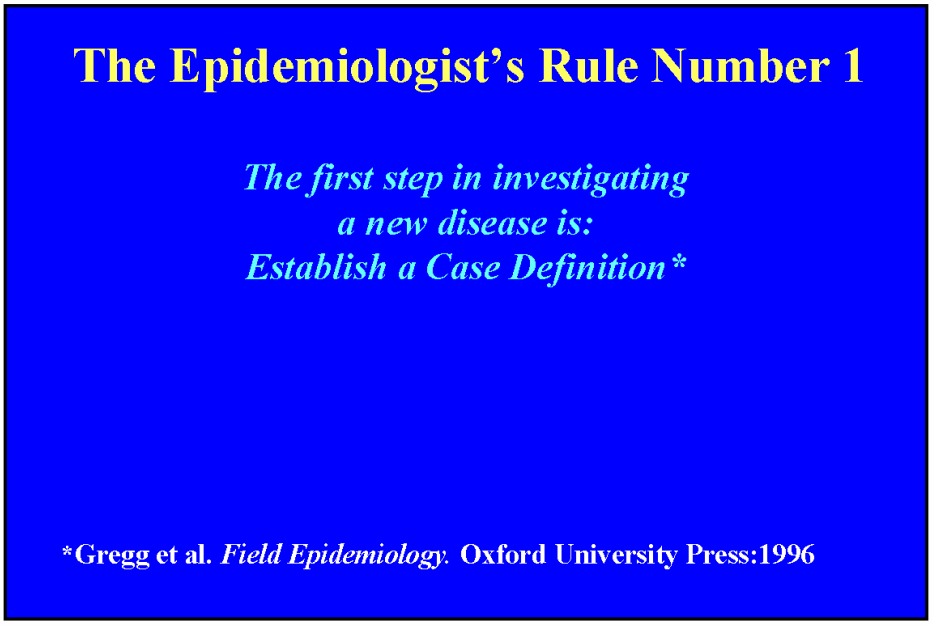
Appendix A

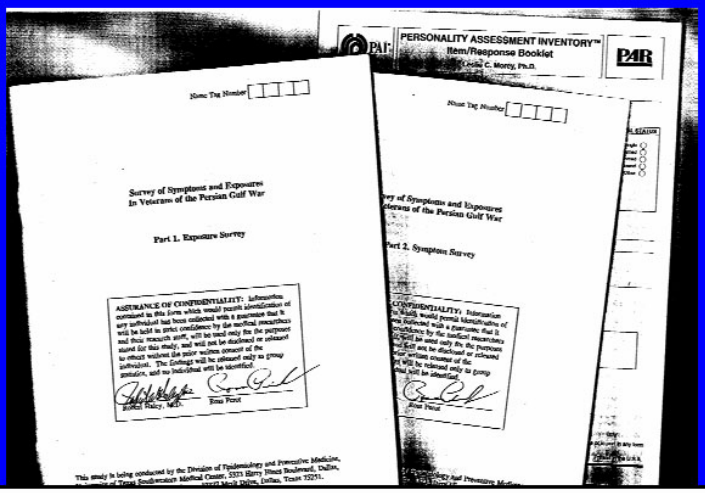
Presentation 1 – Robert Haley



Conducted a Survey in a Reserve Seabees Battalion

24th Reserve Naval Mobile Construction Battalion*

December 1994 – February 1995



Knoxville
Birmingham
Winston-Salem
Charlotte
Atlanta

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

Haley Symptom Questionnaire: Example Question on Paresthesias

2-stage factor analysis
- Symptom factors
- Syndrome factors

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

CIRCLE ONE

Yes . . . 1
No . . . 2

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

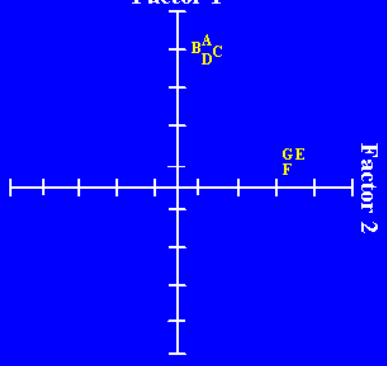
4A. Please indicate what part of your body was affected by this pain, in what month and year it began, and whether it is still a problem for you.

	Was this area involved? YES	NO	If yes, in what month/year did it begin? MONTH	YEAR	Is it still a problem? YES	NO
Scalp	1	2	/		1	2
Face	1	2	/		1	2
Lips	1	2	/		1	2
Tongue	1	2	/		1	2
Chest	1	2	/		1	2
Back	1	2	/		1	2
Hands	1	2	/		1	2
Arms	1	2	/		1	2
Abdomen	1	2	/		1	2
Groin	1	2	/		1	2
Genital area	1	2	/		1	2
Rectal area	1	2	/		1	2
Thighs	1	2	/		1	2
Calves	1	2	/		1	2
Feet	1	2	/		1	2
Other	1	2	/		1	2

specify _____

-6-

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

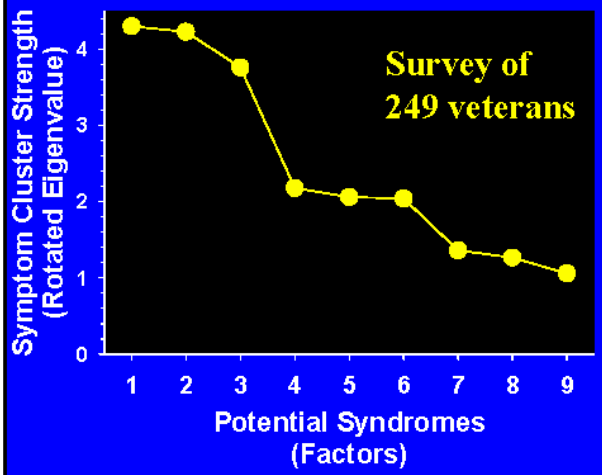


Anatomic location of paresthesias	Correlation with: Symptom Factor 1	Symptom Factor 2
A. Arms	.84*	.19
B. Feet	.82*	.17
C. Hands	.79*	.21
D. Calves	.78*	.19
E. Face	.21	.74*
F. Tongue	.18	.69*
G. Lips	.14	.67*

JAMA 1997;277:215-222.

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

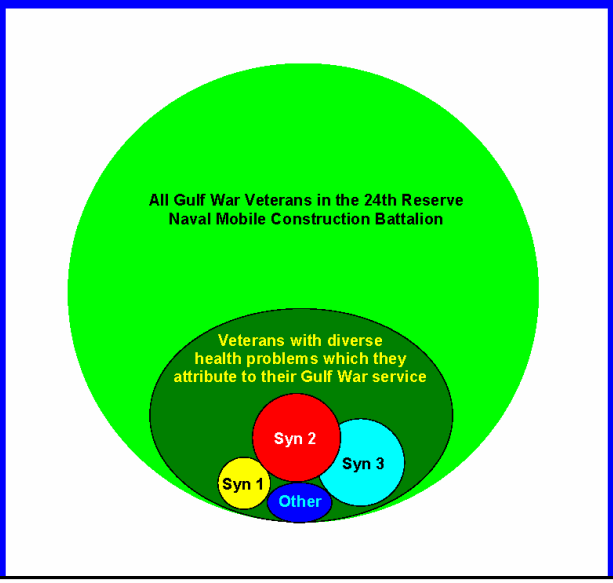
Factor Analysis of 52 Symptom Scales Identifying 6 Possible Gulf War Syndromes



