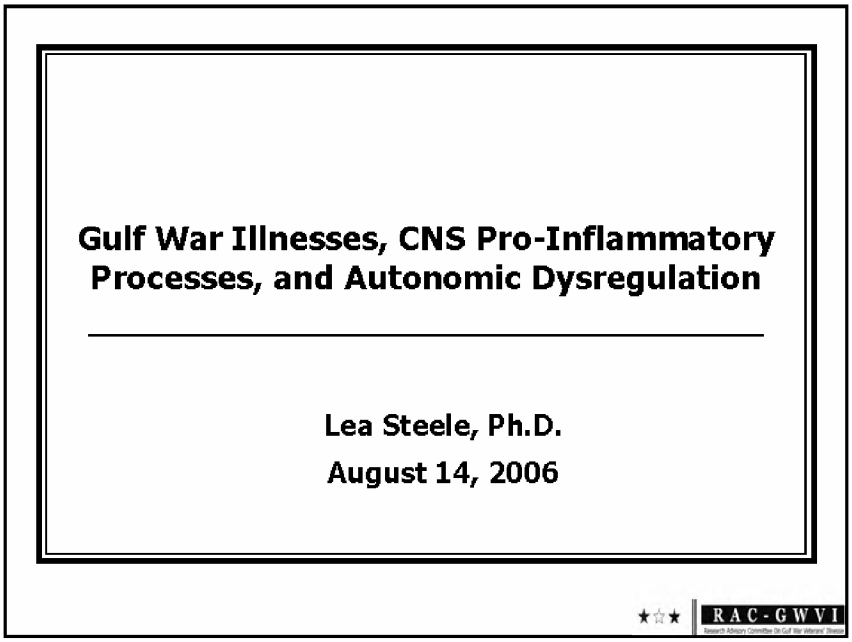
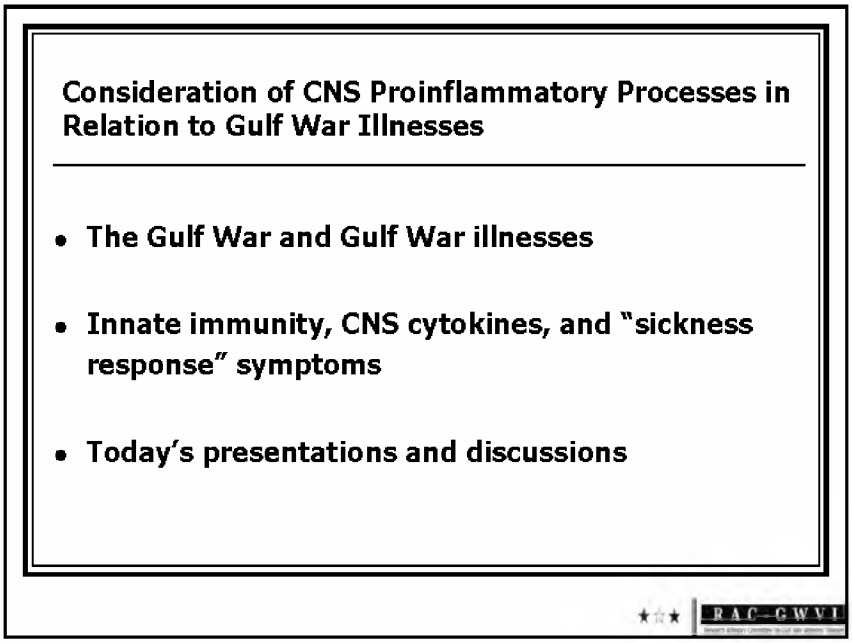
Appendix

Presentation 1 – Lea Steele





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

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

Aug 2, 1990 - Iraq invaded Kuwait

Jan 16, 1991 - Air strikes began

Feb 24, 1991 - Ground combat began

Feb 28, 1991 - Cease fire declared

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

~ 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

- 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

- **"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

- 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

- *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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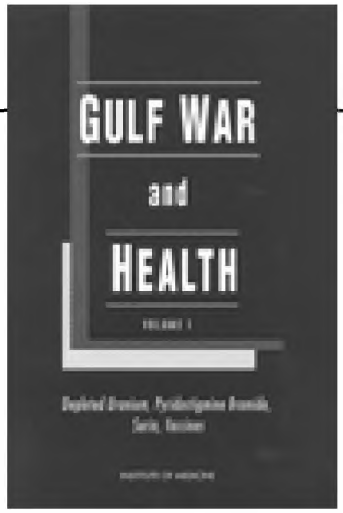
Summary/Panel Reports

- 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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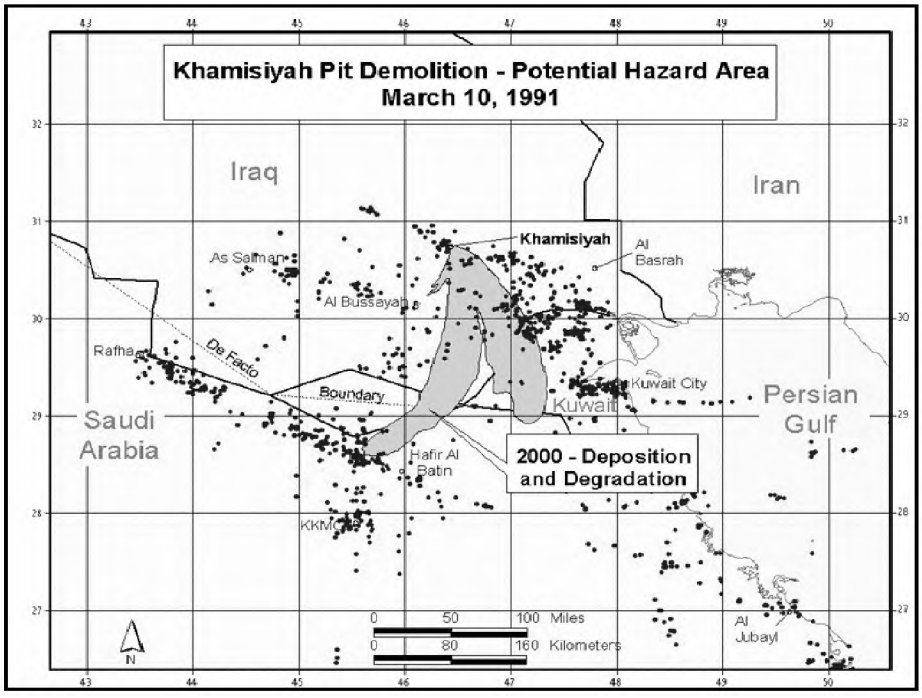

