiotic can effectively treat the symptoms. Call 398-6511, Ext. 1212, for information on the Dayton trials.

**VA center joins study on Gulf War afflictions**

Volunteers will be followed through various therapies.

By James Homan

The study will test the use and cognitive-behavioral therapy, which involves teaching patients to control their own responses to the symptoms. The government is working to determine whether the study will test.

War veterans are suffering from a wide range of ailments, including chronic fatigue, headaches and joint pain. The environmental and health factors that caused these ailments are still being studied. The study will test whether the use of such therapies could alleviate these ailments.

DAYTON DAILY NEWS  
FRIDAY  
SEPTEMBER 21, 1998

**RELATED STORY**  
Cincinnati VA Center testing treatment program, BA

250,000 U.S. troops received the drug during the Gulf War

**NEWS**

VA's Gulf War illnesses program who took pills during the Gulf is James B. of Odessa, Texas. Tuesday he sometimes has headaches and problems in relation to service.

They haven't ruled that out, Silver said. "Of 10 have some sick veterans get the pill or another, conducted by the examined about 1,000 samples on PB, which led for decades to treat spinal disease inpatients of War, it was given to veterans against poison by the nerve agent. Though there still is evidence Iraq had weapons or had weaponized it.

Reverie Alexander, Colonel of Rand Corp., who headed the review, told a Pentagon news conference the suspected Gulf, PB cannot be ruled out as a cause of Gulf War symptoms.

"This does not imply that it is necessarily a causal factor, only that the possibility cannot be discounted," she says.

**NORTH CAROLINA VETERAN** Brian Martin suffers from ailments he attributes to toxins to which he was exposed during the Persian Gulf War.

## COUNTERTOX Research Partners

- ◆ Boonshoft School of Medicine
  - Pharmacology and Toxicology
  - Biochemistry and Molecular Biology
- ◆ Wright-Patterson AFB, Air Force Research Laboratory
- ◆ Dayton VA Medical Center
- ◆ Battelle Biomedical Research Center
- ◆ Rea Clinic
- ◆ Cenomed Inc

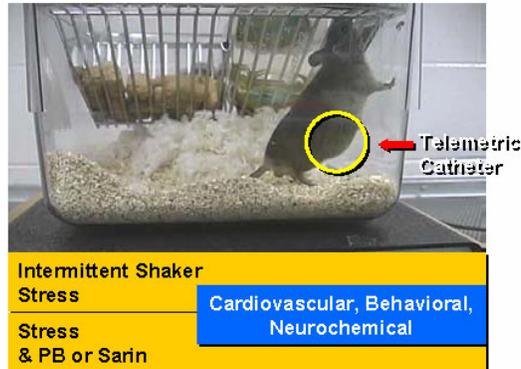
## **Core Research Facilities**

- ◆ Center for Genomics
- ◆ Proteomics Analysis Laboratory
- ◆ Integrative Cardiovascular Laboratory (ICL)
- ◆ Chemical Warfare Agent Research Facility (CWF)
- ◆ Biosafety Laboratories
- ◆ Animal Resources Laboratory
- ◆ Magnetic Resonance Laboratory

## **Major Research Topics: Sarin Exposure**

- ◆ Behavior
- ◆ Autonomic neural function
- ◆ Genomic biomarkers
- ◆ Chemical sensitivity in humans and sarin metabolism
- ◆ Proteomic biomarkers

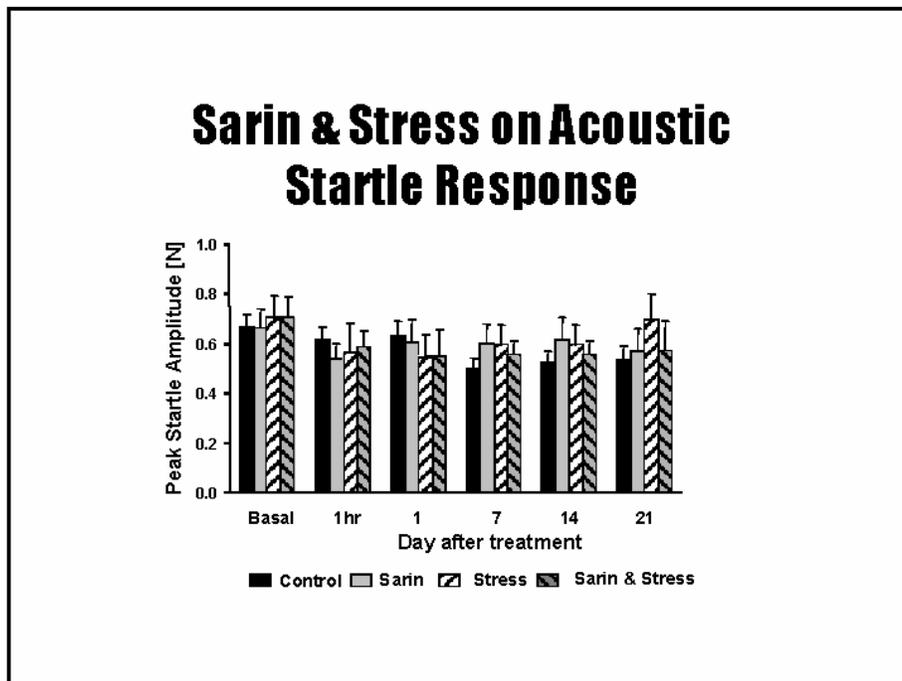
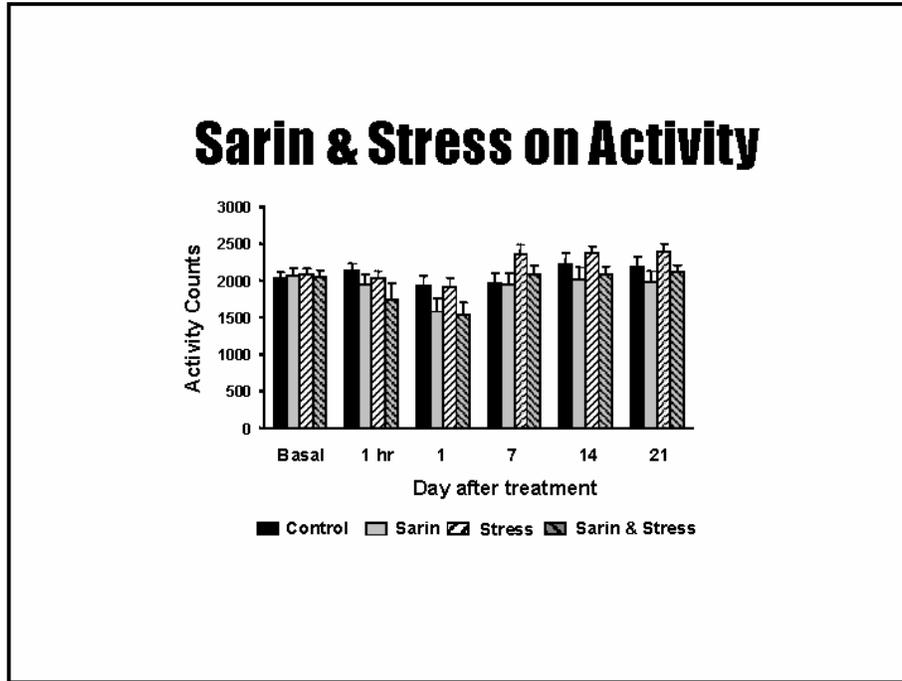
## Experimental Stress Model



