

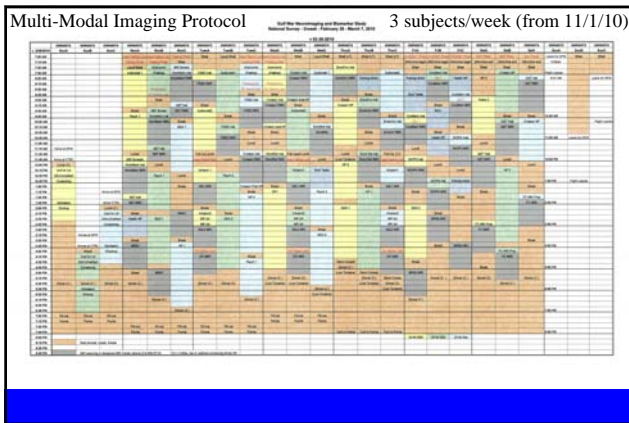
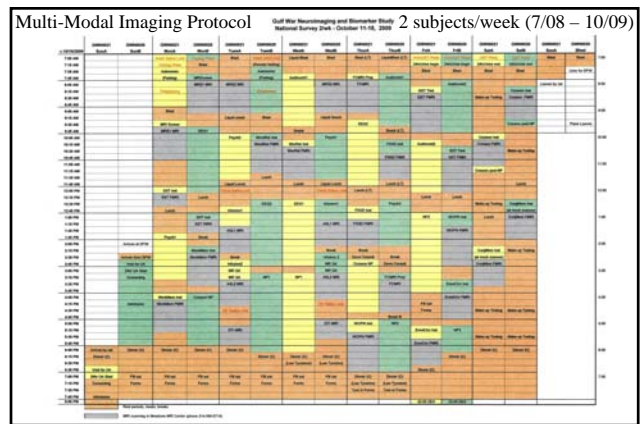
GW Veterans Studied in Neuroimaging & Biomarker Study

| Clinical Group | Seabees Pilot | National Sample | N thru this week |
|----------------|---------------|-----------------|------------------|
| Controls | 16 | 24 | 19 |
| Syndrome 1 | 11 | 22 | 12 |
| Syndrome 2 | 16 | 22 | 16 |
| Syndrome 3 | 11 | 22 | 15 |
| TOTAL | 54 | 90 | 62 |

Hypothesis-
generating

Confirmatory

2/3 complete
(ending 4/31)



Main Topics to Cover

Part 1.
 Imaging Techniques for Possible Diagnosis and Clues to Pathogenesis

Part 2.
 Studies to Discover What the Brain is Doing When GW Veterans Have Symptoms (taking the mystery out of the "mystery illness")

Part 1. Imaging Techniques for Possible Diagnosis and Clues to Pathogenesis

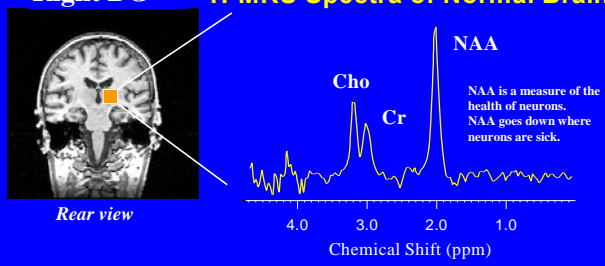
1. Measuring brain chemistry with MR Spectroscopy
2. Measuring rCBF with SPECT or ASL MRI after a cholinergic challenge
3. High resolution EEG
4. Assessing white matter with Diffusion Tensor Imaging (DTI)
5. (PET imaging for neuroinflammation—not funded)

1. Measuring Brain Chemistry with MR Spectroscopy (MRS)



MR Spectroscopy measures chemical concentrations in selected brain regions

Right BG ¹H-MRS Spectra of Normal Brain



Rear view

Chemical Shift (ppm)

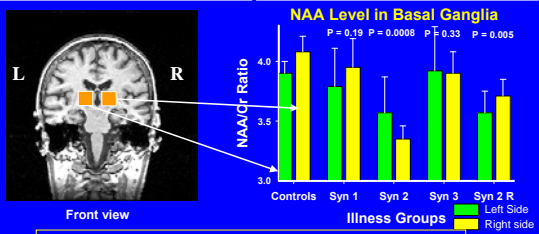
NAA is a measure of the health of neurons. NAA goes down where neurons are sick.

MR Spectroscopy Results in 1998 Seabees Pilot Study

MR Spectroscopy measured the level of *N-acetyl-aspartate (NAA)*, an essential chemical building block inside neurons.

Results revealed a medically significant decrease in NAA, indicating physical damage to neurons.

NAA Level in Basal Ganglia



Front view

Illness Groups

Legend: Left Side (Green), Right Side (Yellow)

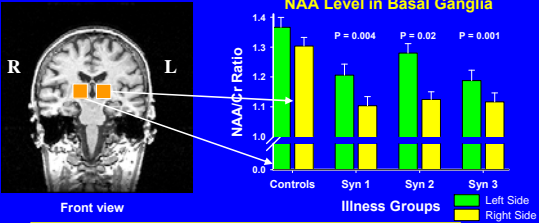
This particular chemical abnormality is not seen in psychological reactions to stress.