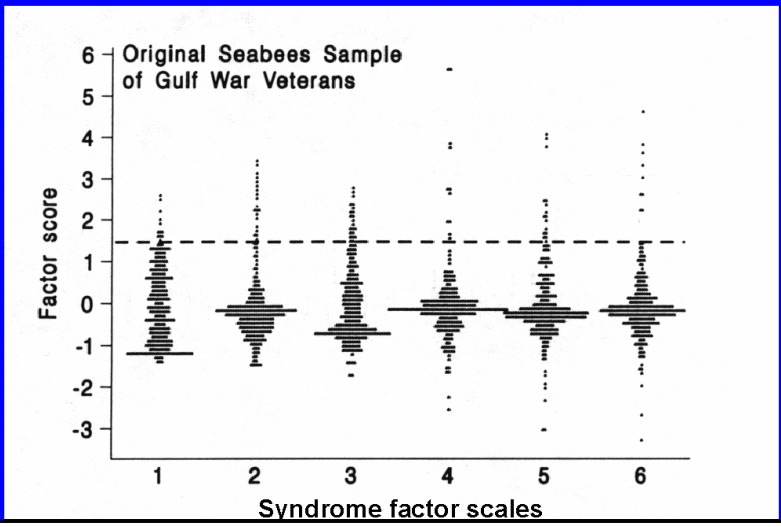
Syndrome Factor	Description	N
1	Impaired Cognition	12
2	Confusion-Ataxia	21
3	Central Pain	22
4-6	Subtypes of Syn 2	36
	Total syndromic	63
	Ill but not syndromic	116
	Remained well	70

JAMA 1997;277:215-222.

Clinical Subgroups of Gulf War Veterans

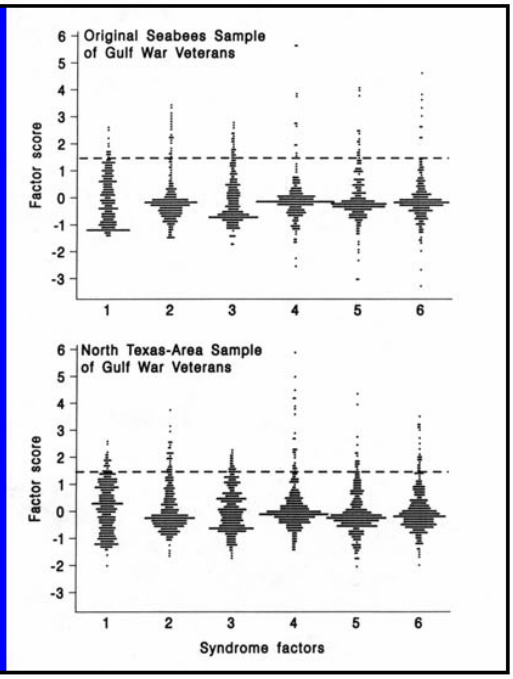


Distribution of the 249 Naval Reservists on each of 6 Syndrome Factor Scales

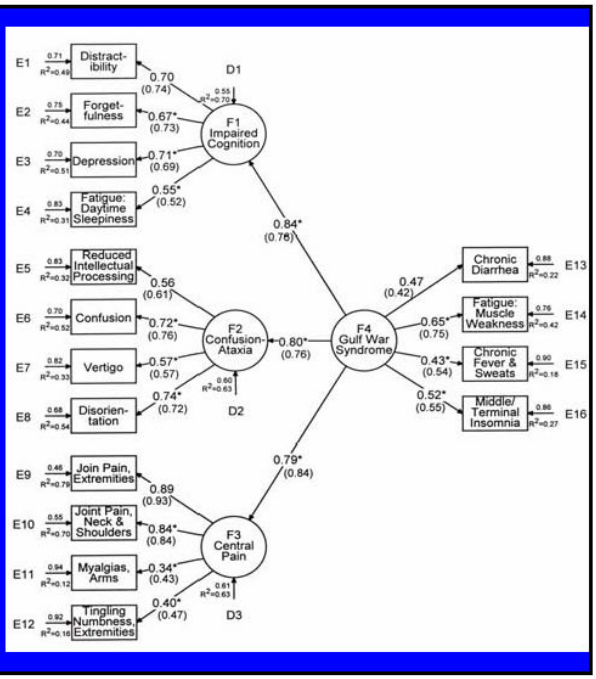


Validation of the Case Definition on 335 Army Veterans Surveyed at the Dallas VA 1997- 1998

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



Structure of Gulf War Syndrome in Two Different GW Veteran Samples



JAMA 1997;277:215-222.
Psych Res 2001;102:175-200.

Comparison of Factor Models from Symptom Surveys of Gulf War Veterans

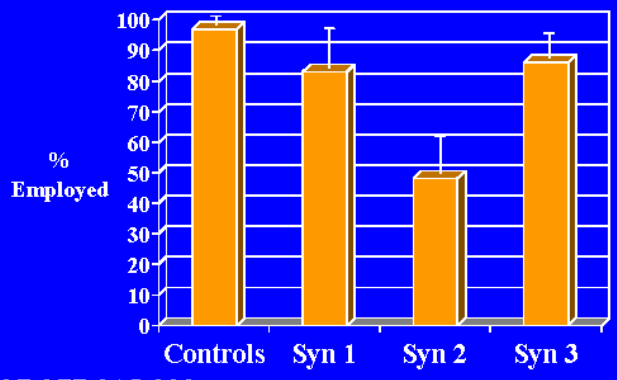
Year	Author	Cognitive	Neurologic	Pain/sensory
1997	Haley	F1	F2	F3
1998	Fukuda	F1	---*	F2
1999	Ismail	F1	---*	F3
2001	Haley	F1	F2	F3
2001	Cherry	F1	F3	F2
2001	Bourdette	F1	F2†	F3
2002	Kang	F1	F2	F3

*Did not measure the symptoms of the neurologic factor.

†"Mixed somatic" symptoms included autonomic symptoms.

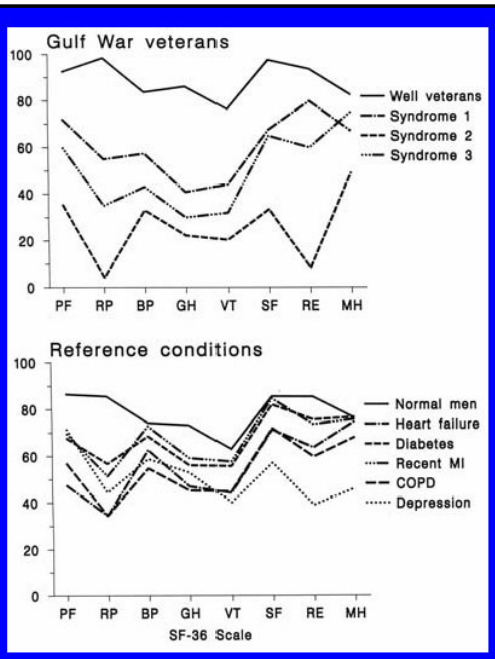
Knoke (2000) and Doebbeling (2000) measured symptoms of standard psychiatric conditions and thus found none of the syndrome factors found by the other groups.

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



JAMA 1997;277:215-222.

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



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

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

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

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

JAMA 1997;277:215-222.