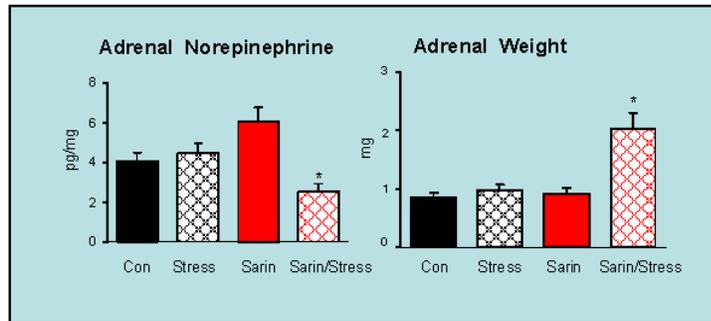
## METHODS

### Stress & Sarin Exposure

- ◆ Mice exposed to intermittent shaker stress for 7 days
- ◆ Sarin was injected sc for 3 days (64  $\mu\text{g}/\text{kg}$ , 0.4 $\times\text{LD}_{50}$ )
- ◆ Groups: Control, Sarin, Sarin & Stress, Stress
- ◆ Evidence for adrenal dysfunction, altered fear potentiation and self-mutilation

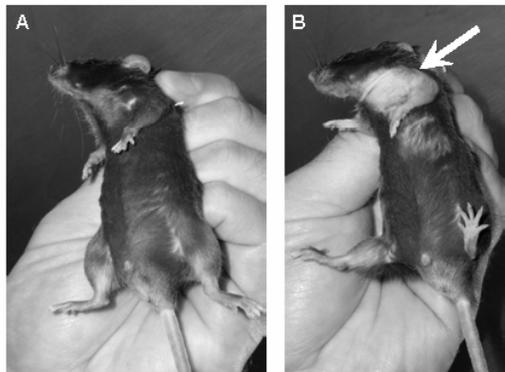


## Sarin and Stress Adrenal Function



Lucot et. al

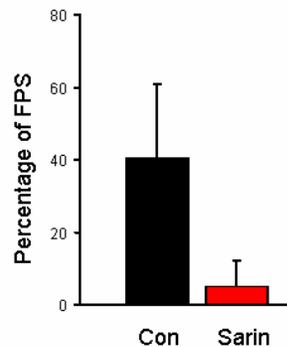
## Sarin & Stress Evidence for Self-Mutilation



Sarin & stress produced self mutilation in a subset of mice (~ 30%). This was not observed in the other groups.

Lucot et. al

## Sarin and Fear Potentiation of Startle Response



Behavioral response prevented in sarin treated mice

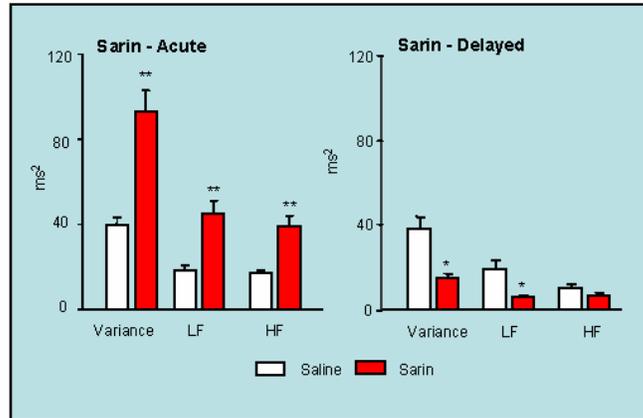
Lucot et. al

## METHODS

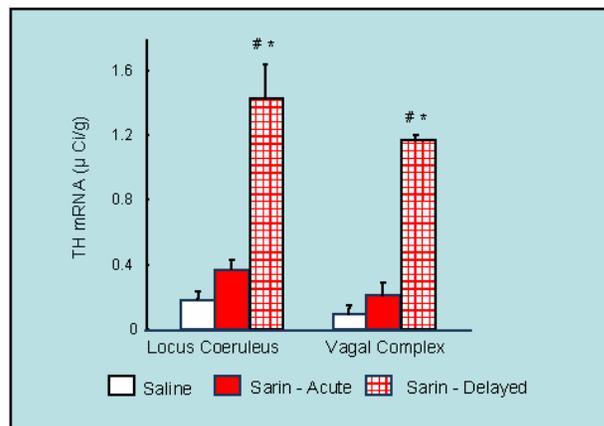
### Sarin & Autonomic Function

- ◆ Mice with telemetric carotid catheters
- ◆ Sarin was administered sc for 2 days (8  $\mu\text{g}/\text{kg}$ , 0.05LD<sub>50</sub>)
- ◆ Cardiovascular parameters measured from 1 to 10 weeks after sarin
- ◆ Evidence for delayed autonomic dysfunction, similar to that seen with heart failure

