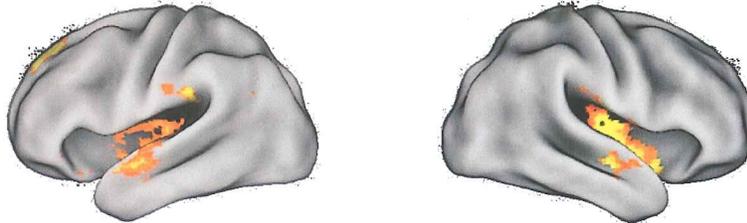


Neuroimaging biomarkers in Gulf War Illness



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Introduction

Approximately 25-30% of the 700,000 U.S veterans who served in the 1st Gulf War have been affected by Gulf War Illness*.

Symptoms include:

- Fatigue
- Joint and muscle pain
- Headaches
- Sleep disturbance
- Gastrointestinal complaints
- Loss of concentration
- Short-term memory loss
- Respiratory difficulties

*RAC-GWVI Gulf War Illness and the Health of Gulf War Veterans, 2008.



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Potential Exposures

Table 4B. SIGNIFICANT multivariable association of deployment characteristics with GWI, by location in theater. Veteran-reported experiences and exposures. Adjusted OR (95% CI)*

	All veterans ^A	Veterans in Iraq and/or Kuwait ^B	Veterans not in Iraq or Kuwait ^C
	144 GWI cases, 160 controls	101 GWI cases, 76 controls	43 GWI cases, 84 controls
SCUD missile exploded within 1 mile	2.18 (1.27, 3.72)*	3.07 (1.53, 6.19)*	1.08 (0.46, 2.54)
Wore uniforms treated with pesticides	2.91 (1.41, 6.01)*	1.35 (0.55, 3.35)	12.74 (2.64, 61.5)*
Used pesticides on skin	1.65 (0.92, 2.95)	2.07 (1.06, 4.05)*	1.62 (0.63, 4.17)
Frequently <4 hr sleep in 24 hr period	2.19 (1.29, 3.73)*	1.99 (0.93, 4.25)	1.56 (0.66, 3.66)
Smoke from oil well fires	1.33 (0.69, 2.55)	2.78 (1.01, 7.66)*	1.36 (0.60, 3.09)
Took Pyridostigmine bromide pills	2.88 (1.68, 4.94)*	3.50 (1.65, 7.41)*	1.44 (0.59, 3.47)

A Adjusted for being within 1 mile of exploding SCUD, wearing uniforms treated with pesticides, taking PB pills, and frequently having < 4 hr sleep in 24 hr.

B Adjusted for being within 1 mile of exploding SCUD missile, using pesticides on skin, and taking PB pills.

C Adjusted for wearing uniforms treated with pesticides. *p < 0.05.

Steele L, Sastre A, Gerkovich MM, Cook MR. *Environ Health Perspect.* 120(1):112-118.



Can Neuroimaging provide insight into symptoms suffered by veterans with GWI?

- Recent research in chronic low back pain, fibromyalgia (FM), and GWI show the potential for functional magnetic resonance imaging (fMRI).
 - Functional Connectivity
 - Connections between "pain brain" regions predict transition from acute to chronic low back pain (Baliki MN, 2012).
 - Voxel Based Morphometry (VBM)
 - Alterations in grey matter density associated with FM (Kuchinad A, 2007).
 - Association between white matter loss & higher levels of sarin/cyclosarin exposure in GW veterans (Heaton KJ, 2007).
 - Blood oxygen level dependent (BOLD) imaging
 - Abnormal central pain processing and cognitive impairment during memory task in GW veterans (Gopinath K, 2012; Odegard TN, 2012).
 - Diffusion Tensor Imaging (DTI)
 - White matter tract abnormalities in Irritable Bowel Syndrome (Chen JY, 2011)



Inducible symptoms using exercise

Recent studies have used acute exercise stressors as a useful model to exacerbate baseline symptoms.