Hypothesis Regarding The Nature of Gulf War Syndrome

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

Hypothesis Regarding The Cause of Gulf War Syndrome

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

Undertook a Series of Clinical Case-Control Studies

Purpose: To attempt to validate the case definition

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

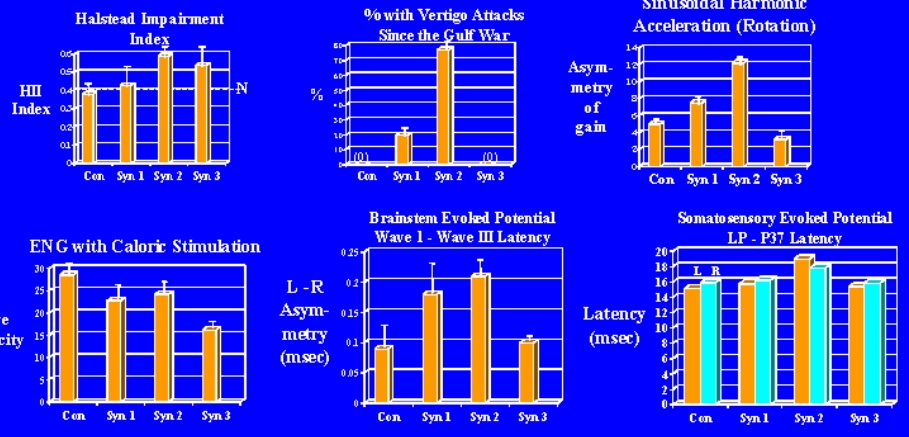
From the 249 Surveyed Veterans Selected Smaller Samples for Case-Control Studies of Brain Function and Serologic Markers

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

Usual Medical Tests Gave Negative Results
No Significant Group Differences

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

Positive Results
On Neurophysiologic Tests



JAMA 1997;277:2223-230.

Jan. 15, 1997 Issue of JAMA

Original Contributions

Is There a Gulf War Syndrome?

Searching for Syndromes by Factor Analysis of Symptoms

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

Evaluation of Neurologic Function in Gulf War Veterans

A Blinded Case-Control Study

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

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

A Cross-sectional Epidemiologic Study

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

Pilot Study with Col. Bill Davis

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

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

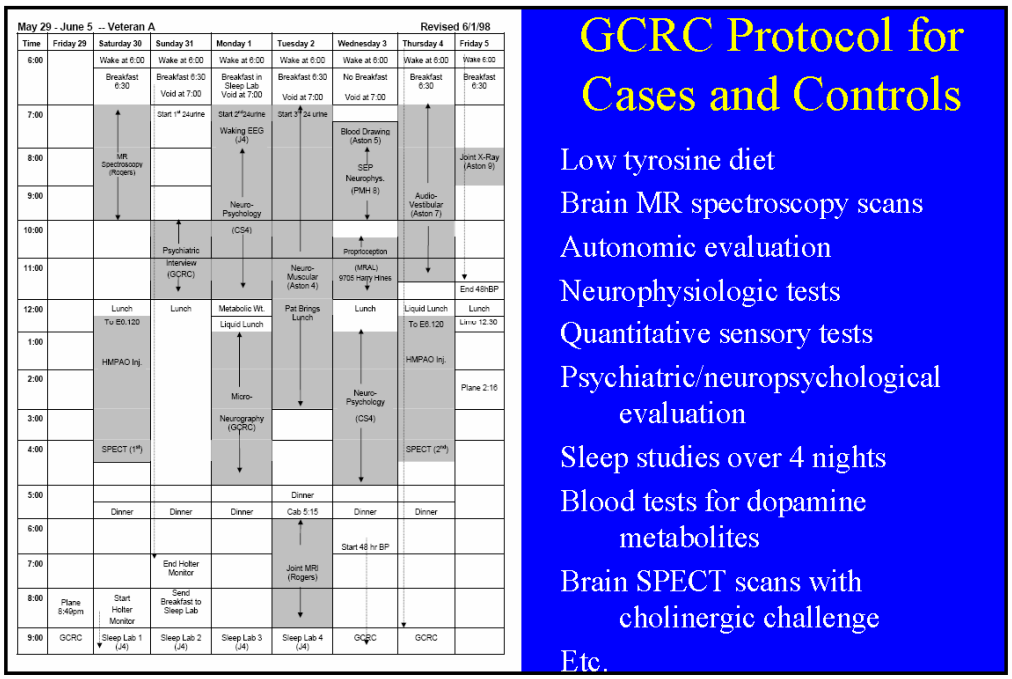
Developed Gulf War neurological illness soon after returning from the Gulf War.

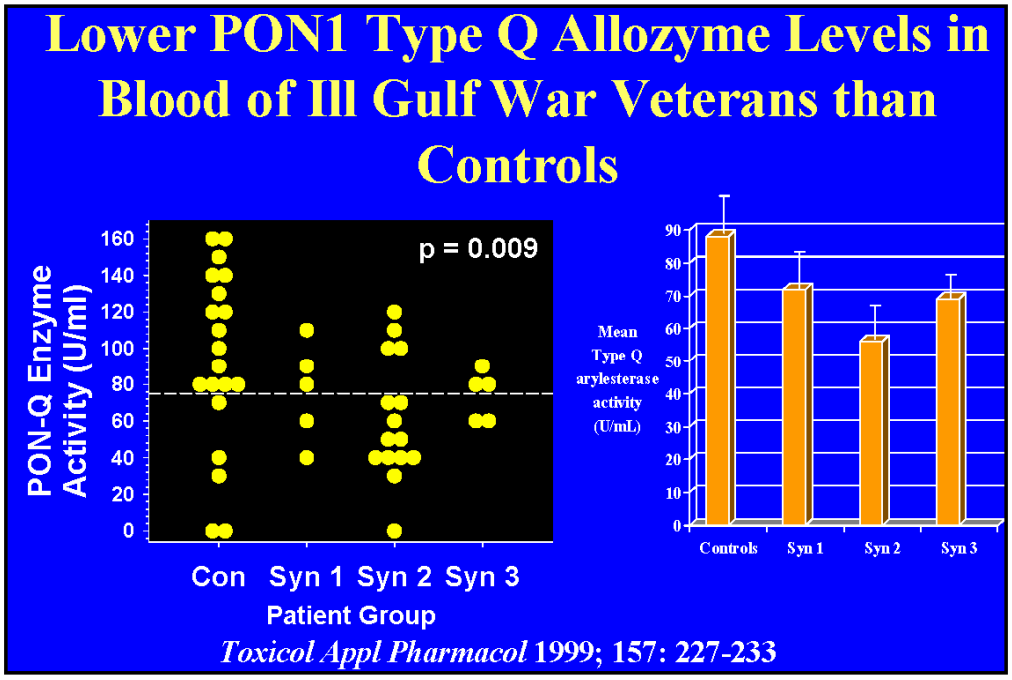
Col. Bill Davis had a twin!