MR Spectroscopy Results in 2007-8 Seabees Re-Study

MR Spectroscopy measured the level of *N-acetyl-aspartate (NAA)*, an essential chemical building block inside neurons.

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NAA Level in Basal Ganglia



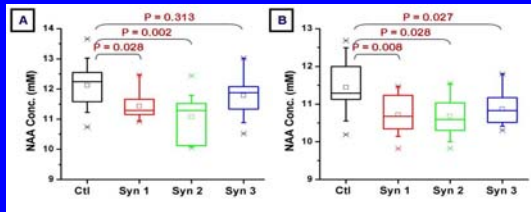
Front view

Illness Groups

Legend: Left Side (Green), Right Side (Yellow)

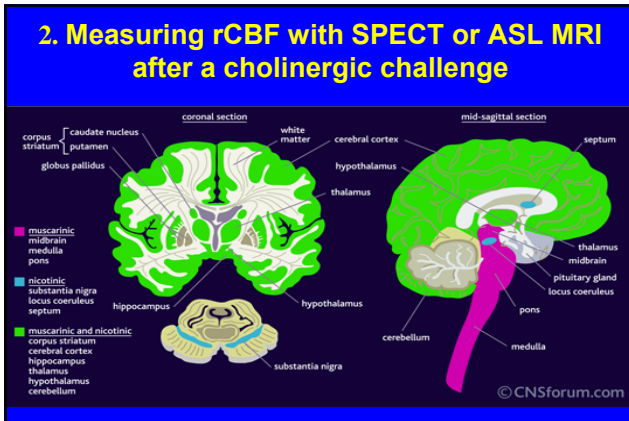
This particular chemical abnormality is not seen in psychological reactions to stress.

New Approach: Absolute metabolite concentrations in BG



Baek et al., ISMRM 2010

- Water as internal reference
- Literature values for water and metabolite T1 and T2
- Water properties could differ between groups



Why regional cerebral blood flow (rCBF) ?

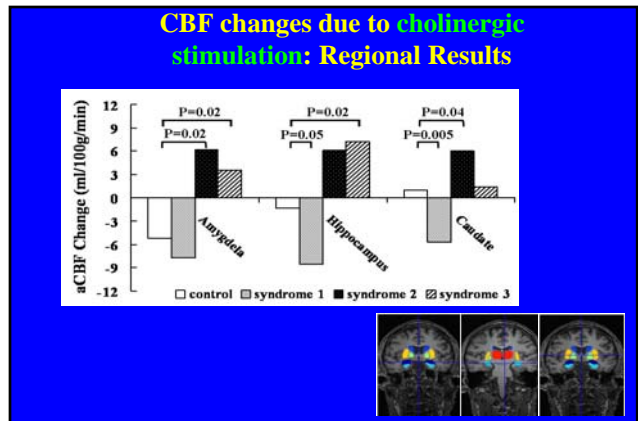
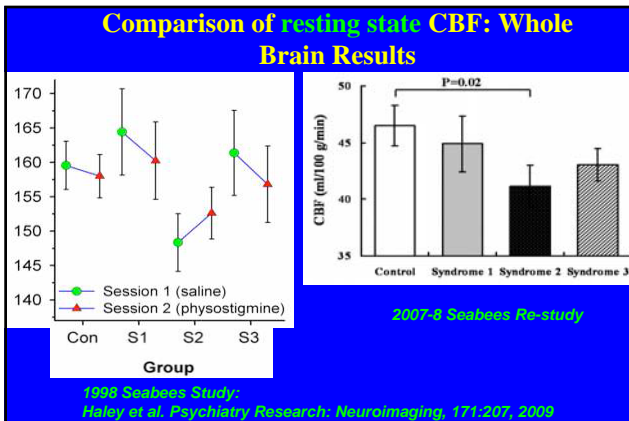
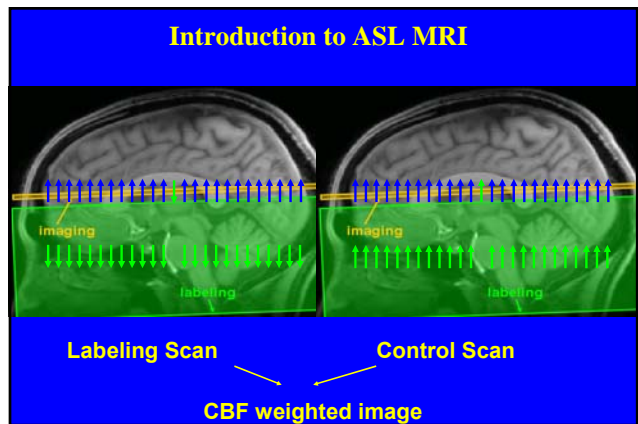
- One plausible etiology of the GWI is the exposure to cholinesterase-inhibiting chemicals. *Henderson et al. 2001.*
- Altered cholinergic system may change neural activity and produce symptoms of GWI.
- So we measured changes in rCBF from a cholinergic challenge with physostigmine to see if cholinergic brain function is intact.