## Delayed Effect of Sarin on Heart Rate Variance and Frequency Domains



## Low Dose Sarin Produces Delayed Changes in Brainstem Amine Function



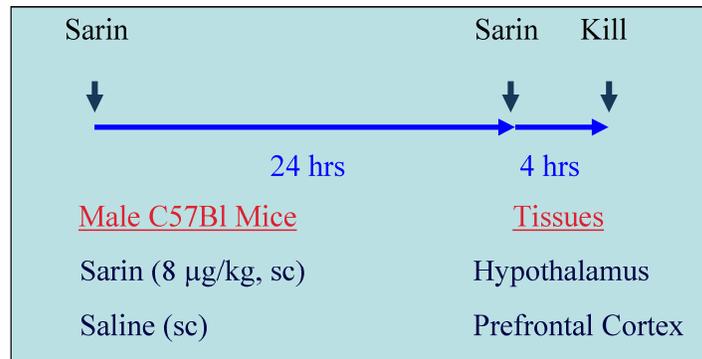
## Gene Array Analysis



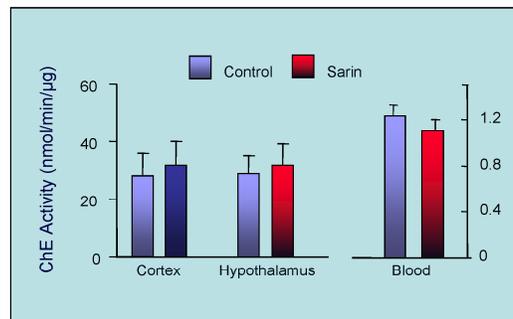
### **Gene Array Method**

- ◆ Affymetrix GeneChip® (oligonucleotide based array)
- ◆ Mouse genome U74A (v2) chip
- ◆ Microarray Suite (v 5.0)
- ◆ Affymetrix Data Mining Tool (v 2.0)
- ◆ GeneSpring (v 6.1)
- ◆ PathwayAssist (v 2.5)

## Experimental Protocol Low Dose Sarin on Genomic Expression



## Low Dose Sarin Brain and Blood Cholinesterase



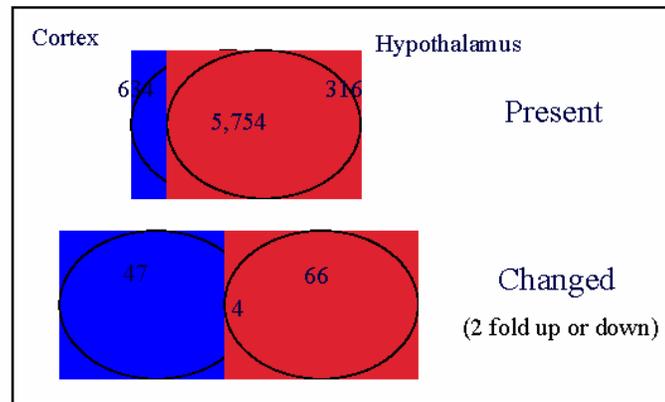
## Data Mining (GeneSpring)

- ◆ Raw intensity and flags (P, M & A) from DMT
- ◆ Normalize all 14 chips to the median of controls
- ◆ Eliminate genes that were not present in at least 2 samples (6,388 in cortex & 6,070 in hypothalamus)
- ◆ Select genes increased or decreased (two-fold) Hcompared to control (70 in cortex & 51 in the hypothalamus)

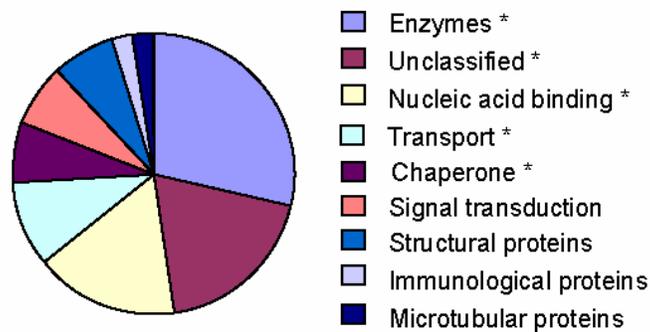
## Low Dose Sarin Effect on Cortex and Hypothalamus

Tissue	Increase	Decrease
Cortex	51	19
Hypothalamus	31	20

## Genomics: Comparison of Hypothalamus to Cortex



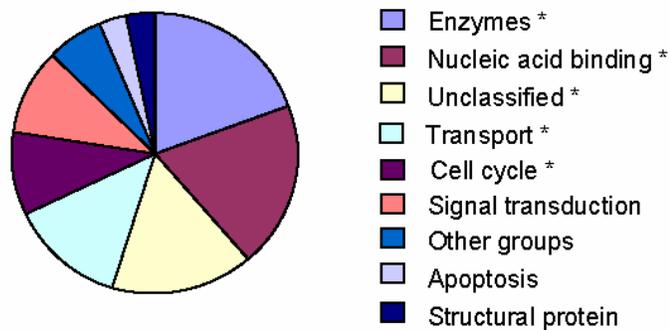
## Classes of Genomic Changes Hypothalamic Increases



## Sarin Increases Hypothalamic:

- ◆ Glial cell derived neurotrophic factor (GDNF)
- ◆ Serotonin transporter (SLC6A4)
- ◆ Tyrosine/Tryptophan activation protein (YWHAQ)
- ◆ PG F2 receptor negative regulator (PTGFRN)
- ◆ Doublecortin (DCX)