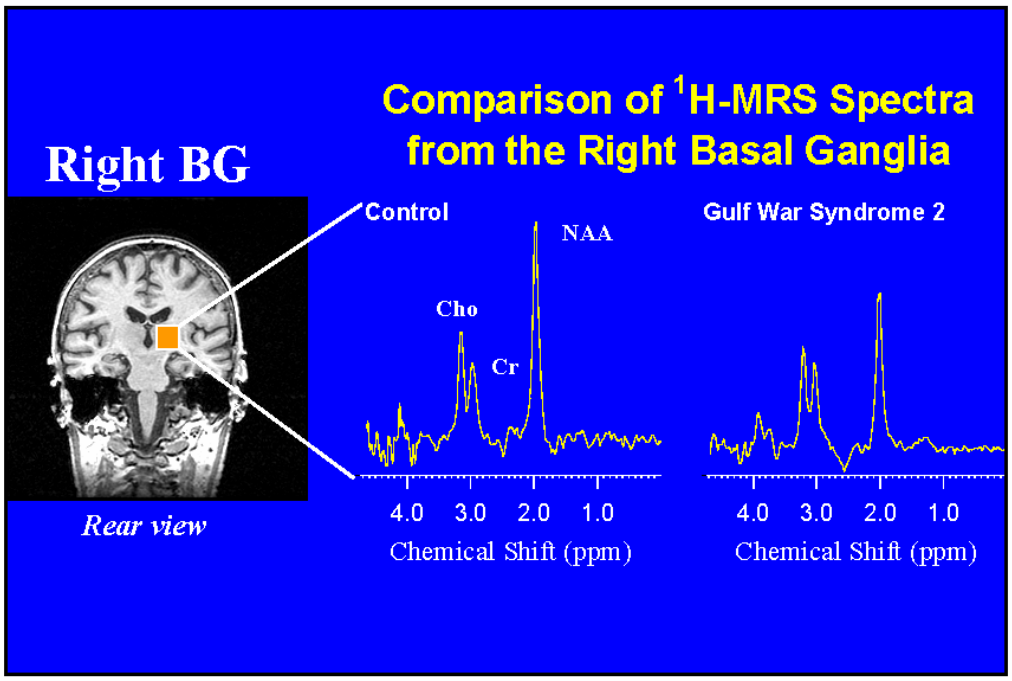
January-June 1998

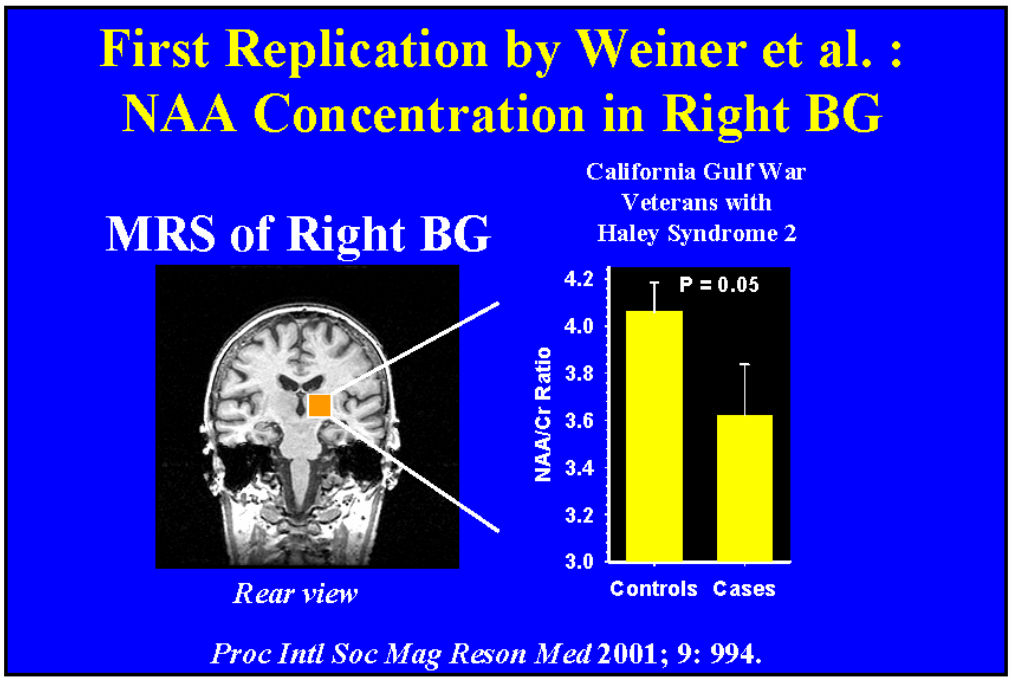
Second Clinical Case-Control Study with More Advanced Tests of Brain Function and Genetic Predisposition

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


May 29 - June 5 -- Veteran A
Revised 6/1/98
GCRC Protocol for Cases and Controls
Low tyrosine diet
Brain MR spectroscopy scans
Autonomic evaluation
Neurophysiologic tests
Quantitative sensory tests
Psychiatric/neuropsychological evaluation
Sleep studies over 4 nights
Blood tests for dopamine metabolites
Brain SPECT scans with cholinergic challenge
Etc.

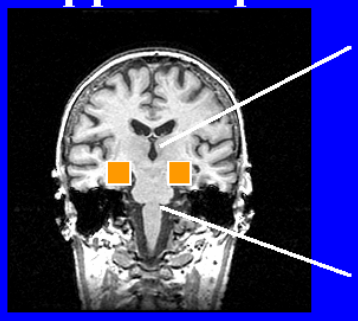

Lower PON1 Type Q Allozyme Levels in Blood of Ill Gulf War Veterans than Controls
PON-Q Enzyme Activity (U/ml)
p = 0.009
Con
Syn 1
Syn 2
Syn 3
Patient Group
Mean Type Q arylesterase activity (U/mL)
Controls
Syn 1
Syn 2
Syn 3
Toxicol Appl Pharmacol 1999; 157: 227-233


Comparison of ^{1}H-MRS Spectra from the Right Basal Ganglia
Right BG
Rear view
Control
NAA
Cho
Cr
4.0 3.0 2.0 1.0
Chemical Shift (ppm)
Gulf War Syndrome 2
4.0 3.0 2.0 1.0
Chemical Shift (ppm)


First Replication by Weiner et al. : NAA Concentration in Right BG
MRS of Right BG
Rear view
California Gulf War Veterans with Haley Syndrome 2
NAA/Cr Ratio
4.2
4.0
3.8
3.6
3.4
3.2
3.0
P = 0.05
Controls
Cases
Proc Intl Soc Mag Reson Med 2001; 9: 994.

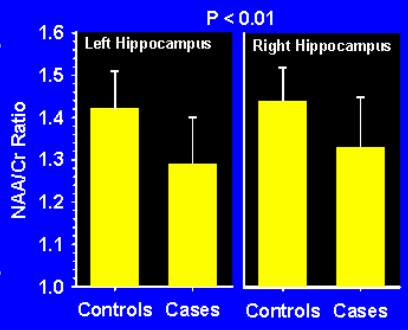
Second Replication by Menon et al. : NAA Concentration in the Hippocampus