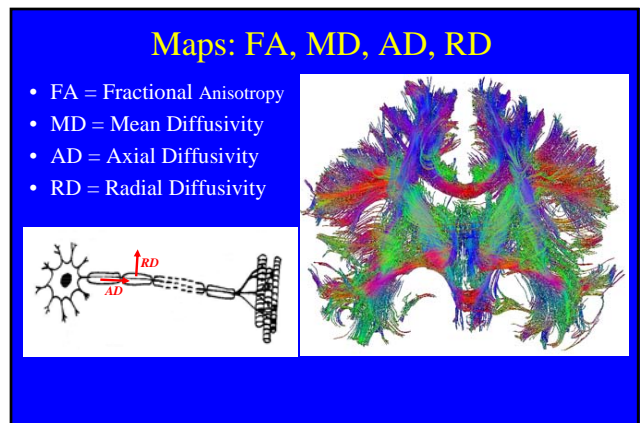
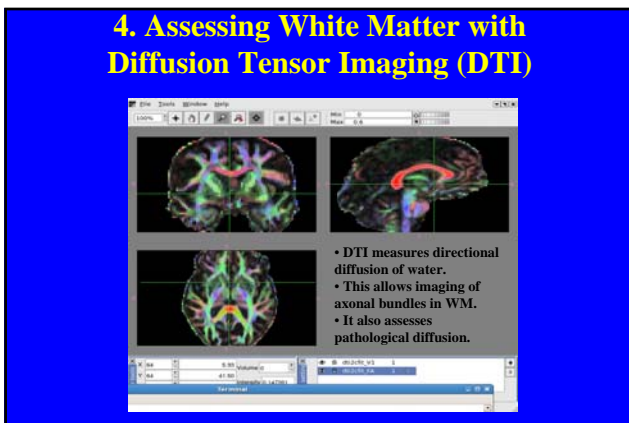
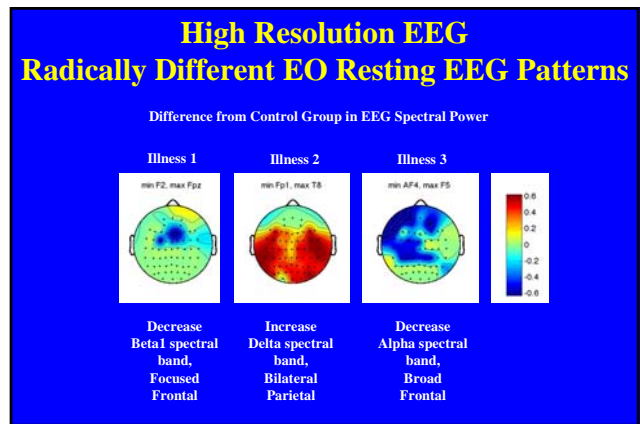
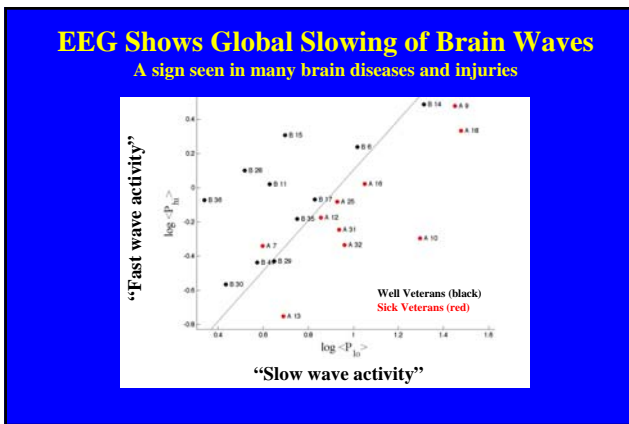
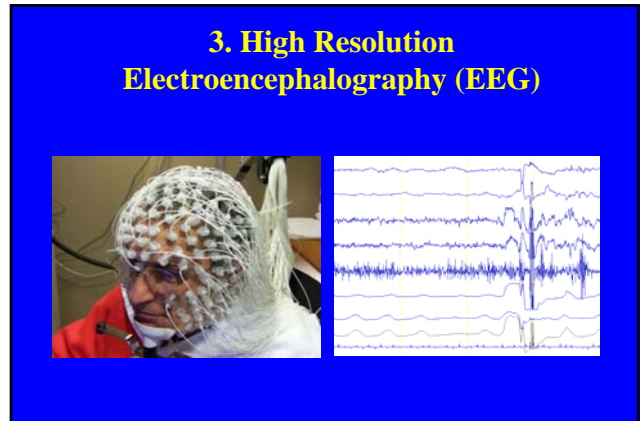
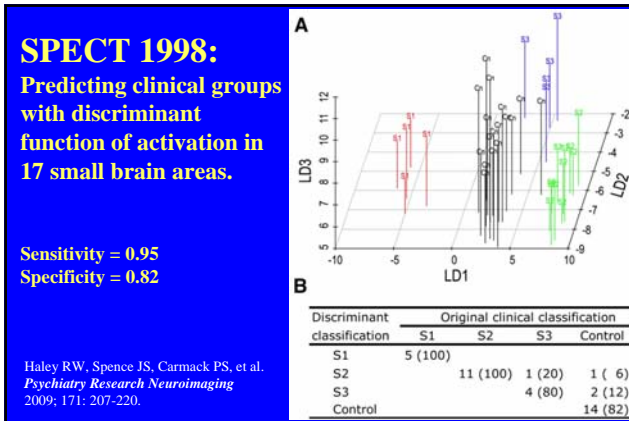
Goals of this study