- Increase in pain sensitivity in GW veterans (Cook DB, 2010).
- Increase in ratings of perceived exertion in GW veterans (Cook DB, 2003).
- Modulation of pain thresholds in FM (Cosek E, 1996).
- Altered gene expression in CFS and GW veterans (Light AR, 2009; Broderick G, 2013).

Are exercise induced symptoms related to central nervous System dysfunction?



The exercise in GWI protocol

Hypothesis: Exercise would exacerbate symptoms & reveal dysfunctional mechanisms in the brain.



Screen→ (baseline status)	fMRI with cognitive test	Day 1 Exercise → Day 2 Exercise ↳ Exercise-induced fatigue ↳	fMRI with cognitive test	Follow-up of fatigue & activity
↳ Exercise-induced cognitive and autonomic dysfunction ↳				

Protocol Highlights

- Subjects completed a 2-back working memory test before & after exercise.
- Widespread pain was experimentally measured using thumb pressure and dolorimetry.
 - Supine and standing heart rate and blood pressure were measured.



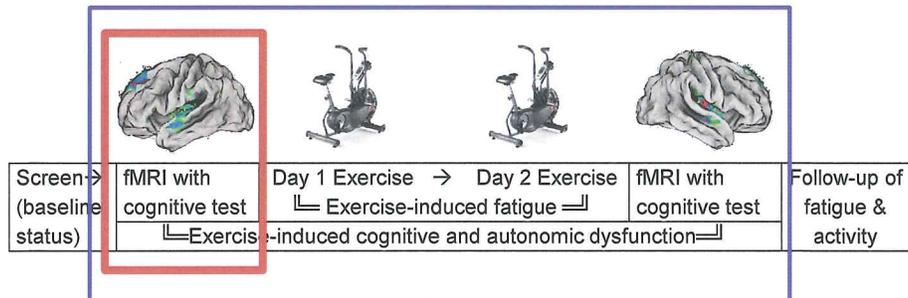
The 2-back working memory test

Viewed	A	B	D	C	C	B	A	D	D	A
Reported	-	-	A	B	D	C	C	B	A	D

- The 2-Back task is a working memory paradigm that requires continual encoding and retrieval of information.
- Stimuli are a series of randomly presented single letters (A, B, C, or D).
- The task was presented using a block design that alternated between 0-Back and 2-Back.
- the 2-Back required pressing the button corresponding to the letter presented 2 letters earlier.



View the protocol in two different ways



1. **Cross-sectional changes:** Identify differences at baseline (red box).
2. **Causal changes:** Compare before and after exercise (purple box).



1st study: “Static” Differences at Baseline

Clinical impression:

Pain and fatigue complaints fluctuated in parallel.

Hypothesis:

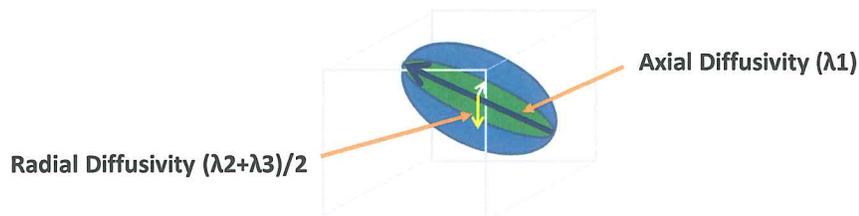
Alterations in white matter may underlie symptoms of pain and fatigue in GW veterans.

Methods:

- 1) Diffusion Tensor Imaging (DTI).
- 2) Measure of baseline hyperalgesia: Strain gauge dolorimeter at the 18 traditional FM tender points.
- 3) Subjective assessments of fatigue and pain.



Diffusion Tensor Imaging (DTI)



DTI: A MRI method that maps the diffusion of water in biological tissues in 3 different directions also known as eigenvectors

Main eigenvectors

- Axial diffusivity (AD): The parallel vector (black arrow) indicates axonal function.
- Radial diffusivity (RD): Two perpendicular vectors (white and yellow arrows) indicate primarily myelin integrity.