Left and Right Hippocampus



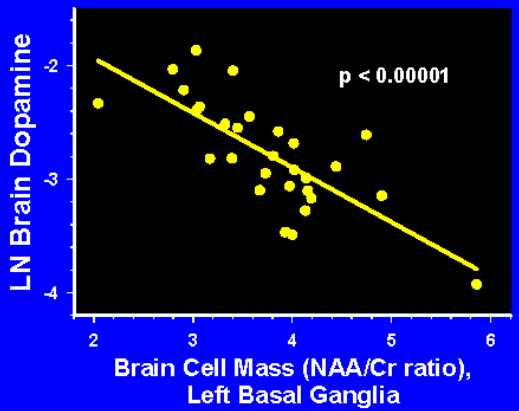
Rear view

Mississippi Gulf War Veterans with Gulf War Illness

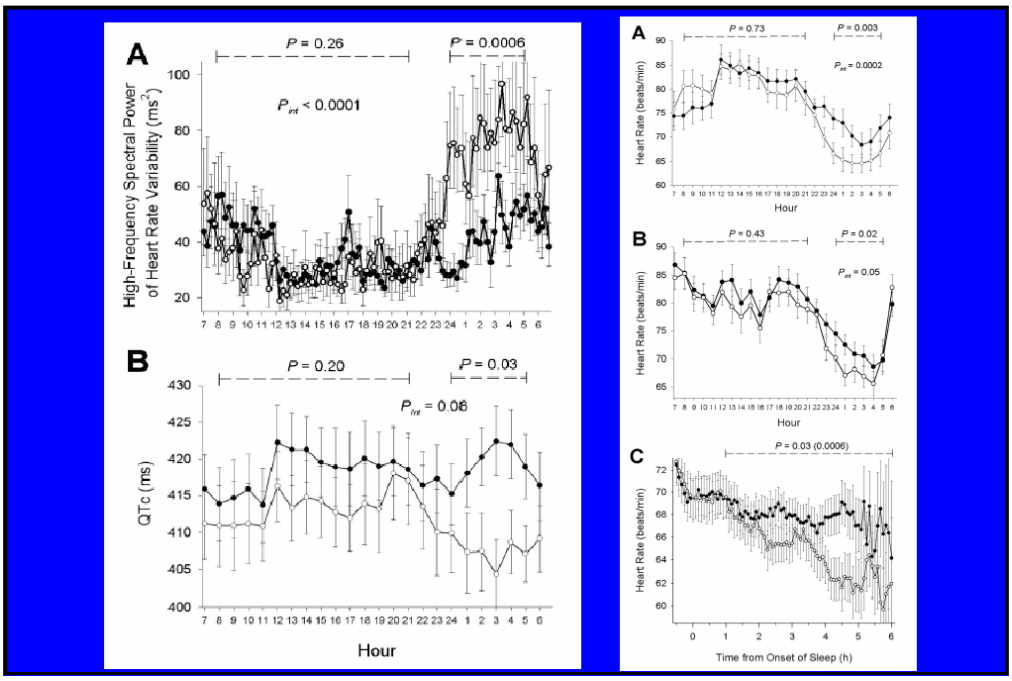


Brain Research 2004; 1009: 189-194.

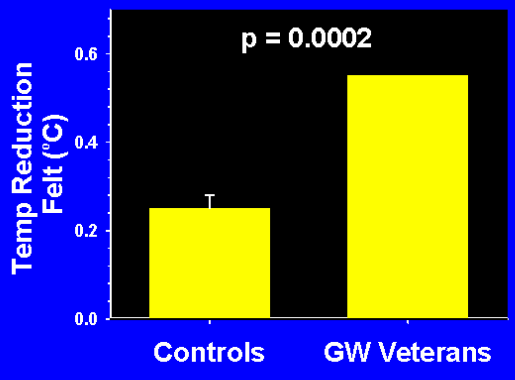
Increased Brain Dopamine Production with Brain Cell Damage



Archives of Neurology 2000; 57: 1280-1285



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

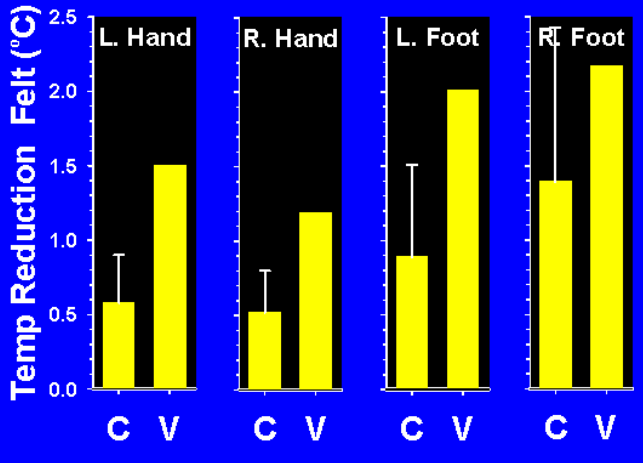


J Neurol, Neurosurg, & Psychiatr 1996; 60: 499-451

Quantitative Sensory Testing: Cooling Threshold

Impaired Cooling Perception Threshold*

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



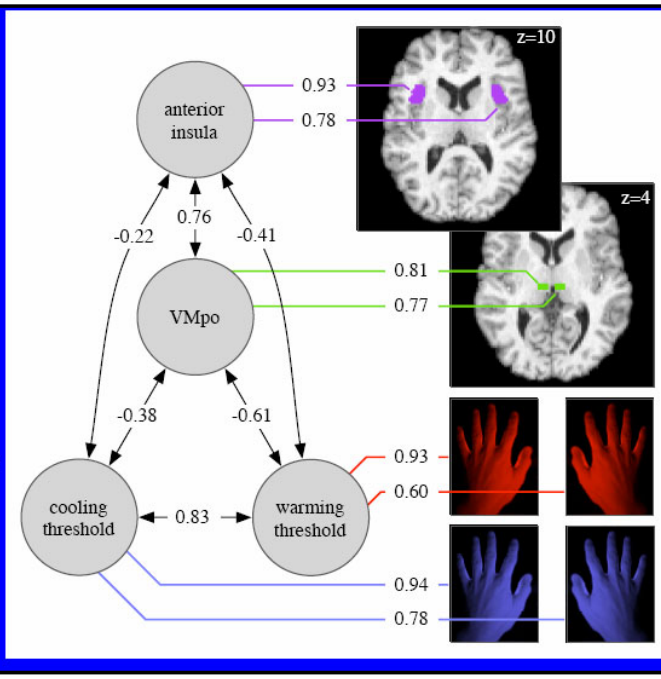
Global Test:
P < 0.0001

C = Control
V = GW Veteran

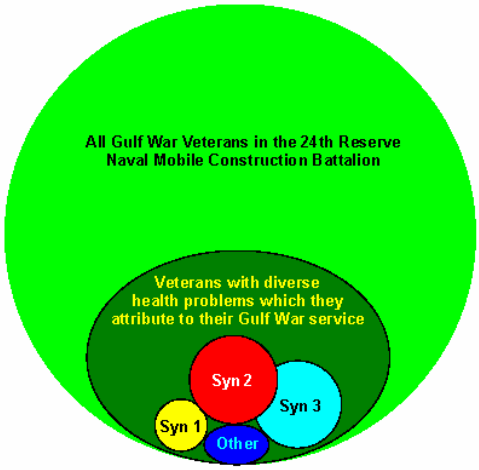
*Similar findings for warming threshold but not vibratory

SEM of Temperature Sensory System Dysfunction

- Goodness of fit indices show extremely good fit.
- T2 rCBF in other brain regions produce poor fitting SEMs.