Table 7. Studies of Chronic Effects of Low-Dose Sarin Exposure in Animals

Study	Year	Animal Model	Major Finding
Burchfiel[64]	1976	monkey	Persistent effects on electroencephalograph readings
Husain[128]	1993	mouse	Delayed development of spinal cord lesions
Jones[149]	2000	rat	Chronic reduction in nicotinic ACh receptor binding in cerebral cortex
Kassa[155]	2000	rat	Chronic alteration in immune function (lymphocyte proliferation, bactericidal activity of macrophages)
Kassa[157]	2000	rat	Persistent changes in DNA and protein metabolism in liver tissues
Kassa[156]	2001	rat	Subtle chronic signs of neurotoxicity and immunotoxicity with repeated exposures
Kassa[161]	2001	rat	Impaired spatial memory
Conn[57]	2002	rat	No persistent effects on reported indices of temperature regulation and motor activity
Henderson[111]	2002	rat	Delayed, persistent changes in cholinergic receptors in brain areas associated with memory loss and cognitive changes
Hulet[170]	2002	guinea pig	Persistent failure to habituate on functional test battery
Screnin[163]	2002	rat	Persistent increase in cerebral blood flow in specific areas
Kaira[151]	2002	rat	Suppression of immune response (antibody-forming cells and T cell responses) mediated by the autonomic nervous system
Roberson[168]	2002	guinea pig	Chronic depression of AChE activity, persistent behavioral changes (disordered activity, increased rearing behavior)
Husain[127]	2003	mouse	Persistent reductions in respiratory exchange, blood AChE activity and BChE activity, NTE activity in various tissues
Screnin[162]	2003	rat	Down-regulation of muscarinic receptors in hippocampus, decreased habituation
Kassa[152-154]	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

What does all this tell us about Gulf War illnesses?

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Epidemiologic Studies: General Findings

- 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"

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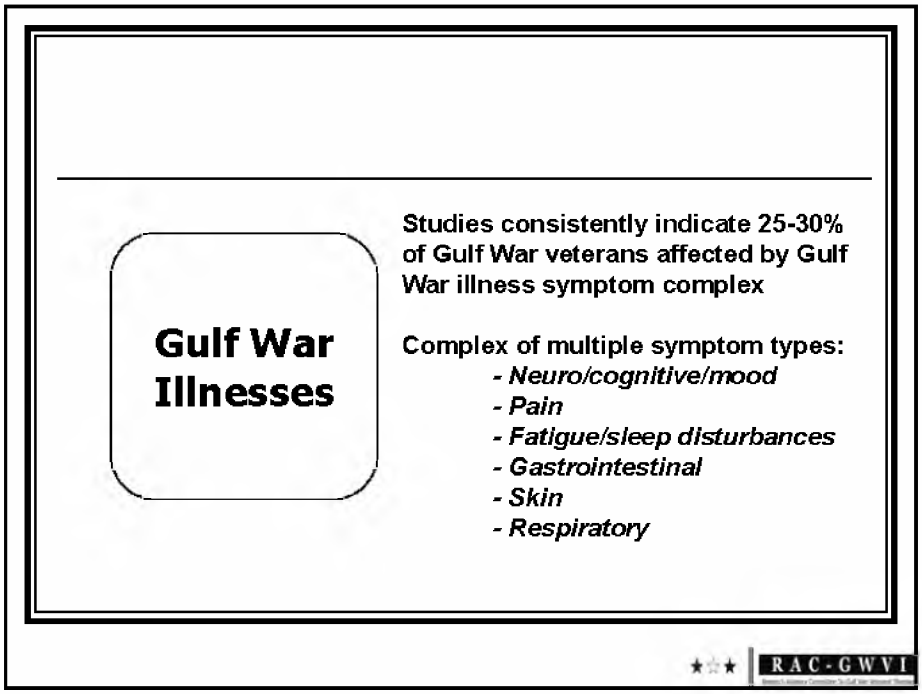
Table 3. Prevalence Estimates of Multisymptom Illness in Gulf and Non-Gulf Veterans

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

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


Gulf War Illnesses
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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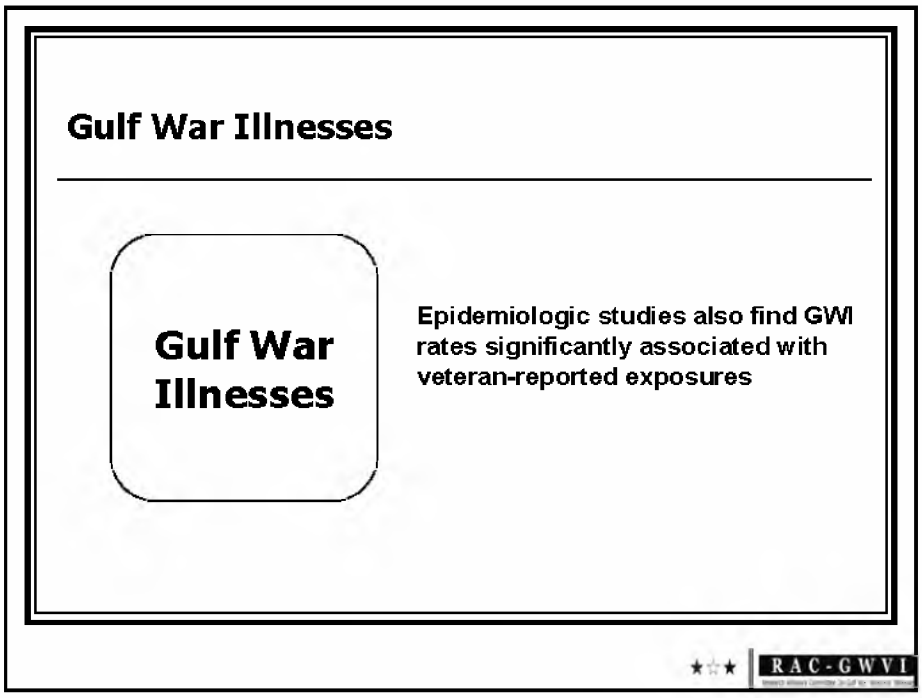
Gulf War Illnesses
Gulf War Illnesses
Epidemiologic studies also find GWI rates significantly associated with veteran-reported exposures
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Gulf War Exposures in relation to GWI: Summary of Epidemiologic Evidence