## Classes of Genomic Changes Hypothalamic Decreases



## **Sarin Decreases Hypothalamic:**

- ◆ PG D2 synthase (PTGDS)
- ◆ Voltage gated K<sup>+</sup> channel (KCND2)
- ◆ Artemin of Glial cell line-Derived Neurotrophic Factor (GDNF) family (ARTN)
- ◆ Ankyrin repeat & SOCS box containing (ASB3)

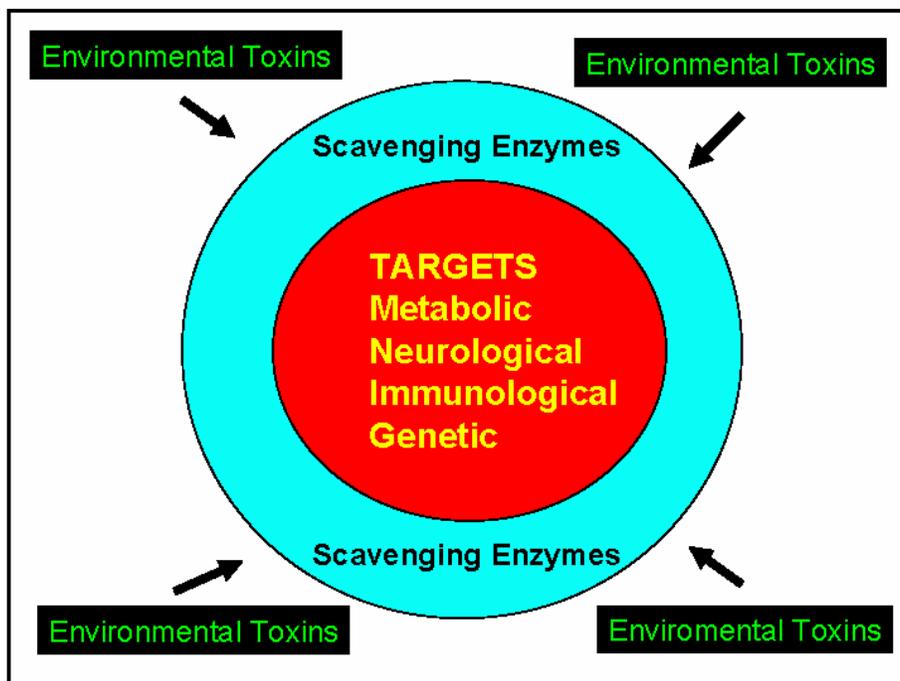
## **Low Dose Sarin & Genomics CONCLUSIONS**

- ◆ There are brain changes in genetic expression at a sarin dose that does not change AChE activity
- ◆ Cortex and hypothalamus respond differently to acute sarin exposure.
- ◆ Detailed analysis (genomic and proteomic) will provide mechanistic insights into low dose sarin toxicity

## Chemical Metabolism in Humans with Chemical Sensitivity

- ◆ Hypothesis: Chemical sensitivity is linked to deficiencies in scavenging enzymes.
- ◆ Corollary: Chemically sensitive individuals should have altered levels of scavenging enzymes

Alter et. al



## Activities Measured

Formaldehyde  $\longrightarrow$  Formate

- 1) Aldehyde Dehydrogenase (ALD): NAD
- 2)  $\alpha$ -Alcohol Dehydrogenase ( $\alpha$ ADH): NAD, GSH