- Try to re-confirm previous SPECT findings using both SPECT and a different technique.
- Search for a cost-effective method that is suitable for large scale study or routine screening.

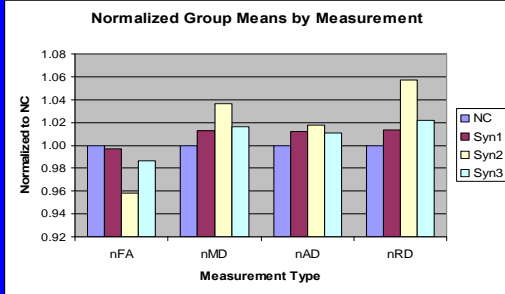
Measure Regional CBF with:

- Single Photon-Emission Computed Tomography (SPECT)
- Arterial Spin Labeling (ASL) MRI

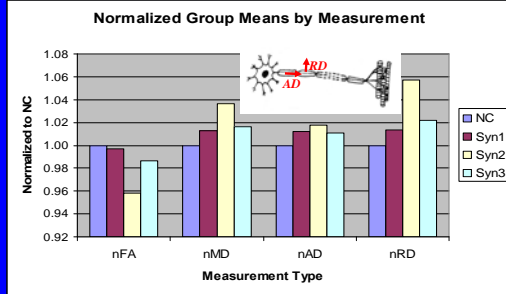




Means by Group



Means by Group



Part 2. Studies to Discover What the Brain is Doing When GW Veterans Have Symptoms

Taking the mystery out of the
“mystery illness”

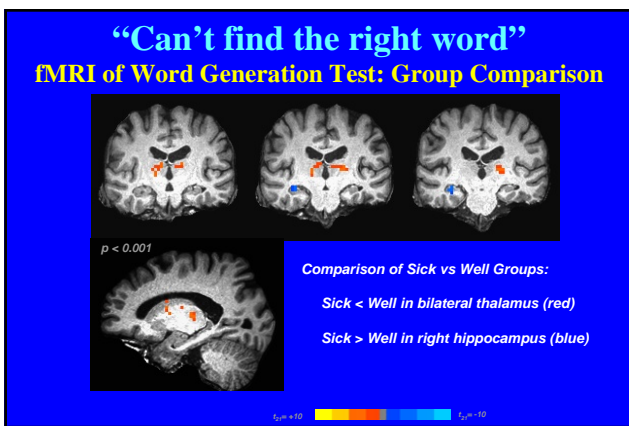
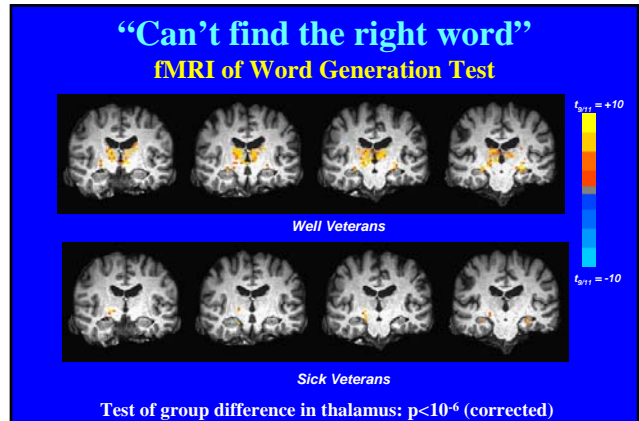
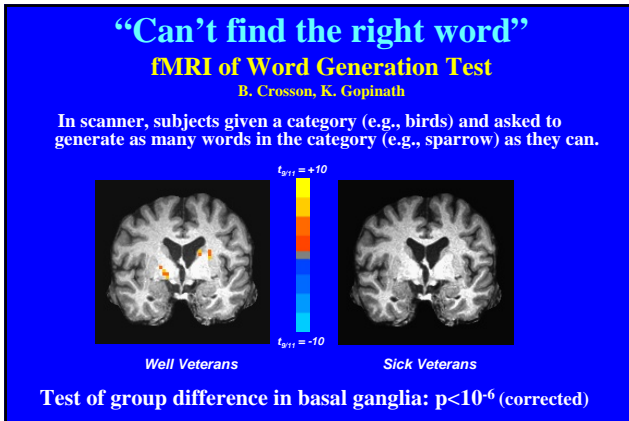
Gulf War Veterans' Illnesses – Key Features