Average of All three vectors:

- Fractional Anisotropy (FA): Sensitive to microstructural alterations.
- Mean Diffusivity (MD): Sensitive to necrosis and swelling.



Demographics

Rayhan RU, Stevens BW, Timbol CR, Adewuyi O, et al. (2013) Increased Brain White Matter Axial Diffusivity Associated with Fatigue, Pain and Hyperalgesia in Gulf War Illness. PLoS ONE 8(3): e58493.

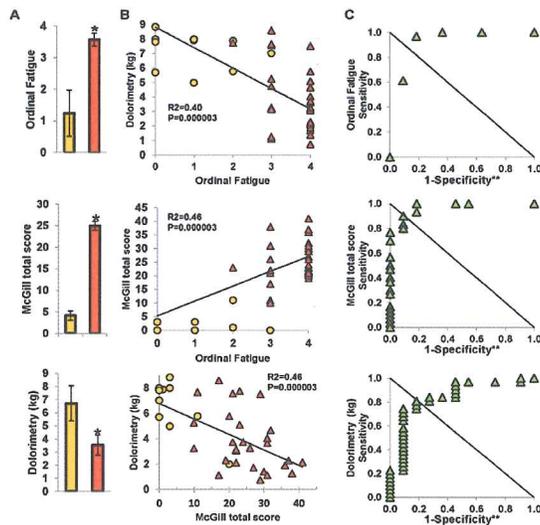
	Controls	CMI
Age	45.6 yr [41.2 to 50.5]	45.9 yr [43.2 to 48.4]
Gender		
Male	11	25
Female	9	6

doi:10.1371/journal.pone.0058493.t001

- We use the 1998 Chronic Multi-symptom Illness (CMI) and the 1994 Chronic Fatigue Syndrome (CFS) criteria (Fukuda K, 1994; Fukuda K, 1998) to assess potential in comorbidity.
- All veterans met the criteria for CMI and CFS.



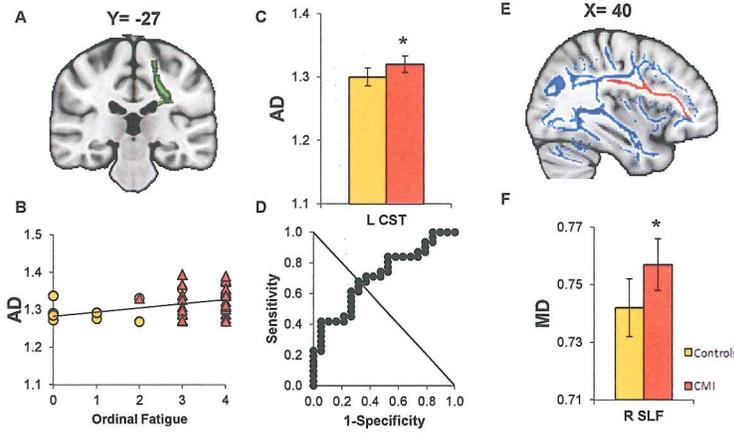
Correlation of Subjective Variables



Rayhan RU, Stevens BW, Timbol CR, Adewuyi O, et al. (2013) PLOS ONE 8(3): e58493.



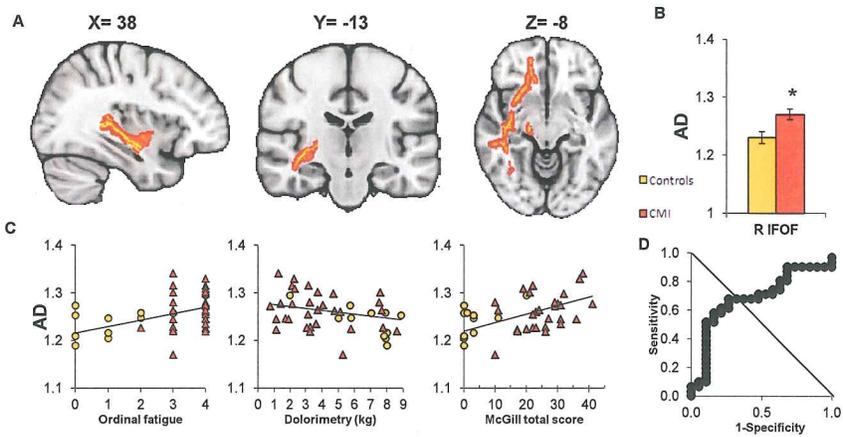
Increased Axial and Mean Diffusivity



A) Left Corticospinal Tract and increased AD
E) Right Superior Longitudinal Fasciculus and increased Mean Diffusivity (MD)