Paraoxon  $\longrightarrow$  P - Nitrophenol + Diethyl Phosphate

Paraoxonase:  $\text{Ca}^{2+}$

Phenyl Acetate  $\longrightarrow$  Phenol + Acetate

Arylesterase:  $\text{Ca}^{2+}$

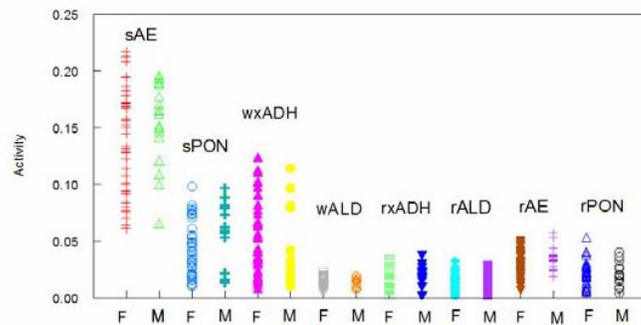
## Sample Population

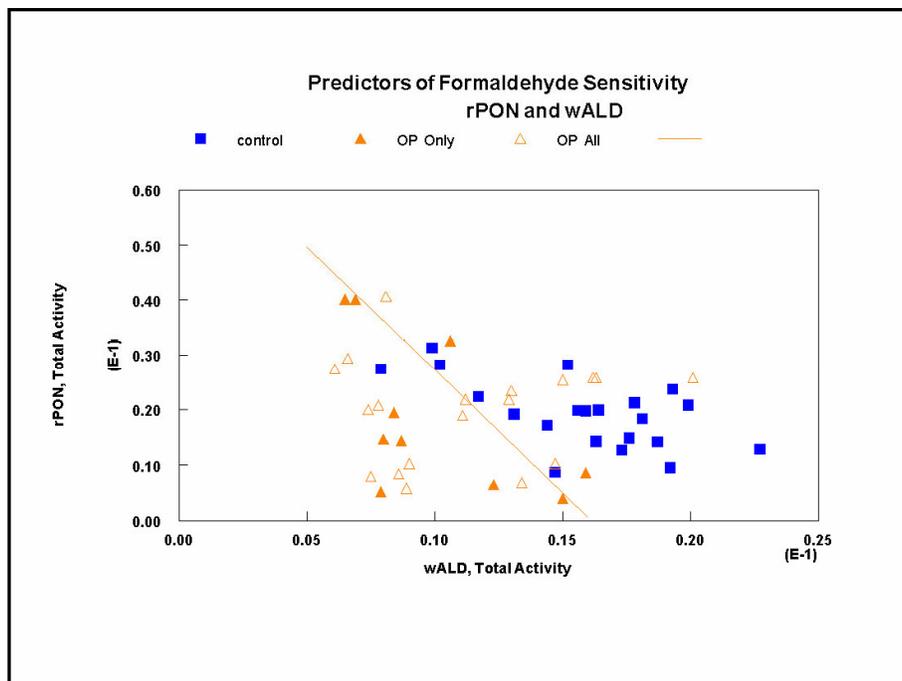
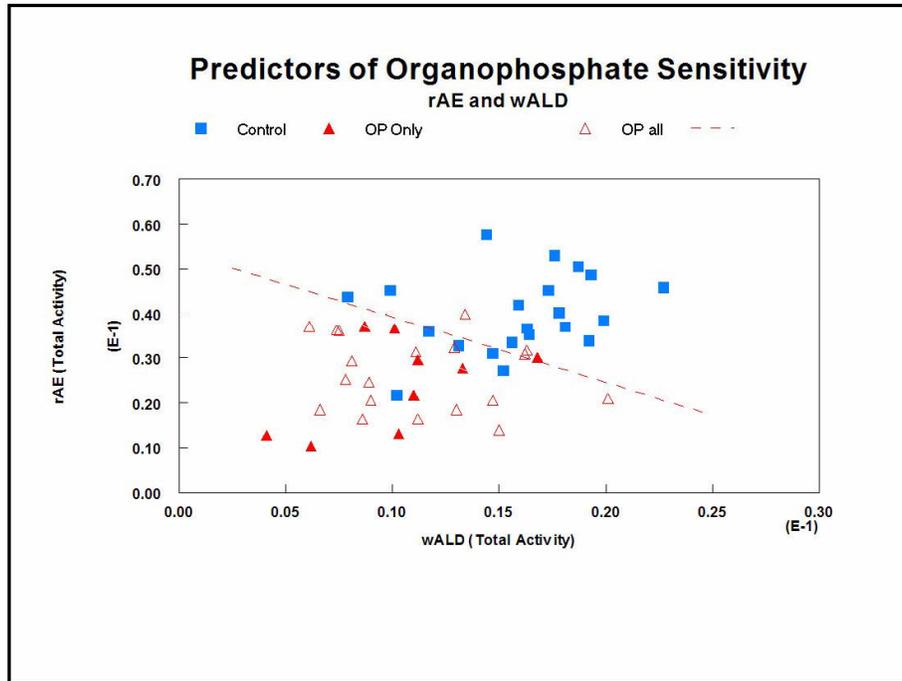
- ◆ Blood samples from chemically sensitive (formaldehyde or organophosphate) persons
- ◆ Rea Clinic, Dallas, TX
- ◆ Experiments were not performed directly with humans

## Abbreviations

- ◆ Chi Alcohol Dehydrogenase: xADH
- ◆ Aldehyde Dehydrogenase: ALD
- ◆ Paraoxonase: PON
- ◆ Arylesterase: AE
- ◆ Red blood cell: r
- ◆ White blood cell: w
- ◆ Serum: s
- ◆ Organophosphate: OP

Gender Dependence of Activity





## **Biomarkers for Chemical Sensitivity**

- ◆ NO age dependence
- ◆ NO gender dependence
- ◆ Ethnic dependence – little data
- ◆ BIOCHEMICAL MARKERS FOR ORGANOPHOSPHATE AND FORMALDEHYDE SENSITIVITY



**Presentation 11 – Janet Harris**

Department of Defense Congressionally Directed Medical Research Programs



**US Army Medical Research and Materiel Command (USAMRMC)**

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**Overview of the Congressionally Directed Medical Research Programs (CDMRP)**

**Presented to**  
**Research Advisory Committee on Gulf War Veterans' Illnesses**

**Presented by**  
**Janet Harris, Ph.D., R.N.**  
**Colonel, US Army Nurse Corps**  
**Director**

**15 August 2006**

*US Army Medical Research and Materiel Command*



1

Department of Defense Congressionally Directed Medical Research Programs



**Introduction**

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- ◆ **Overview of the CDMRP**
  - ❖ **Origins and Funding History**
  - ❖ **Structural Features**
- ◆ **Peer Reviewed Medical Research Program (PRMRP) Funded Gulf War Focused Research**
- ◆ **Gulf War Veterans' Illnesses Research Program (GWVIRP)**
  - ❖ **Overview and Funding Priorities**

*US Army Medical Research and Materiel Command*



2

Department of Defense Congressionally Directed Medical Research Programs



## CDMRP Philosophy

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### THE CENTRAL THEME IS INNOVATION

- ◆ **Vision:** Find and fund the best research to eradicate diseases and support the warfighter for the benefit of the American public
- ◆ **Mission:** We provide hope by promoting innovative research, recognizing untapped opportunities, creating partnerships, and guarding the public trust