	Unadi OR	Adi OR	Adi ResultsConsist	Dose/resp
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

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

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

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

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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*

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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-γ, IL-2 (unstimulated CD4);
  elevated IL-10 (stimulated CD4)
- Zhang/Natelson:
  Elevated IL-2, IL-10, IFN-γ in PBLs

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Extensive Amount of Information Available

Gulf War experiences and exposures

Gulf War Illnesses

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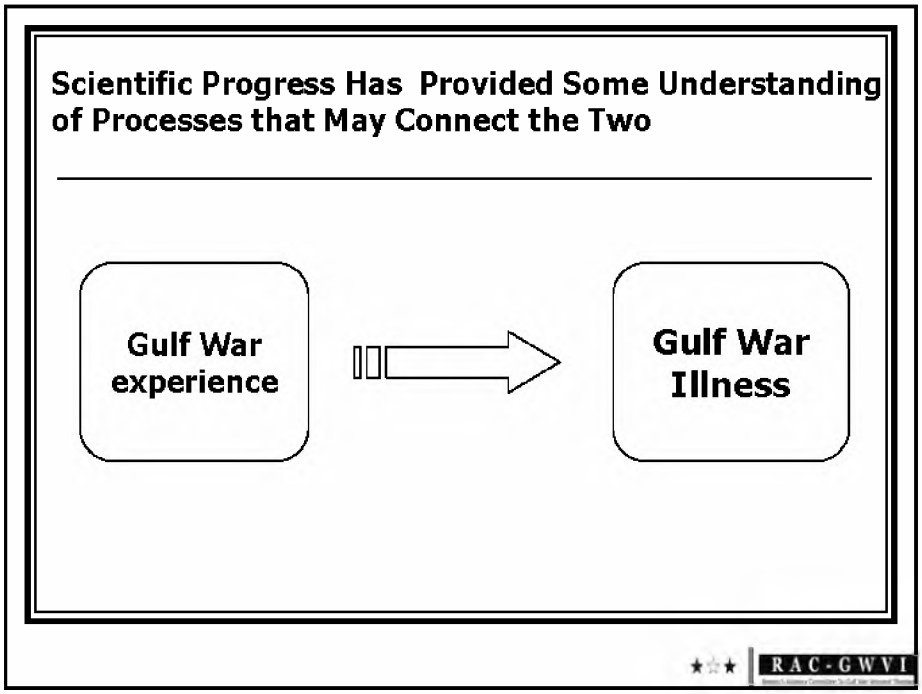
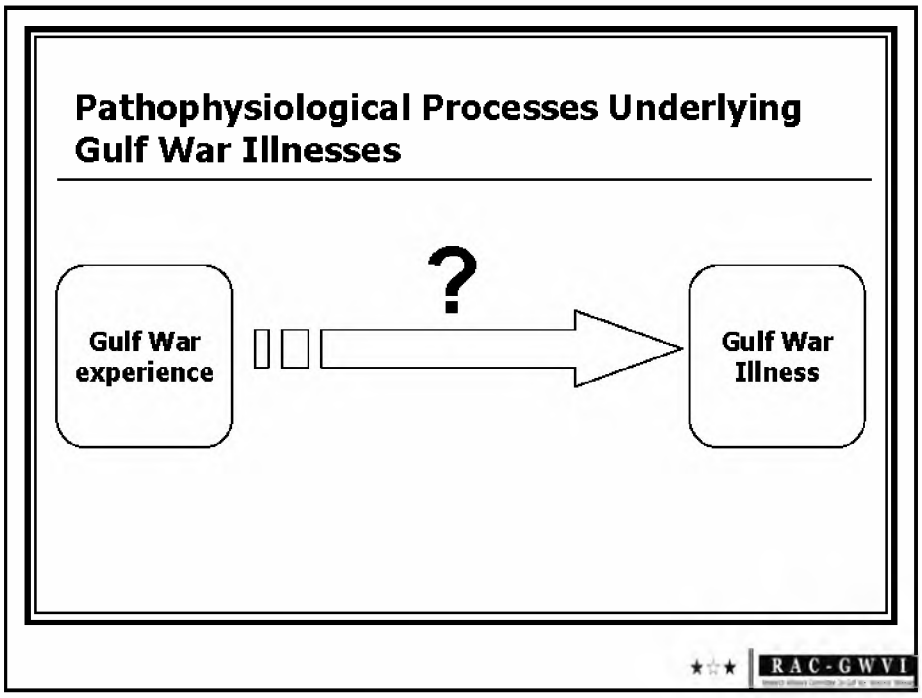

Scientific Progress Has Provided Some Understanding of Processes that May Connect the Two
Gulf War experience
Gulf War Illness
RAC-GWVI


Pathophysiological Processes Underlying Gulf War Illnesses
?
Gulf War experience
Gulf War Illness
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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?*
- *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

- *What pathophysiological process(es) underlie this complex of multiple types of symptoms in multiple systems?*
- ***How linked to 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

- *What pathophysiological process(es) underlie this complex of multiple types of symptoms in multiple systems?*
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- ***Why have symptoms persisted 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

- *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 so few objective indicators of disease?***