Increased AD in right Inferior Frontal Occipital Fasciculus

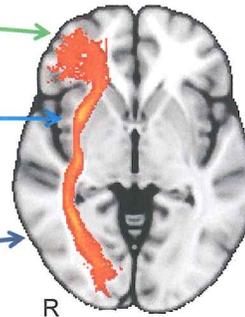


What does the right IFOF connect?

Ventromedial prefrontal cortex (vmPFC)
Orbitofrontal cortex (OFC)

Anterior Insula

Posterior fusiform, cuneus,
lateral cortices of the
occipital lobe.

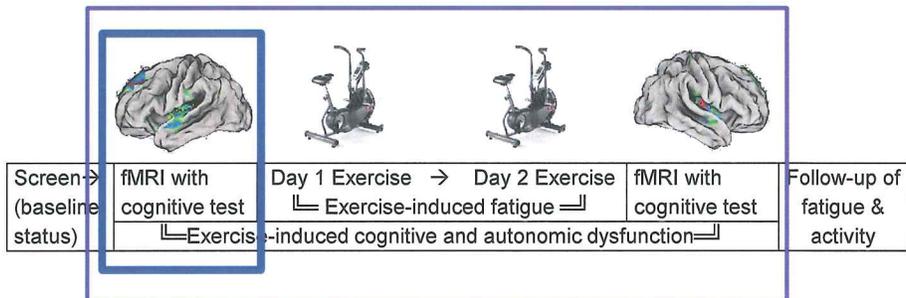


R

Representative transverse Slice of Brain



View the protocol in two different ways



1. **Cross-sectional changes:** Identify differences at baseline (red box).
2. **Causal changes:** Compare before and after exercise (purple box).



2nd study: "Dynamic" differences due to physiological stressors

Hypothesis:

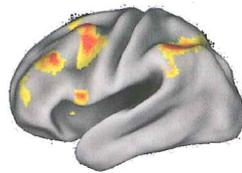
Stressing the system will reveal the underlying central nervous system regions responsible for post-exertional in GWI.

Methods:

- 1) Blood oxygen level dependent (BOLD) flow during 2-back cognitive testing.
- 2) Voxel Based Morphometry (VBM)
- 3) Supine and standing blood pressure, heart rate and pain measurements throughout the four day protocol.

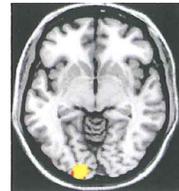


BOLD fMRI and VBM



BOLD fMRI

1. Neural activity increases in brain
2. Blood flow increases ("reactive hyperemia")
3. Oxygenated hemoglobin kicks out deoxygenated hemoglobin in areas.
4. Gradient occurs leading to the signal Intensity that indirectly represents neural activity in the brain.

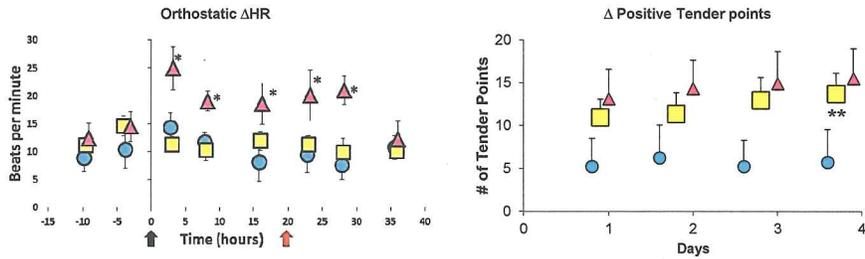


Voxel Based Morphometry (VBM)

1. Involves voxel wise comparison of gray or white matter between groups.
2. Spatially normalize all the subjects in the study into the same stereotaxic space.
3. Segment the grey matter, white matter and cerebrospinal fluid.
4. Study specific template was used to normalize images in standard MNI template.