3

US Army Medical Research and Materiel Command



Department of Defense Congressionally Directed Medical Research Programs



## CDMRP History

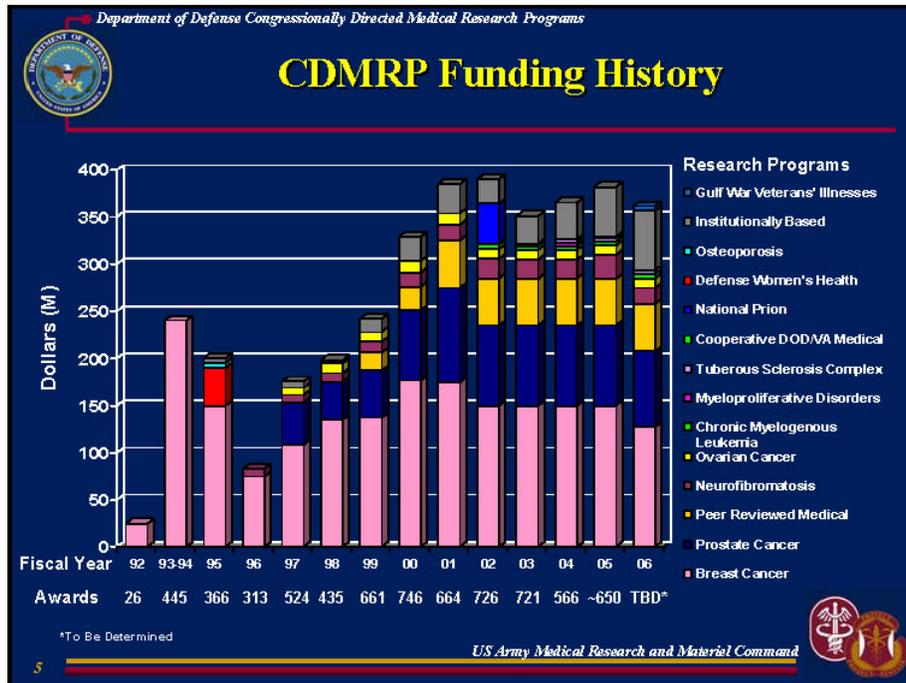
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- ◆ In 1993, grassroots advocacy heightened political awareness of breast cancer.
- ◆ Federal budgetary opportunities spurred Congress to appropriate \$210 million (M) to the Department of Defense (DOD) budget for breast cancer research.
- ◆ Program assigned to the USAMRMC; subsequent consultation with the Institute of Medicine for programmatic advice.
- ◆ Additional research programs added, including neurofibromatosis in FY96, ovarian cancer and prostate cancer in FY97, defense health (now called PRMRP) in FY99, prion, tuberous sclerosis complex, and chronic myelogenous leukemia in FY02, and myeloproliferative disorders in FY04.
- ◆ These and other programs are managed by the CDMRP.

4

US Army Medical Research and Materiel Command





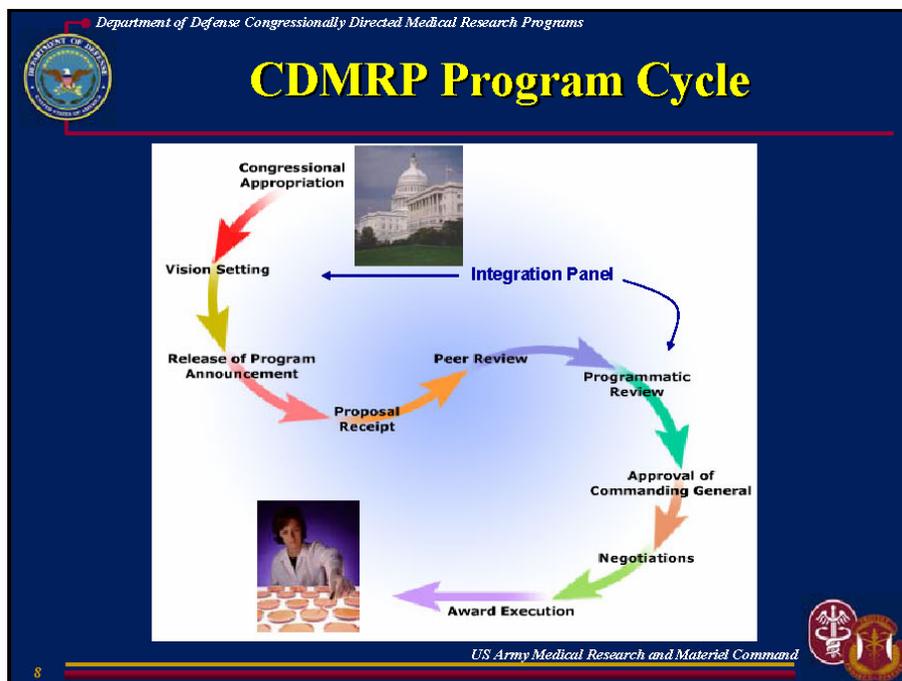
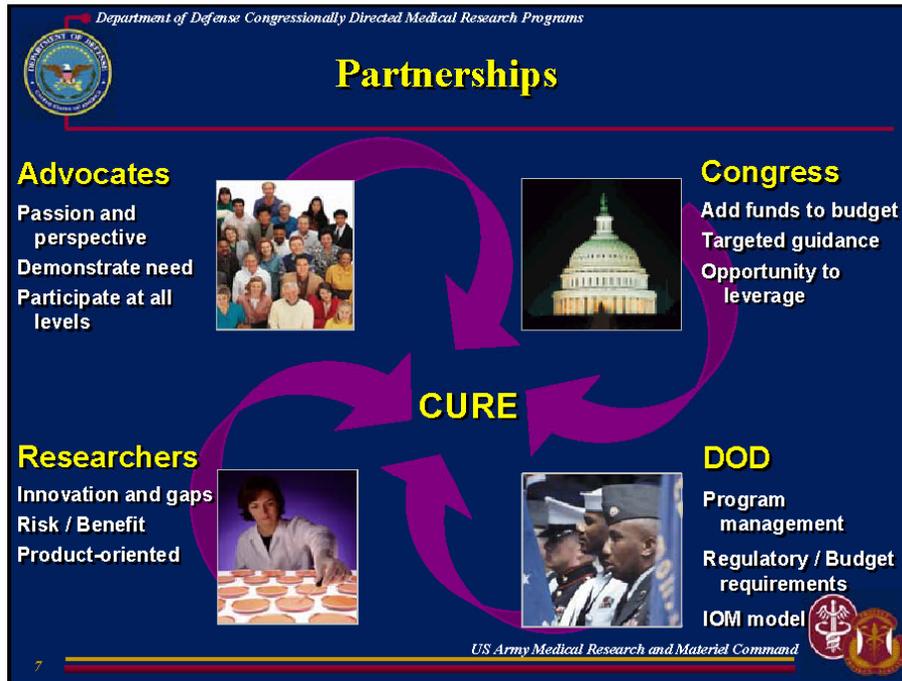
Department of Defense Congressionally Directed Medical Research Programs