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

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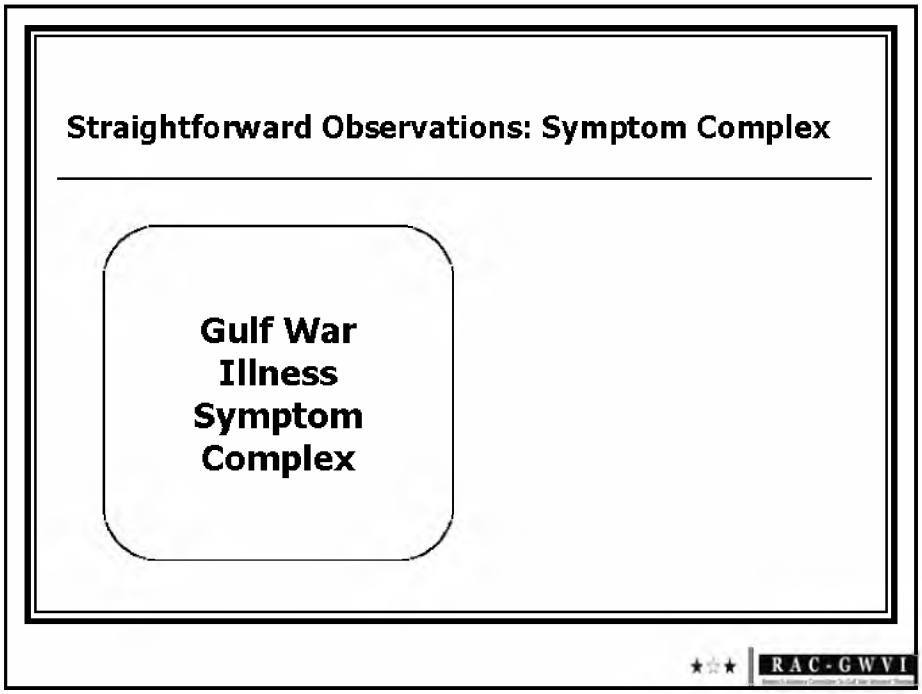
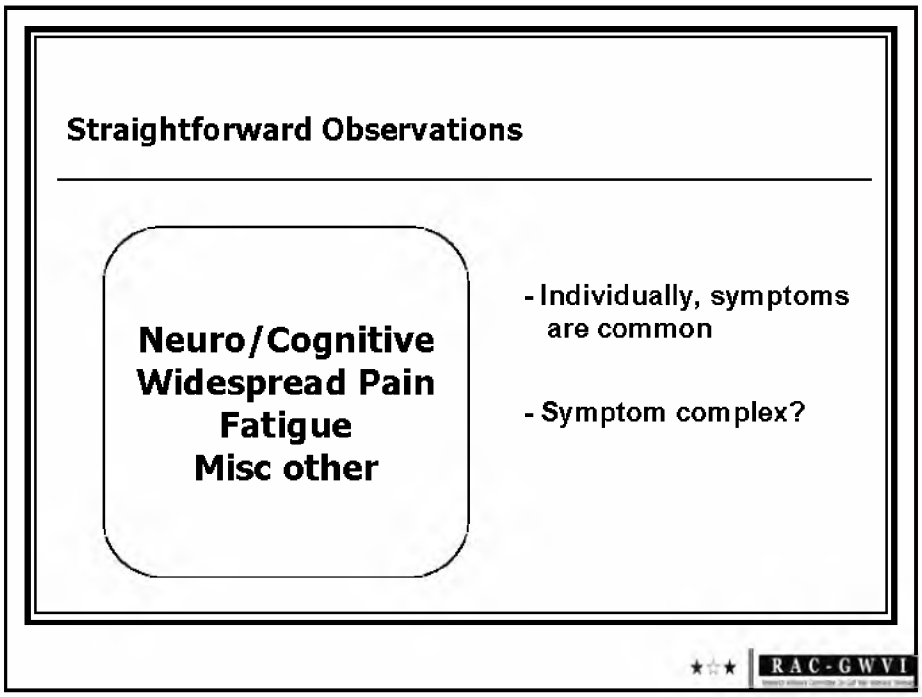

Straightforward Observations: Symptom Complex
Gulf War
Illness
Symptom
Complex
RAC-GWVI