Clinical Subgroups of Gulf War Veterans



October 2002
Alteration of Cholinergic Receptors in Rats by Low-Level Sarin

Rogene Henderson
Senior Scientist
Lovelace Respiratory Research Institute
Albuquerque, NM

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

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

Response of Rats to Low Levels of Sarin

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

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

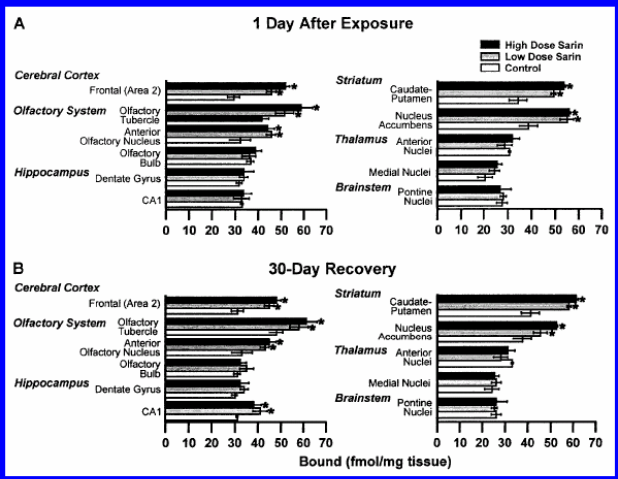
Toxicology and Applied Pharmacology **184,** 82–87 (2002)
doi:10.1006/taap.2002.9497

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

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

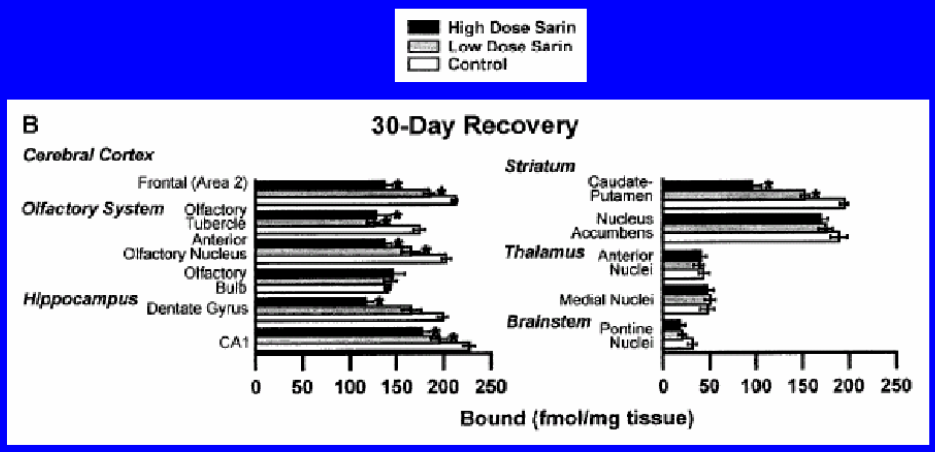
Lovelace Respiratory Research Institute, Albuquerque, New Mexico 87108

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



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

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

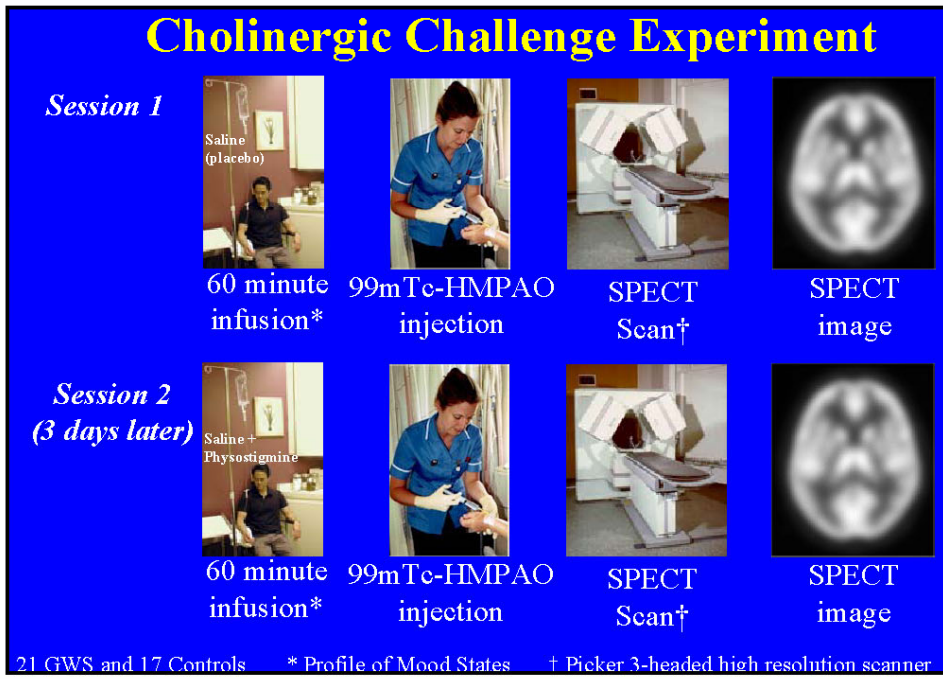


Henderson et al. ***Toxicology Applied Pharmacology*** 2002;184:67-87

If soldiers suffered brain cell damage from sarin nerve gas (a cholinergic stimulant), we might expect to see an abnormal brain cell response to an experimental cholinergic challenge.

- So we performed an experiment to see how the cholinergic stimulant *physostigmine* would affect regional cerebral bloodflow (rCBF) measured by 99mTc-HMPAO-SPECT scans.
- The same 21 cases (5, 11, 5) and 17 controls

Cholinergic Challenge Experiment



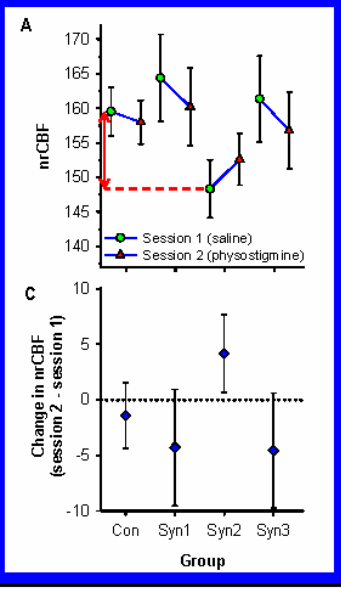
21 GWS and 17 Controls * Profile of Mood States † Picker 3-headed high resolution scanner

The Statistical Development Team

Wayne Woodward Bill Schucany Dick Gunst Robert Haley

Pat Carmack Jeff Spence

Global Hypothesis Tests



% diff. =
-11.21
159.60

7.0%

Model of baseline nrCBF (session 1)

Source of variation in Session 1 rCBF	Numerator df	Denominator df	F	Pr > F
Group	3	34	4.77	0.0070
Structure	15	510	284.00	<0.0001
Group X Structure	45	510	1.31	0.0904

Contrast	Mean Difference	Std Err	df	Pr > |t|
Syn1 vs Con	4.87	4.86	34	0.3234
Syn2 vs Con	-11.21	3.70	34	0.0046
Syn3 vs Con	1.82	4.86	34	0.7104
Syn2 vs Syn1	-16.09	5.15	34	0.0037
Syn2 vs Syn3	13.03	5.15	34	0.0163
Syn1 vs Syn3	-3.05	6.04	34	0.6170

Model of change in nrCBF (session 2 - session 1)

Source of variation in change of rCBF	Numerator df	Denomiator df	F	Pr > F
Group	3	34	2.13	0.1145
Structure	15	510	2.48	0.0016
Group X Structure	45	510	1.67	0.0052