## CDMRP Structural Features

- ◆ Funds added to the DOD budget by Congress
- ◆ Respond to targeted guidance from Congress
- ◆ Two-tier formal review of proposals – Institute of Medicine Model
- ◆ Vision is adapted yearly to accommodate rapid change
- ◆ Highly flexible model to address research gaps
- ◆ Consumer advocate representation



US Army Medical Research and Materiel Command



Department of Defense Congressionally Directed Medical Research Programs



## Two-Tier Review Process

<h3><u>Peer Review</u></h3> <ul style="list-style-type: none"><li>◆ Evaluation of scientific merit</li><li>◆ Criteria-based evaluation</li><li>◆ Proposals evaluated within related disciplines</li></ul> <p><b>Products:</b></p> <ul style="list-style-type: none"><li>◆ Proposal scoring</li><li>◆ Summary statements</li></ul>	<h3><u>Programmatic Review</u></h3> <ul style="list-style-type: none"><li>◆ Evaluation of programmatic relevance</li><li>◆ Comparison-based evaluation</li><li>◆ Proposals evaluated across multiple disciplines</li></ul> <p><b>Product:</b></p> <ul style="list-style-type: none"><li>◆ List of funding recommendations for the Commanding General</li></ul>
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9

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## Consumers and the CDMRP

Consumers have been active participants in our program since its inception in the early 1990s

- ◆ Human Use Review Boards
- ◆ Stakeholders Meetings
- ◆ Integration Panels
- ◆ Peer Review Panels



10

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## Value of Consumer Advocacy Involvement

- ◆ **Contributes unique professional and personal experiences**
- ◆ **Adds perspective, passion, and a sense of urgency**
- ◆ **Ensures that human dimensions of disease are incorporated into scientific considerations, program policy, investment strategy, and research focus**



11

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## Filling Research Gaps and Taking Risks

- ◆ **Fund meritorious research that other agencies will not risk funding, supporting more creativity and innovation**
- ◆ **Look for innovative research that can leapfrog advances rather than fund next incremental step**
- ◆ **Fund early concepts, pursue new research (to produce preliminary data needed for traditional research funding)**

12

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## Relevance to DOD Mission

- ◆ Multidisciplinary research that can impact the military mission
  - ❖ Cell death and battlefield deaths
  - ❖ Angiogenesis
  - ❖ Biosensors
  - ❖ Vaccines
  - ❖ Telemedicine
- ◆ Impact of research on DOD beneficiaries
- ◆ PRMRP – “. . . direct relevance to military health”
  - ❖ Sleep Management
  - ❖ Laser Eye Injury
  - ❖ Alcohol Abuse Prevention Research
  - ❖ Smoking Cessation
  - ❖ **Gulf War Illness**

13  US Army Medical Research and Materiel Command

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## PRMRP Studies on Gulf War Veterans' Illnesses

- ◆ “Longitudinal Health Study of Gulf War Veterans”
  - ❖ Han Kang, 2001, VA Medical Center, Washington, DC
  - ❖ Assess the health status of Gulf War veterans and non-Gulf War veterans by comparing chronic medical conditions, post-traumatic stress disorder and other psychological conditions
- ◆ “Neuropsychological Functioning in Gulf War Veterans Exposed to Pesticides and Pyridostigmine Bromide”
  - ❖ Maxine Krengel, 2003, Boston University
  - ❖ Evaluate the role of pesticides in the development of central nervous system symptoms reported by Gulf War veterans and assess the additive and/or synergistic effects of combinations of chemical exposures and stress

14  US Army Medical Research and Materiel Command

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## PRMRP-Funded Awards Studying Gulf War-Related Issues

- ◆ **“Prospective Study of ALS Mortality among World War II, Korea, and Vietnam Veterans”**
  - ◆ Alberto Ascherio, 2004, Harvard University School of Public Health
  - ◆ Examine whether men who served in the military during World War II, Korea, Vietnam, or the Gulf War have a higher risk of ALS (amyotrophic lateral sclerosis) mortality as compared with men who did not serve
- ◆ **“Neurotoxicity from Chronic Exposure to Depleted Uranium”**
  - ◆ Stephen Lasley, 2001, University of Illinois at Chicago
  - ◆ Gulf War veterans who retained fragments of depleted uranium (DU) have exhibited lowered performance on neurocognitive tests
  - ◆ Determine whether chronic exposure to DU impairs neuronal processes underlying cognitive function via alterations induced at hippocampal glutamatergic synapses that directly modulate Ca<sup>2+</sup>-mediated cellular processes
- ◆ **“Development of Strategies to Treat and Prevent Norovirus Infections”**
  - ◆ Xi Jiang, 2003, Cincinnati Children's Hospital Medical Center
  - ◆ Outbreak of Norovirus during the first Persian Gulf War
  - ◆ Develop new strategies for prevention and treatment of Norovirus

15

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## Overview of the FY06 Gulf War Veterans' Illnesses Research Program (GWVIRP)

- ◆ The Defense Appropriations Act of 2006 (Public Law 109-359) provides \$5M to fund peer-reviewed medical research
- ◆ The intent of this appropriation is to support research on the chronic illnesses affecting veterans of the 1991 Gulf War
- ◆ **Priority Topic Areas**
  - ◆ Identification of mechanisms underlying Gulf War illnesses
  - ◆ Chronic effects of neurotoxic substances to which veterans were exposed during deployment
  - ◆ Studies that expand on earlier research identifying neurological and immunological abnormalities in ill Gulf War veterans
  - ◆ Identification of promising treatments

16

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## Overview of the FY06 GWVIRP Continued