Straightforward Observations
Neuro/Cognitive
Widespread Pain
Fatigue
Misc other
- Individually, symptoms are common
- Symptom complex?
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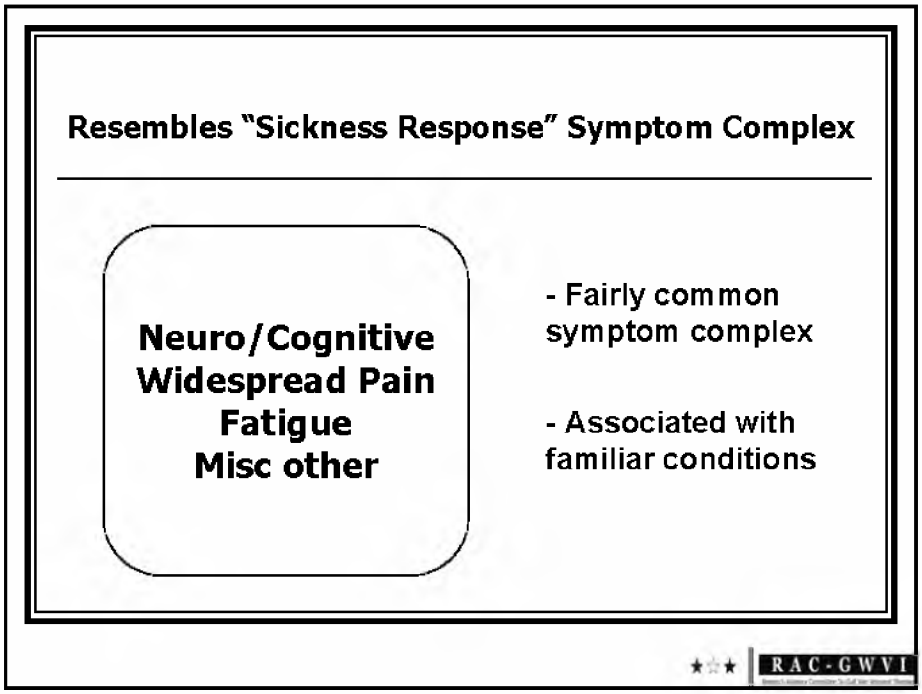
Resembles "Sickness Response" Symptom Complex
Neuro/Cognitive
Widespread Pain
Fatigue
Misc other
- Fairly common symptom complex
- Associated with familiar conditions
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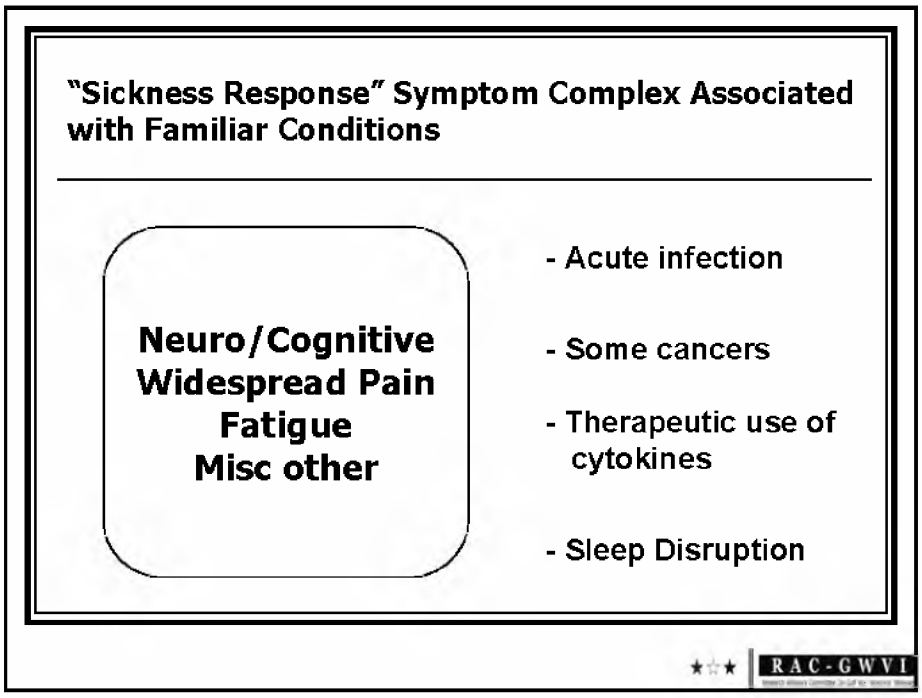