- Memory deterioration
- Attention/concentration problems
- Impaired word finding
- Constant body pain
- Subtle motor control problems
- Chronic fatigue
- Personality change

Typical Symptoms of Gulf War Syndrome

- Can't find the right word
- Can't remember things
- Can't concentrate or pay attention
- Constant body pain, tingling or numbness
- Feeling tired, fatigued all the time
- Easily angered, irritable

Symptom:
“Can't Find the Right Word”



Symptom:

“Can’t Remember Things”

Study 1

“Can’t remember things”

fMRI Task of Learning and Remembering Words, Objects, Faces and Nature Scenes

W. Ringe

| STIMULI | LEARN | SEEN MANY | SEEN ONCE |
|---------------|-------|-----------|-----------|
| WORDS | FACT | NIGHT | FACT |
| OBJECTS | | | |
| FACES | | | |
| NATURE SCENES | | | |



Symptom:
“Can’t Remember Things”

Study 2

“Can’t remember things”
fMRI of Working Memory and Executive Function
B. Rypma, M. Motes

- Commonly reported symptoms in GW illness:
 - Impaired concentration
 - Impaired “short-term memory” (executive working memory functions)
- These symptoms may be due to:
 - Cholinergic system damage
 - Disproportionally affects the frontoparietal circuit, which is known to mediate working memory.

“Can’t remember things”
fMRI of Working Memory and Executive Function

Method: In scanner, subjects given a set of 2, 4 or 7 letters to remember over a brief delay (8s), and then scan is done while they recall whether a probe letter was in the memory set.

Result: Well veterans use executive function in dorsal PFC and parietal storage. Sick veterans use ventral PFC for rehearsing (work-around).

Item Recognition Task
Reaction Time Increases with Memory Set Size

Working Memory Measure

Patient Groups’ Activation Differences

| | Encoding | Maintenance | Response |
|---|----------|-------------|----------|
| Controls vs. Sd 1 Cognitive Impairment | | | |
| Controls vs. Sd 2 2-Cognition/Ataxia | | | |
| Controls vs. Sd 3 Anthrax/neuropathy | | | |

Hypotheses

- Encoding-related activity greater in healthy controls than in patients
- Retrieval-related activity greater in patients than in healthy controls
- Healthy subjects emphasize encoding processes to optimize performance
- Patients emphasize retrieval related strategies

Hypotheses

- Sd 1 Cognitive Impairment Group: Reduced efficiency; overall activity reductions compared to controls
- Sd 2 Confusion/Ataxia Group: Reduced efficiency; strategy differences
- SD 3 Pain Group: Reduced efficiency; strategy differences

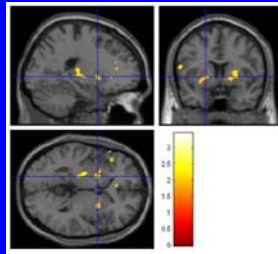
Symptom: “Can’t Concentrate or Pay Attention”

“Can’t concentrate or pay attention” fMRI of Continuous Performance Test (CPT)*

M. Posamentier

*Conners' Continuous Performance Test (CPT)

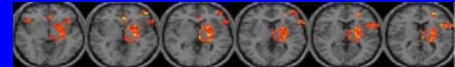
- CPT Not-X Task
 - In scanner, subject presses a button at every letter, but at not “X.”
 - Group A failed to inhibit at “X” significantly more often than Group B.
- CPT is abnormal in all studies done.



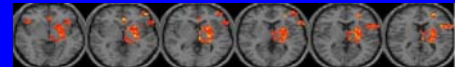
Sick < Well in basal ganglia

fMRI Findings for 3 Syndrome Groups on the CPT Paradigm

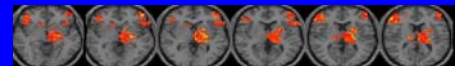
Controls vs. Sd 1
Cognitive Impairment



Controls vs. Sd 2
Confusion/Ataxia



Controls vs. Sd 3
Central Pain



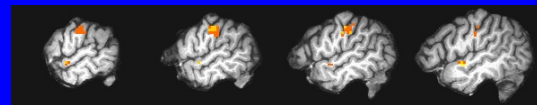
All 3 illness groups showed reduced activity in basal ganglia and prefrontal cortex regions compared with Well.