Phenotype Identification



- **Stress Test Associated Reversible Tachycardia (START; Pink Triangles).**
- **Orthostatic tachycardia (Δ HR)** was defined as a change in heart rate of 30 bpm upon standing.
- Type 3 analysis of deviance and pairwise comparisons with Bonferroni corrections ($*P < 0.0001$).
- **Stress Test Originated Phantom Perception (STOPP; Yellow Squares).**
- Increase in Positive Tender Points following exercises. ($**P = 0.008$; Paired t-test, Bonferroni corrected).

Controls (cyan circles) did not exhibit any alterations in symptoms.



Demographics after subgrouping

	Controls	STOPP	START
<i>n</i> =	10	18	10
Males	80%	72%	90%
Age	48.9 [\pm 6.1]	45.8 [\pm 3.5]	44.4 [\pm 5.2]
BMI	29.5 [\pm 3.7]	31.5 [\pm 3.6]	28.5 [\pm 3.7]

Mean [\pm 95% CI]. No significant difference between subgroups with respect to exposures (Table S1)**.

**Rayhan RU, Stevens BW, Raksit MP, Ripple JA, Timbol CR, et al. (2013) Exercise Challenge in Gulf War Illness Reveals Two Subgroups with Altered Brain Structure and Function. PLOS ONE 8(6): e63903.



Principle Component Analysis of Fatigue

PCA loading per query	START		STOPP	
	Component 1 †	Component 2 ††	Component 1 ‡	Component 2 ††
Poor memory	0.921 †	0.038	0.246	0.657 ††
Difficulty concentrating	0.801 †	-0.557	0.608 ‡	0.491
Slips of the tongue	0.767 †	-0.195	0.151	0.843 ††
Finding correct word	0.564 †	0.208	0.008	0.921 ††
Feeling sleepy or drowsy	0.179	0.040	0.145	0.089
Lacking energy	-0.614	-0.266	0.722 ‡	0.057
Problems starting things	-0.589	0.943 ††	-0.410	0.505
Feel week	-0.128	0.835 ††	0.866 ‡	-0.234
Less strength	0.102	0.624 ††	0.812 ‡	0.044
Problems with tiredness	-0.445	0.573 ††	0.583 ‡	0.179
Resting more	-0.280	0.553 ††	0.815 ‡	-0.078
Signifiant Eigenvalues	3.779	2.761	4.116	2.159
Variance (%)	34.4%	25.1%	37.4%	19.6%
Cumulative Variance (%)	34.4%	59.5%	37.4%	57.1%
Monte Carlo Simulation Means	3.269970	2.452196	2.354251	1.844100
95th Percentile	**3.728901	**2.691814	**2.662237	**2.095816
Component correlation	0.137		0.178	

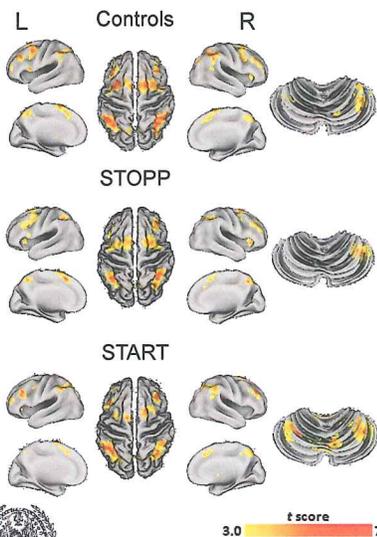
START load a primary Cognitive Construct whereas STOPP load a primary physical fatigue construct.