"Sickness Response" Symptom Complex Associated with Familiar Conditions
Neuro/Cognitive
Widespread Pain
Fatigue
Misc other
- Acute infection
- Some cancers
- Therapeutic use of cytokines
- Sleep Disruption
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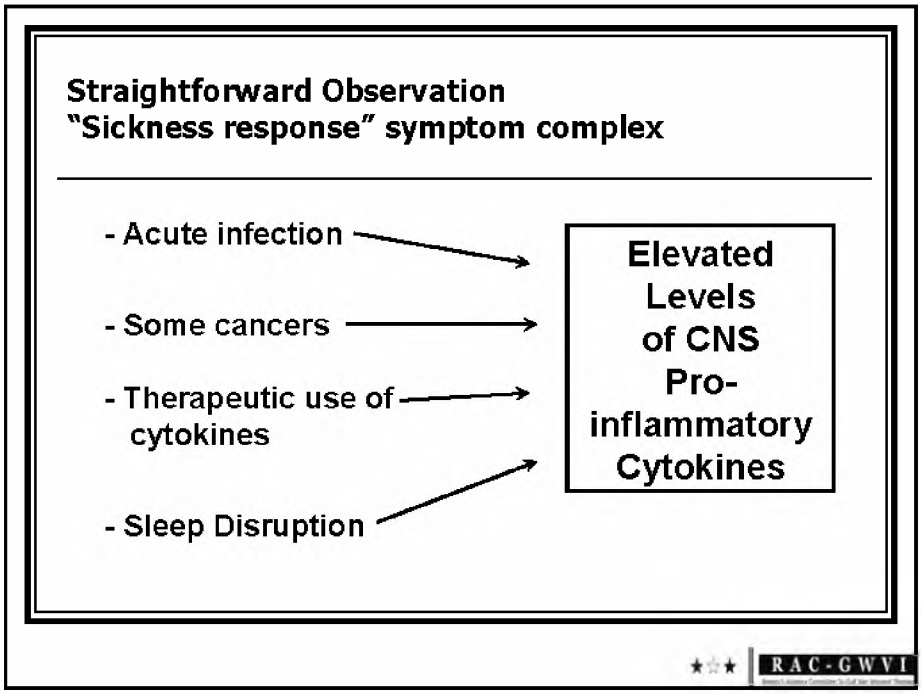
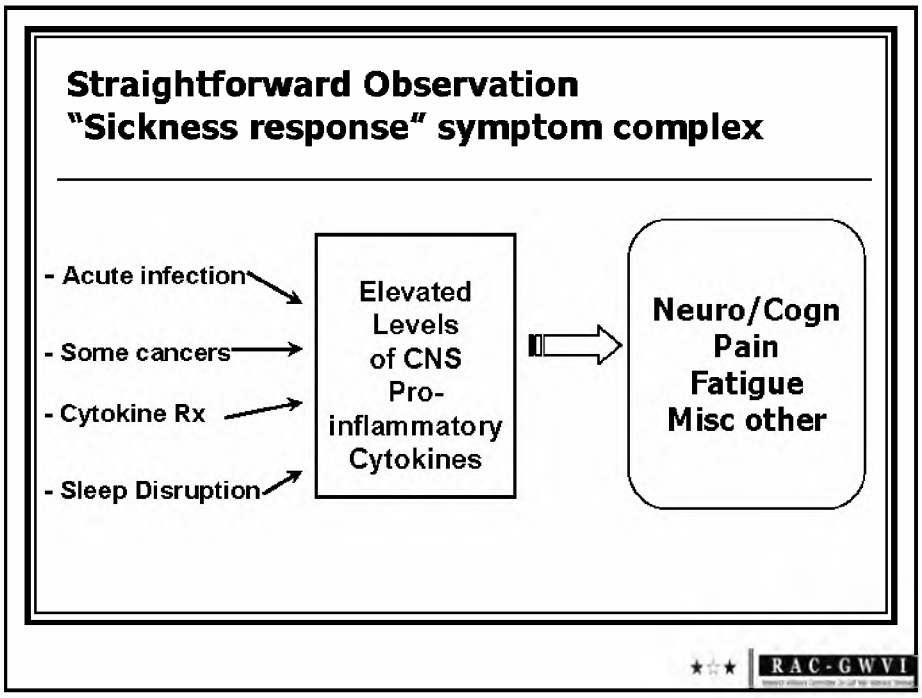
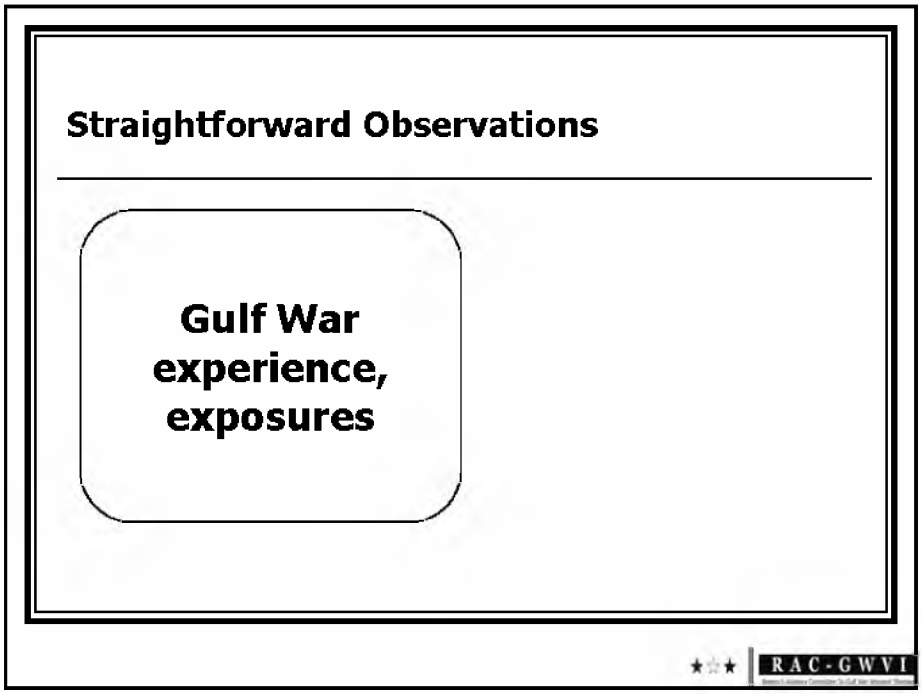
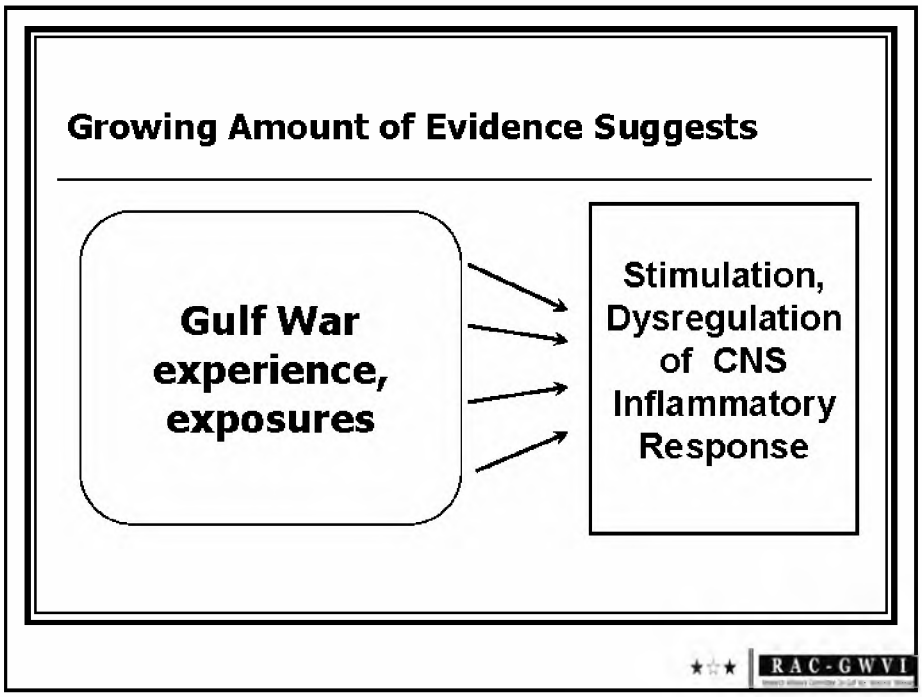