Symptom: “Constant Body Pain, Tingling or Numbness”

“Constant body pain, tingling or numbness”

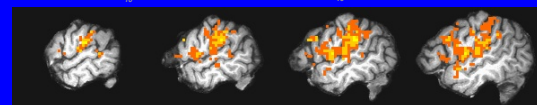
fMRI of Heat Pain Stimulation

K. Gopinath



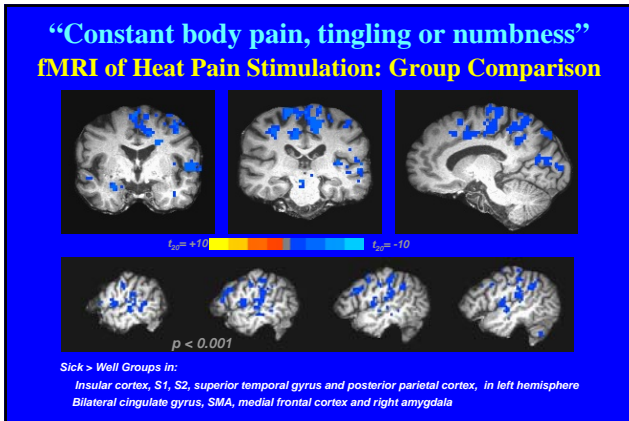
$p < 1 \times 10^{-5}$

Well Veterans



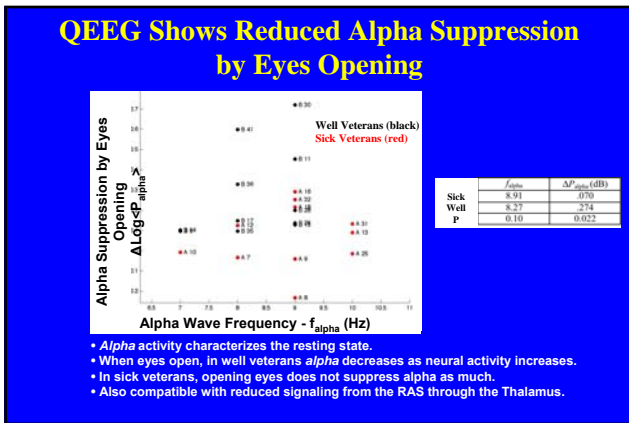
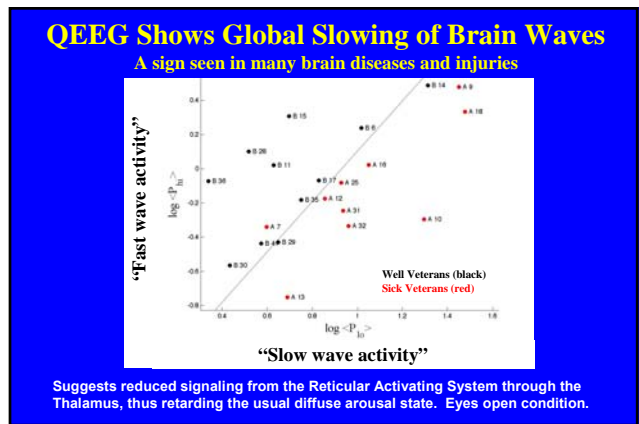
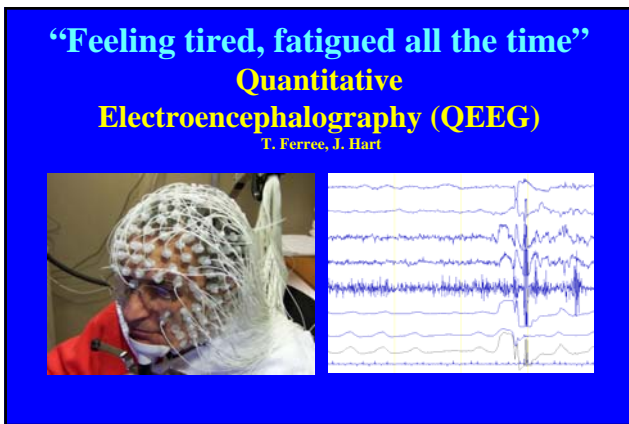
$p < 1 \times 10^{-4}$

Sick Veterans



Symptom:
“Feeling Tired or Fatigued All the Time”

Study 1



Symptom:
“Feeling Tired or Fatigued All the Time”

Study 2

“Feeling tired, fatigued all the time”

fMRI of Functional Connectivity in Resting State

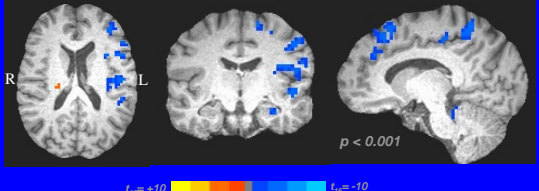
R. Briggs, K. Gopinath

- Series of MR scans taken with subject in resting state.
- Analysis places a “seed” in a given brain structure and measures correlation of activation of other brain structures with the “seed” structure.
- Reveals the amount of interaction between the “seed” structure and the other brain structures.