Blocks in which Significant Difference in Baseline rCBF: Syn 2 < Controls

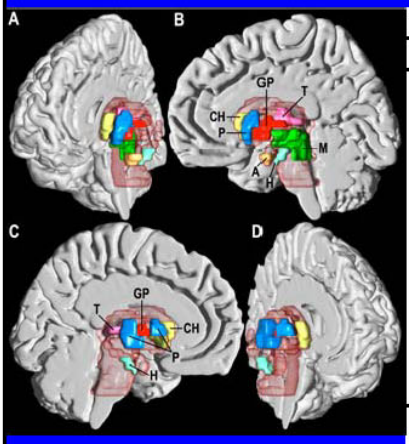
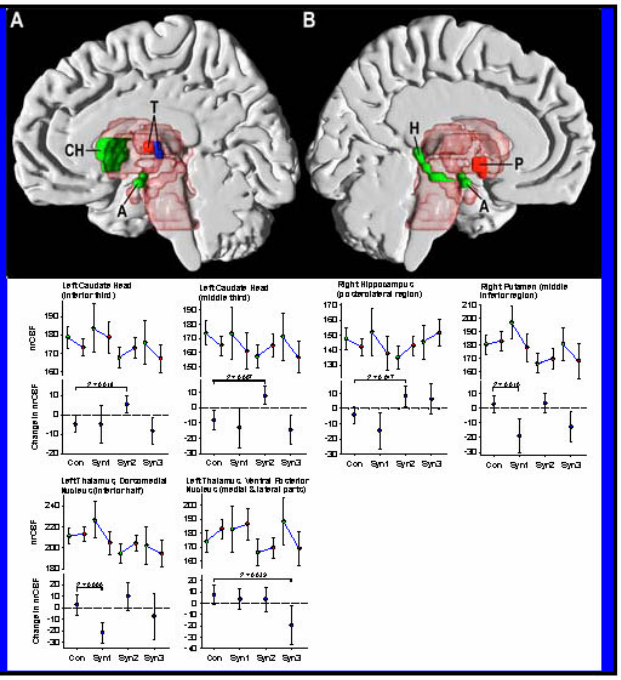


Table S3. Deep brain blocks in which baseline nrCBF was significantly lower in syndrome 2 than controls

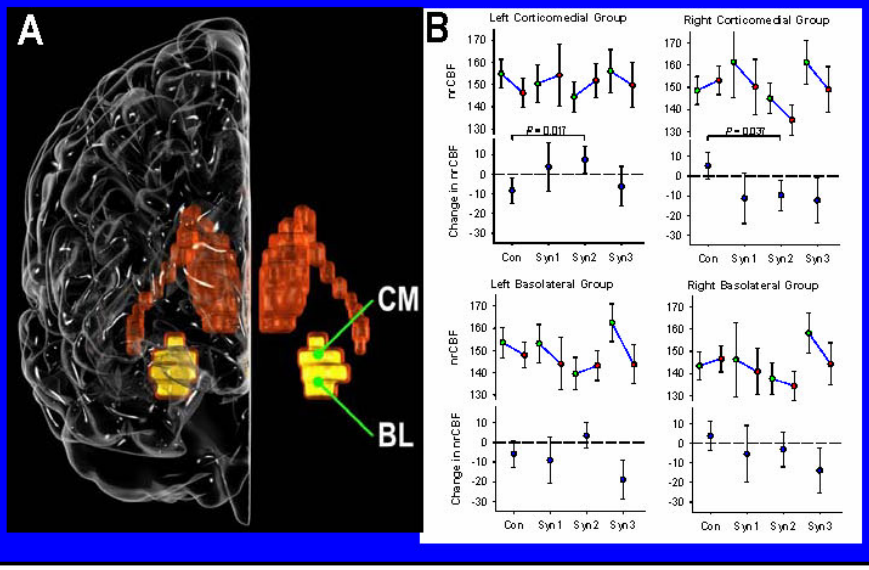
			Left hemisphere		Right hemisphere	
Block	Structure	Location in Structure	% diff.	*P*	% diff.	*P*
1	caudate	Inferior third of caudate head	6.2	0.0469	6.9	0.0356
2	caudate	Middle third of caudate head	9.5	0.0348	7.3	0.0247
1	globus pallidus	Anterior inferior	9.0	0.0214		
2	globus pallidus	Posterior inferior	8.5	0.0306		
3	globus pallidus	Superior	9.0	0.0067	8.9	0.0123
1	midbrain	Posterior inferior	8.9	0.0255		
3	midbrain	Posterior superior	9.8	0.0227		
4	midbrain	Anterior superior	10.3	0.0354		
1	putamen	Anterior inferior	7.8	0.0212		
2	putamen	Anterior superior			6.5	0.0475
3	putamen	Middle inferior			7.8	0.0460
5	putamen	Posterior inferior			9.6	0.0095
6	putamen	Posterior superior			9.8	0.0039
3	thalamus	Dorsomedial nucleus (inferior segment)	7.8	0.0474		
4	thalamus	Ventromedial posterior nucleus	9.3	0.0088		
5	thalamus	Dorsomedial nucleus (superior)	10.6	0.0105		
6	thalamus	Pulvinar (inferior medial)	10.4	0.0105		
7	thalamus	Pulvinar (inferior lateral)			10.1	0.0259
12	thalamus	Ventral posterior nucleus (medial & lateral)			8.4	0.0199

P values are corrected for multiple comparisons by the Dunnett method. The percentage difference (% diff.) was defined as nrCBF of the control group minus nrCBF in the syndrome 2 group divided by nrCBF in the control group. The percentage difference from the control group was 7.0% over all 78 deep brain blocks.