Straightforward Observation
"Sickness response" symptom complex
- Acute infection
- Some cancers
- Therapeutic use of cytokines
- Sleep Disruption
Elevated Levels of CNS Pro-inflammatory Cytokines
RAC-GWVI


Straightforward Observation
"Sickness response" symptom complex
- Acute infection
- Some cancers
- Cytokine Rx
- Sleep Disruption
Elevated Levels of CNS Pro-inflammatory Cytokines
Neuro/Cogn
Pain
Fatigue
Misc other
RAC-GWVI


Straightforward Observations
Gulf War experience, exposures
RAC-GWVI


Growing Amount of Evidence Suggests
Gulf War experience, exposures
Stimulation, Dysregulation of CNS Inflammatory Response
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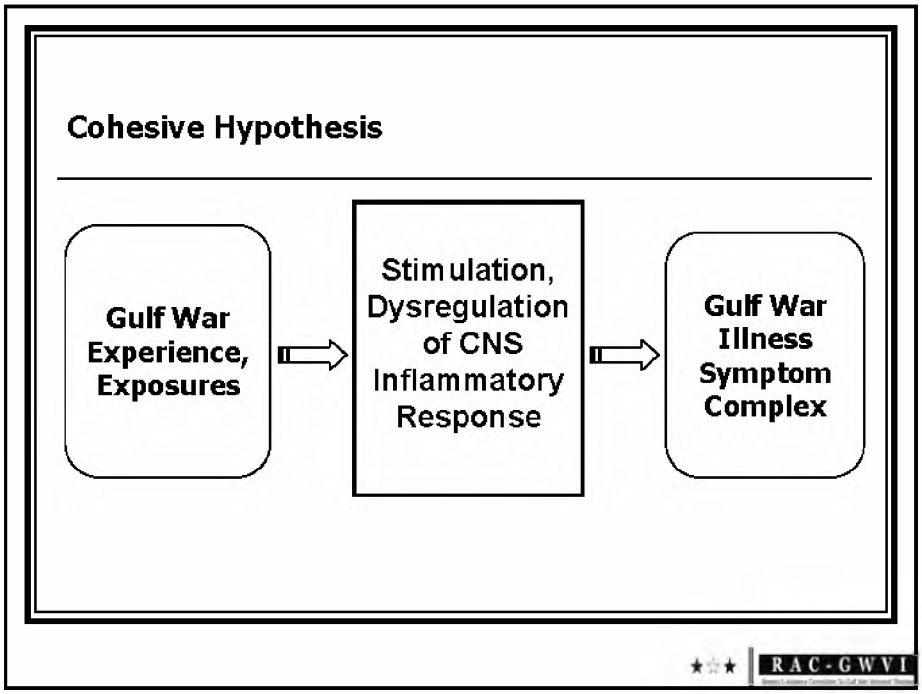
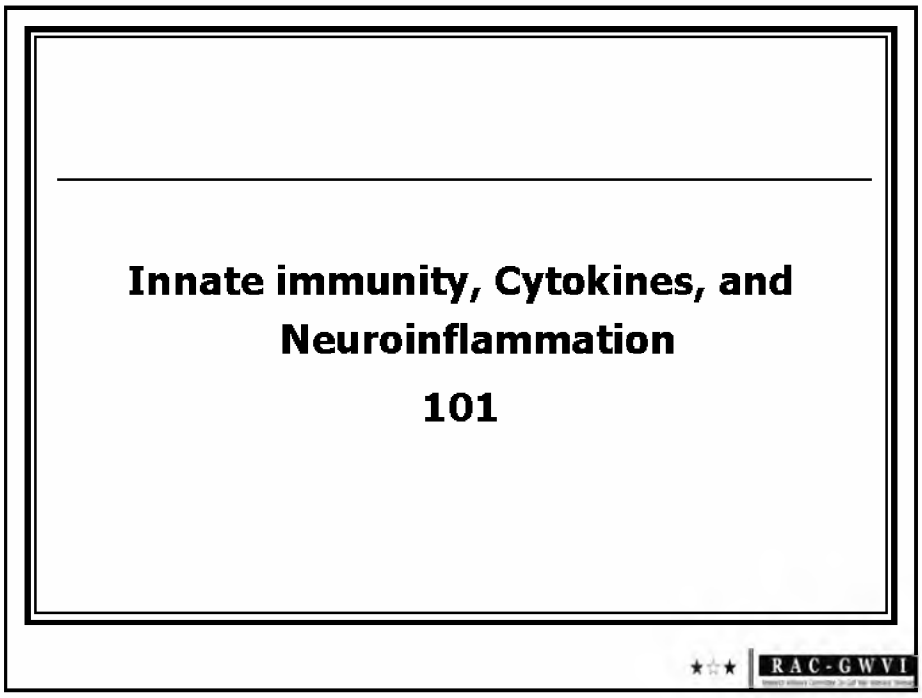

Cohesive Hypothesis
Gulf War Experience, Exposures
Stimulation, Dysregulation of CNS Inflammatory Response
Gulf War Illness Symptom Complex
RAC-GWVI


Innate immunity, Cytokines, and Neuroinflammation
101
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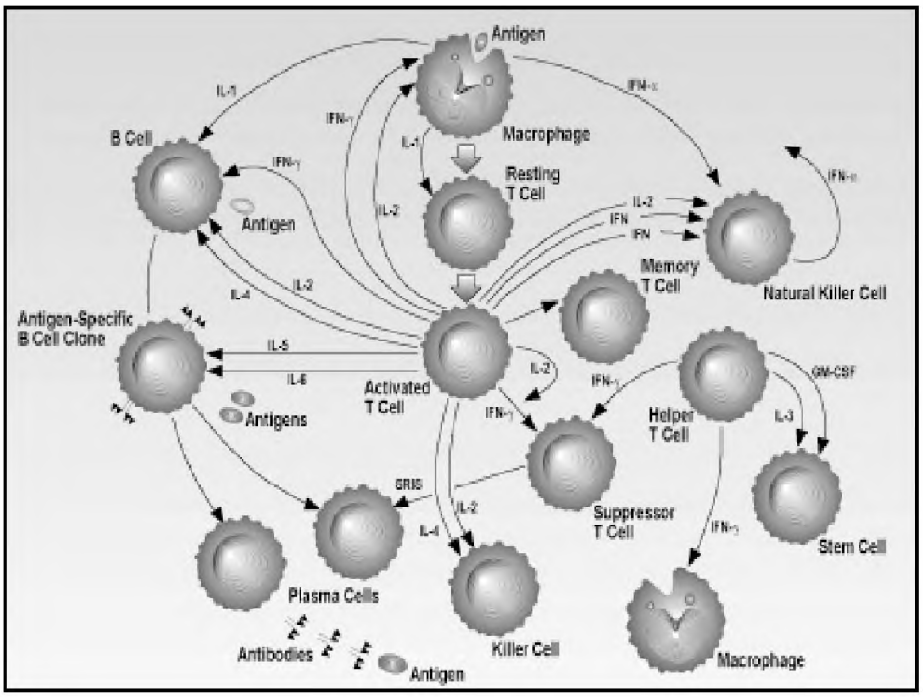

Innate Immunity

- **First line of defense: "nonspecific" response to infection, injury, foreign substances**
- **In periphery, innate immune response can lead to inflammation, adaptive immune response via multiple interrelated processes that protect the body from diverse "insults"**
- **Stimulates inflammatory/immune cascade**

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Cytokines

- **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

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

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Immunity in the Central Nervous System