“Feeling tired, fatigued all the time”

fMRI of Functional Connectivity in Resting State

Seed in: Left Dorsal Striatum (Group Comparison)

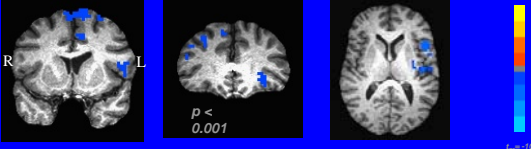


Sick > Well: Increased connectivity (blue) with medial frontal cortex, parietal cortex, sup. temp. gyrus, post-central gyrus and insula.
Sick < Well: Decreased connectivity (red) with right thalamus

“Feeling tired, fatigued all the time”

fMRI of Functional Connectivity in Resting State

Seed in: Left Ventral Striatum (Group Comparison)



Sick > Well : Increased connectivity (blue) with ventrolateral prefrontal cortex, insula, precentral, cingulate, SMA and sup. frontal
Sick < Well : Decreased connectivity (red) with bilateral thalamus
Indicates constant hyper-arousal / hyper-vigilance

Symptom:


“Easily Angered, Irritable”

“Easily Angered”

fMRI of Fronto-Striatal Systems in Mood States

W. Ringe

- In scanner, subjects view positive, neutral and negative pictures (right).
- MRI scans taken during periodic self-rated mood checks.

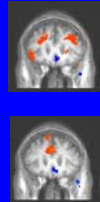


“Easily Angered”

fMRI of Fronto-Striatal Systems in Mood States

W. Ringe

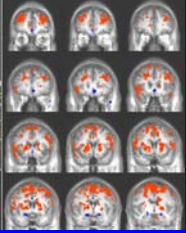
- In scanner subjects view positive, neutral and negative pictures
- MRI scans taken during periodic self-rated mood checks
- Usual findings:
 - Non-depressed subjects activate the Dorsal Striatum – Dorso-Lateral PFC pathway (DSDL)
 - Depressed subjects activate the Ventral Striatum – Ventro-Medial PFC pathway (VSVM)



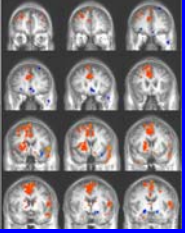
“Easily Angered”

fMRI of Fronto-Striatal Systems in Mood States

Well Veterans



Sick Veterans

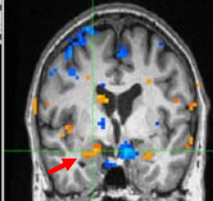


Well Veterans activate the DSDL, and Sick Veterans the VSVM. Will correlate this with other findings to try to explain the depression.

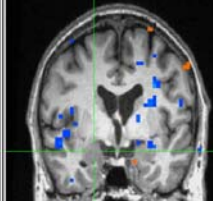
“Easily Angered”

fMRI of Fronto-Striatal Systems in Mood States

Well Veterans



Sick Veterans



In Well Veterans the stimulus activates the Amygdala bilaterally, part of the VSVM. In Sick Veterans the same stimulus does not activate the Amygdala (P < 0.05).


Currently running MDD patients through this paradigm. The literature says that MDD patients show Amygdala hyperactivity, not hypoactivity as in GWS subjects.

Gulf War Veterans' Illnesses – Key Features

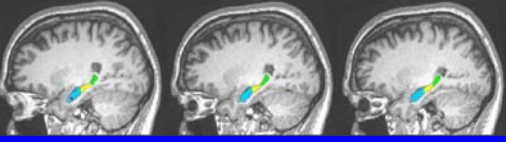
- **Memory deterioration**
- Attention/concentration problems
- Impaired word finding
- Constant body pain
- Subtle motor control problems
- Chronic fatigue
- Easily angered

Activation of the Right Hippocampus During fMRI Test of Facial Memory

Normal Memory Function in the Well Veteran Group

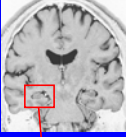
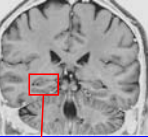
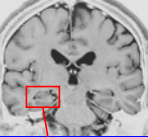
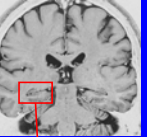






Diminished Memory Function in All 3 Illness Groups

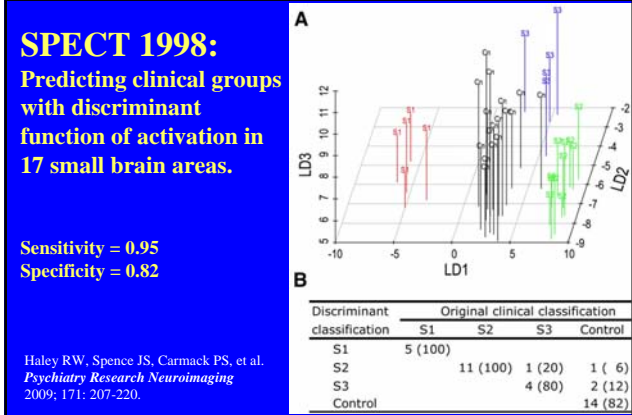


What Pathology Might Underlie the Hippocampal Dysfunction?