- ◆ **Primary Objectives**
  - ❖ Elucidation of pathophysiological mechanisms underlying Gulf War Veterans' Illnesses which may subsequently be targeted to develop treatments for these conditions
  - ❖ Identification and evaluation of treatments which currently exist and which hold promise for treating these illnesses

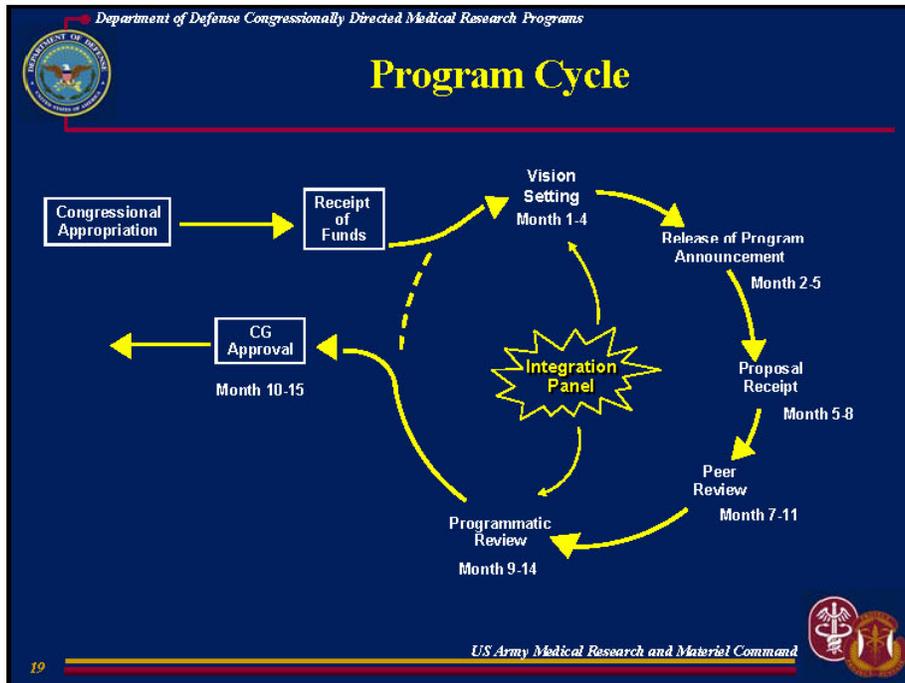
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## GWVIRP Anticipated Award Mechanisms

- ◆ **Exploration - Hypothesis Development Award**
  - ❖ Expected to lead to high-risk, potentially high-gain research endeavors that will garner future funding through CDMRP or other funding agencies
- ◆ **Investigator-Initiated Research Award**
  - ❖ Mechanism encourages basic or clinical research dealing with Gulf War Veterans' Illnesses

18  US Army Medical Research and Materiel Command



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## GWVIRP Joint Programmatic Review Panel

<p><b>Research Advisory          Committee on Gulf War          Veterans' Illnesses</b></p> <ul style="list-style-type: none"> <li>◆ Dr. Carolee Barlow</li> <li>◆ Dr. James P. O'Callaghan</li> <li>◆ Dr. Lea Steele</li> <li>◆ Mr. Anthony Hardie</li> </ul>	<p><b>PRMRP          Joint Programmatic          Review Panel</b></p> <ul style="list-style-type: none"> <li>◆ Maj David Watson, <i>US Air Force</i></li> <li>◆ CAPT David Neri, <i>US Navy</i></li> <li>◆ COL Bruno Petruccelli, <i>US Army</i></li> </ul>
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20

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## CDMRP Enabling the Nation to Cure

- ◆ Mission-oriented and solution-driven
- ◆ Research programs managed to encourage innovation
- ◆ Responsive to changing needs and opportunities
- ◆ Proven stewardship

21

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## Web Site – <http://cdmrp.army.mil>

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- Neurofibromatosis
- Tubercous Sclerosis Complex
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- Dism Diseases
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- Synopsis of Current Program Announcements
- Forms Library
- Program Announcement Archives
- Frequently Asked Questions

**Consumer Involvement**

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- Why Should I Get Involved in Peer Review?
- How Do Consumers Participate in Peer Review?
- How Do I Apply?
- Consumer Milestones
- Consumer Reviewer Inclusion Information for Funding Opportunities
- Frequently Asked Questions

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Identifying the Risk of Breast Cancer Recurrence in Patients Treated with Tamoxifen

Getting to the Bones of Prostate Cancer

Squalamine and Cisplatin: Potential Ovarian Cancer Therapeutic Agents

A New Model for Merlin Localization and Function

22

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**Presentation 12 – William Goldberg**

# Gulf War Update



William J. Goldberg PhD  
Portfolio Manager  
Gulf War Research  
Veterans Health Administration  
Department of Veterans Affairs



# GULF WAR BIOREPOSITORY TRUST

## GW Biorepository Trust

### ■ Scope

- Brain
- Spinal Cord
- Blood/Serum
- Other organs if deemed appropriate

### ■ Purpose

- Tissue bank for the ALS Registry
- Tissue collection from Gulf War Veterans at VAMC's
- Collect high quality biological specimens linked to clinical information from consenting Gulf War era veterans and controls

## GW Biorespository Trust

### ■ Progress to date

- IRB approval obtained for the Biorespository Trust
- Project Coordinator hired
- IRB amendments submitted at Boston and Durham for collection of postmortem tissue and linkage to the ALS Registry
- Tissue collection procedures have been submitted to IRB for approval
- Specialized shipping services (24/7) established
- Specialized equipment for dissection of CNS tissue (brain & spinal cord) has been purchased
- In process of contracting with New York Brain bank to process ALS brains and Spinal Cord until site at Tucson is ready to take over
- In process of establishing a national network of pathologists and assistants



# PORTFOLIO UPDATE

## FY 06 Gulf War Portfolio

- ~ \$13.7 million for new and ongoing projects.
- 64 total projects supported in FY06
- 19 new projects initiated in FY06