- 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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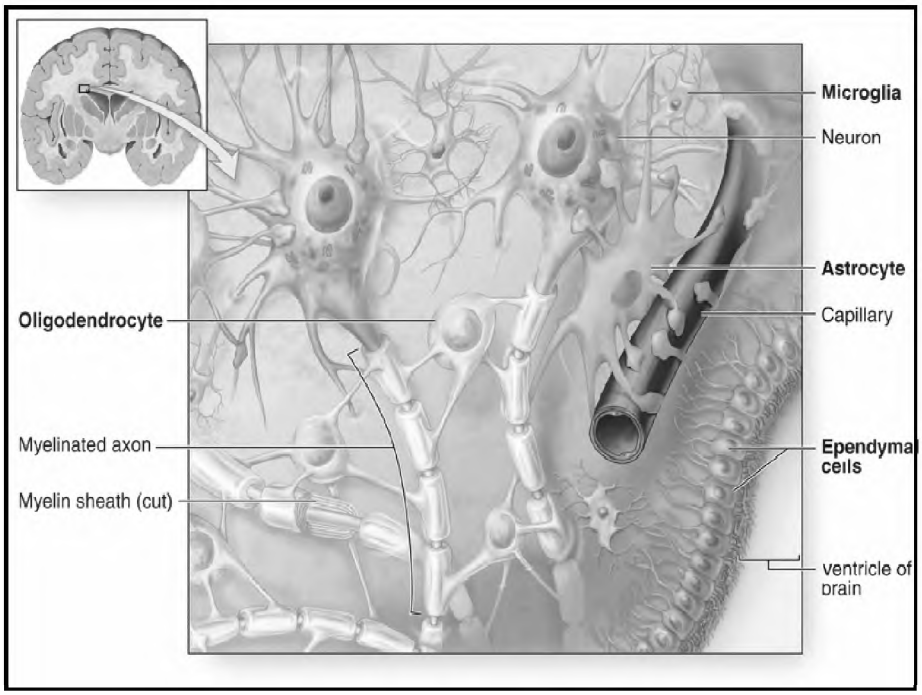

CNS insult → Microglia activation

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)

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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

- **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

- **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

- **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**
- **Chronic pain models**
- **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

- **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

- **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

- **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

- **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**

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Today's Presentations and Discussions

Discussion:

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

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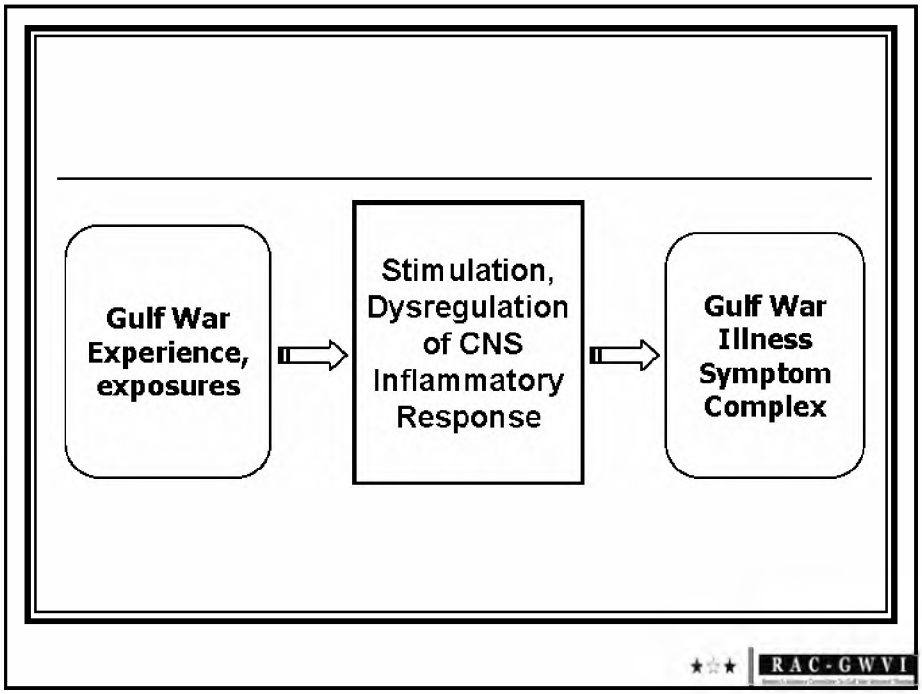

Hypothesis……

- **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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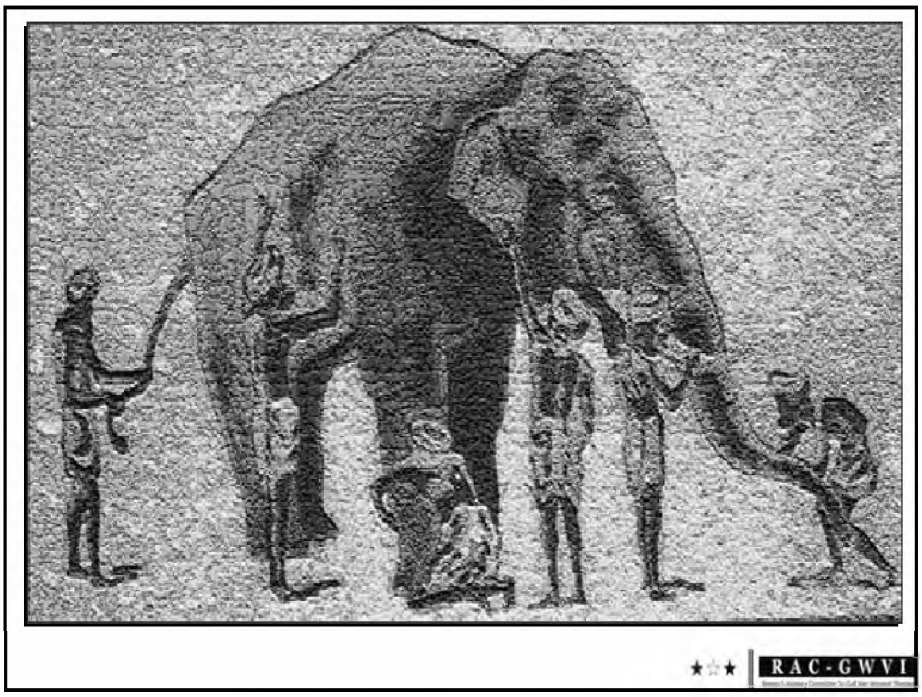
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