High Resolution Brain MRI Detected Actual Holes in the Memory Control Center

| Well Veterans | 3 Sick Veterans | | |
|--|---|---|---|
|  |  |  |  |
|  <p>No cavities</p> |  |  |  |

- 19 of 49 (49%) of the sick veterans had a cavity >4 mm in length
- 1 of 14 (7%) of the well veterans (p < 0.001).

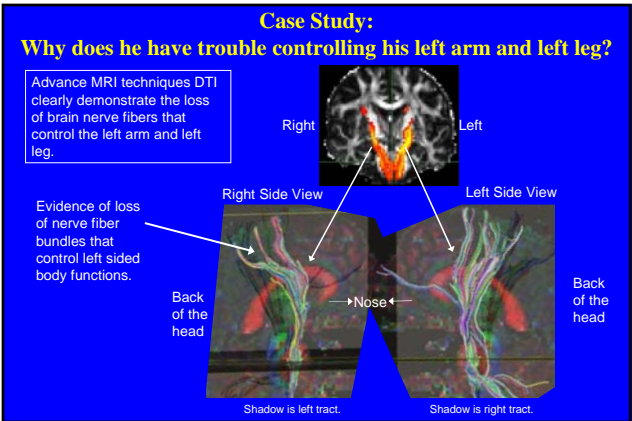
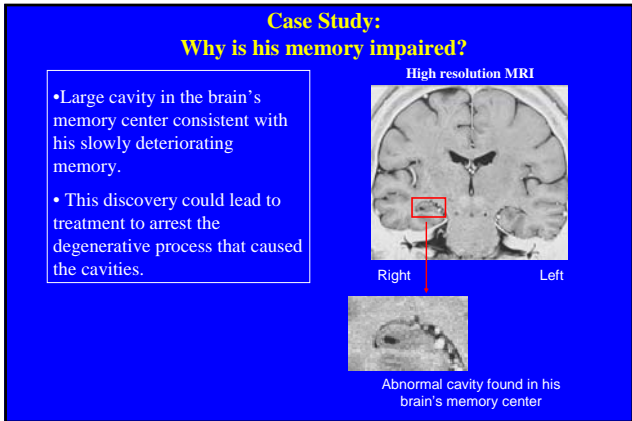
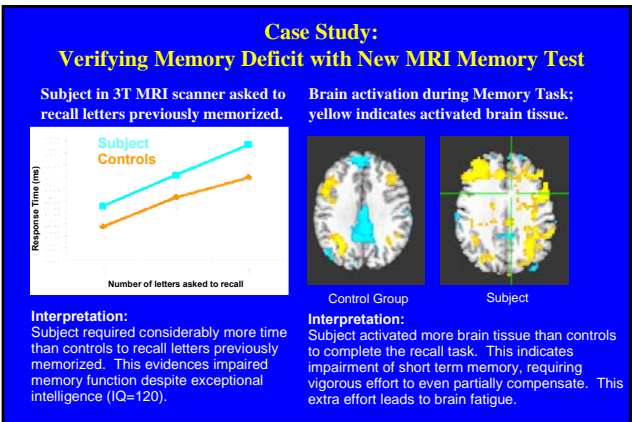


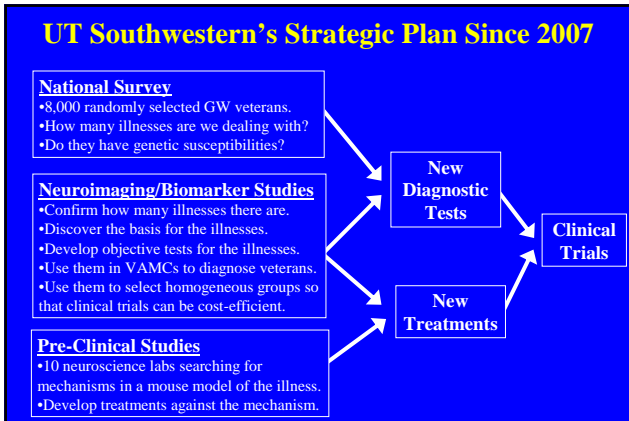
Implications for Diagnosis and Treatment

- Brain imaging tests appear to distinguish veterans with GWI from well veterans and at least 2, possibly 3, variants of GWI.
- Important to distinguish the variants because Sd1 and Sd2/3 appear to show opposite effects on many tests.
- A subset of tests may constitute a sensitive/specific diagnostic test for GWI and provide homogeneous groups for powerful clinical trials of treatment.
- Definitive conclusions await attempted replication of the findings in a representative sample of GW veterans, presently near completion.

Case Study of a Sick Veteran Recently Selected Randomly from the National Survey and Given the 6-Day Testing Protocol Just Last Week

- A former General Officer who was forward deployed in the 1991 Gulf War.
- Mid 50s, high IQ, high functioning
- Deteriorating memory causing him to give up lucrative consulting work.
- Worsening control of left hand and leg function causing him to give up serious athletic participation.
- A VA physician recently told him, "There is no such thing as Gulf War illness; there is nothing wrong with you."





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