Blocks with Significant Physostigmine Effect on nrCBF



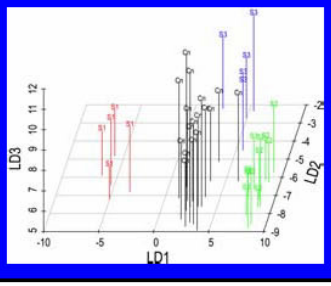
Physostigmine Effects in the Amygdala



Discriminant Model Predicting the 4 Clinical Groups from nrCBF of 17 Blocks

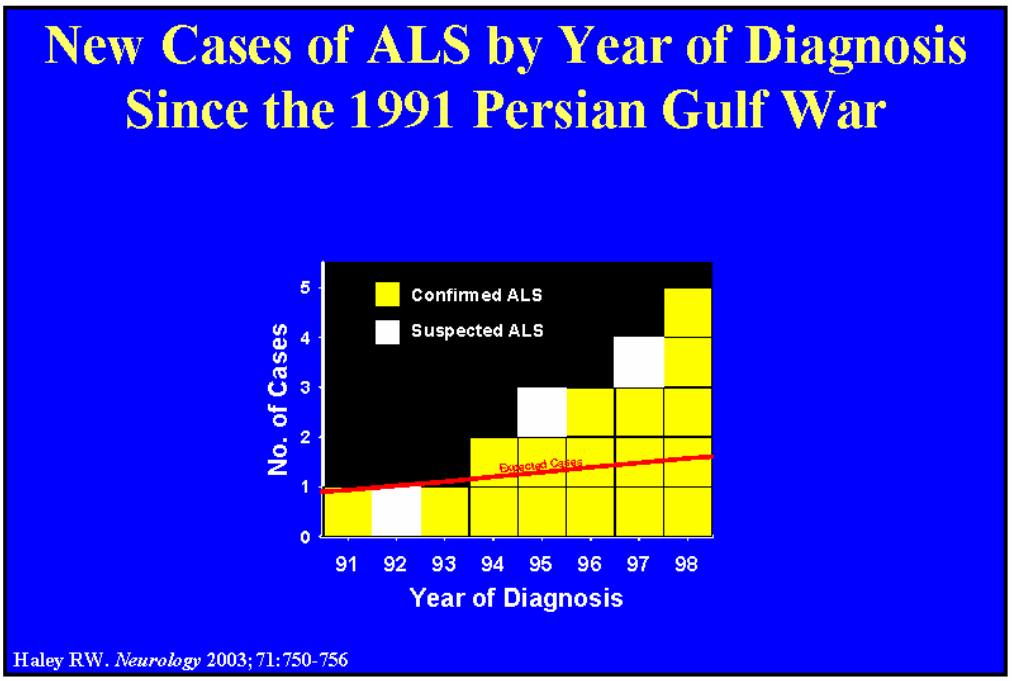
Hemisphere	Deep brain Structure	Approximate block location	SPECT Session	Discriminant loadings LD1	LD2	LD3
Right	Hippocampus	Posterolateral (subventricular)	Stimulated	0.203	0.017	-0.024
Right	Putamen	Anterior inferior segment	Stimulated	0.188	-0.099	0.008
Right	Caudate	Caudate head (middle third)	Baseline	0.179	0.105	0.052
Left	Amygdala	Corticomedial nuclear group	Stimulated	0.158	-0.041	-0.050
Right	Thalamus	VMPO and VP nuclei	Baseline	0.093	-0.173	0.022
Left	Amygdala	Corticomedial nuclear group	Baseline	0.079	0.037	0.042
Left	Thalamus	Pulvinar (inferior segment)	Baseline	0.059	-0.216	0.029
Right	Caudate	Caudate head (middle third)	Stimulated	0.040	-0.025	0.021
Right	Thalamus	LPN and DMN (sup. post.)	Baseline	0.026	0.141	-0.019
Left	Thalamus	AN, DMN (sup. ant.), VLN (sup.)	Stimulated	-0.023	-0.047	0.001
Right	Putamen	Posterior inferior segment	Stimulated	-0.031	0.052	-0.011
Right	Thalamus	Pulvinar (inferior segment)	Baseline	-0.061	0.183	-0.025
Right	Thalamus	Pulvinar (inferior segment)	Stimulated	-0.109	0.069	-0.022
Left	Thalamus	Pulvinar (inferior segment)	Stimulated	-0.143	-0.018	0.020
Right	Putamen	Anterior superior segment	Baseline	-0.161	-0.054	-0.027
Right	Amygdala	Corticomedial nuclear group	Stimulated	-0.201	0.081	0.053
Right	Putamen	Anterior inferior segment	Baseline	-0.221	-0.012	-0.019

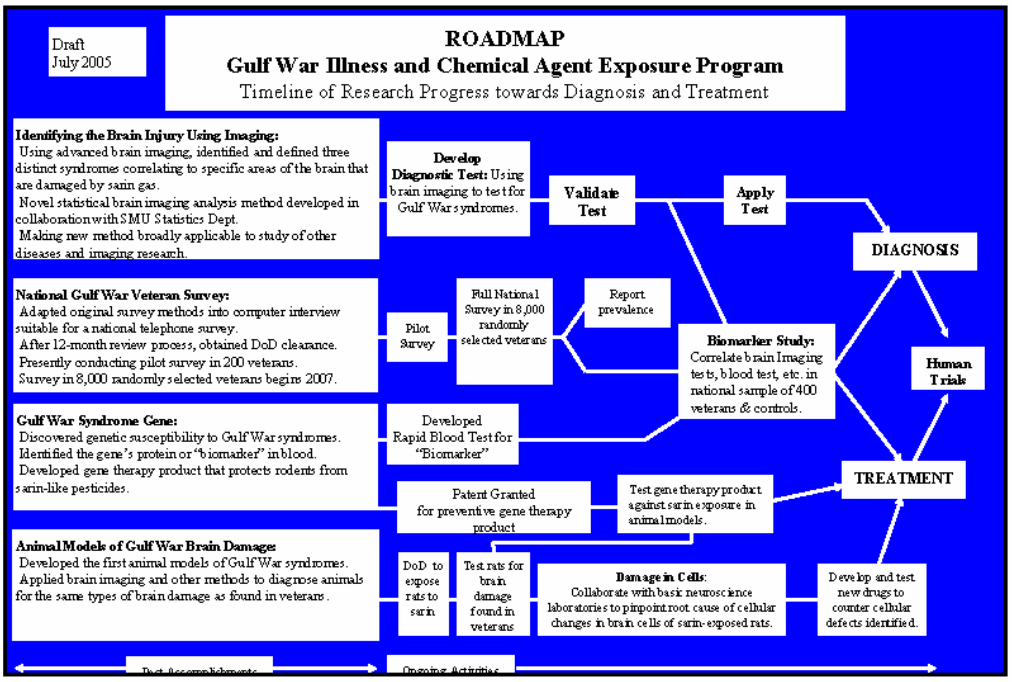
Holdout classification



Discriminant classification	Original clinical classification S1	S2	S3	Control
S1	5 (100)			
S2		11 (100)	1 (20)	1 (6)
S3			4 (80)	2 (12)
Control				14 (82)

Sensitivity = 0.95
Specificity = 0.82


New Cases of ALS by Year of Diagnosis
Since the 1991 Persian Gulf War
Confirmed ALS
Suspected ALS
Expected Cases
No. of Cases
0
1
2
3
4
5
91
92
93
94
95
96
97
98
Year of Diagnosis
Haley RW. Neurology 2003; 71:750-756


Draft
July 2005
ROADMAP
Gulf War Illness and Chemical Agent Exposure Program
Timeline of Research Progress towards Diagnosis and Treatment
Identifying the Brain Injury Using Imaging:
Using advanced brain imaging, identified and defined three distinct syndromes correlating to specific areas of the brain that are damaged by sarin gas.
Novel statistical brain imaging analysis method developed in collaboration with SMU Statistics Dept.
Making new method broadly applicable to study of other diseases and imaging research.
Develop Diagnostic Test: Using brain imaging to test for Gulf War syndromes.
Validate Test
Apply Test
DIAGNOSIS
National Gulf War Veteran Survey:
Adapted original survey methods into computer interview suitable for a national telephone survey.
After 12-month review process, obtained DoD clearance.
Presently conducting pilot survey in 200 veterans.
Survey in 8,000 randomly selected veterans begins 2007.
Pilot Survey
Full National Survey in 8,000 randomly selected veterans
Report prevalence
Biomarker Study:
Correlate brain Imaging tests, blood test, etc. in national sample of 400 veterans & controls.
Human Trials
Gulf War Syndrome Gene:
Discovered genetic susceptibility to Gulf War syndromes.
Identified the gene's protein or "biomarker" in blood.
Developed gene therapy product that protects rodents from sarin-like pesticides.
Developed Rapid Blood Test for "Biomarker"
Patent Granted for preventive gene therapy product
Test gene therapy product against sarin exposure in animal models.
TREATMENT
Animal Models of Gulf War Brain Damage:
Developed the first animal models of Gulf War syndromes.
Applied brain imaging and other methods to diagnose animals for the same types of brain damage as found in veterans.
DoD to expose rats to sarin
Test rats for brain damage found in veterans
Damage in Cells:
Collaborate with basic neuroscience laboratories to pinpoint root cause of cellular changes in brain cells of sarin-exposed rats.
Develop and test new drugs to counter cellular defects identified.
Past Accomplishments
Ongoing Activities