Chalder T, Berelowitz G, Pawlikowski T, et al. J Psychosom Res (1993)

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Pre-Exercise working memory (WM) BOLD



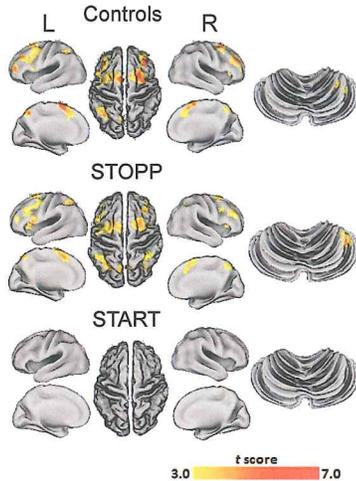
	Controls (N=10)				STOPP (N=18)				START (N=10)			
	Size	x	y	z	Size	x	y	z	Size	x	y	z
Left Cerebrum												
Superior frontal G.					1472	-6	18	48				
Middle frontal G.	631	-32	12	58								
Middle frontal G.	250	-42	30	34					238	-52	22	28
Inferior Parietal L.	613	-48	-48	48	540	-36	-52	42	462	-48	-50	46
Precuneus	122	-6	-60	48								
Anterior Insula					95	-32	20	2				
Caudate body					122	-16	14	6				
Right Cerebrum												
Superior frontal G.	937	20	8	70	291	38	44	30	147	24	16	62
Middle frontal G.	150	36	32	32	635	26	8	60	187	38	36	34
Medial frontal G.									113	4	28	40
Superior parietal L.									543	40	-60	50
Inferior parietal L.	1098	34	-46	44	926	40	-48	46				
Precuneus					386	8	-60	48				
Anterior Insula					188	-32	24	-2				
Left Cerebellum												
Pyramis									121	-20	-62	-30
Right Cerebellum												
Culmen									143	34	-60	-26
Tuber					252	34	-58	-30				

Using the AFNI program AlphaSim significant clusters were identified. Controls subjects had activation patterns consistent with normal working memory. START subjects activated WM regions and compensatory activity (yellow) in the bilateral cerebellar vermis. STOPP subjects activated working memory regions, the cerebellar tuber, bi-lateral anterior insula (red) and compensatory caudate body. ($P < 0.05$, AlphaSim).



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Post-Exercise working memory (WM) BOLD

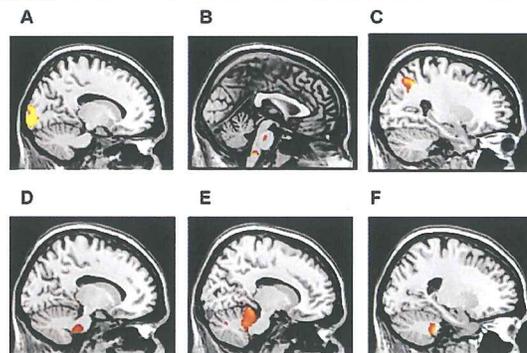


	Controls (N=10)				STOPP (N=18)				START (N=10)			
	Size	x	y	z	Size	x	y	z	Size	x	y	z
Left Cerebrum												
Superior frontal G.	2144	-6	8	64								
Middle frontal G.	419	-36	40	36								
Middle frontal G.	238	-38	46	10	131	-46	26	34				
Medial frontal G.					2185	-6	18	46				
Inferior frontal G.					367	-54	12	10				
Superior parietal					111	-8	66	54				
Inferior parietal L.	217	-44	-48	56								
Precuneus	122	-2	-62	48								
Right Cerebrum												
Superior frontal G	575	40	40	32								
Middle frontal G.					227	34	30	32				
Medial frontal G.												
Superior parietal L					139	10	-64	54				
Inferior parietal L												
Precuneus												
Right Cerebellum												
Tonsil					128	38	-54	-32				

Controls subjects had activation patterns suggesting exercise did not affect their performance. STOPP subjects shifted their activity to the ventromedial prefrontal cortex following exercise. ($P < 0.05$, AlphaSim). START subjects did not show any significant regions of activity.



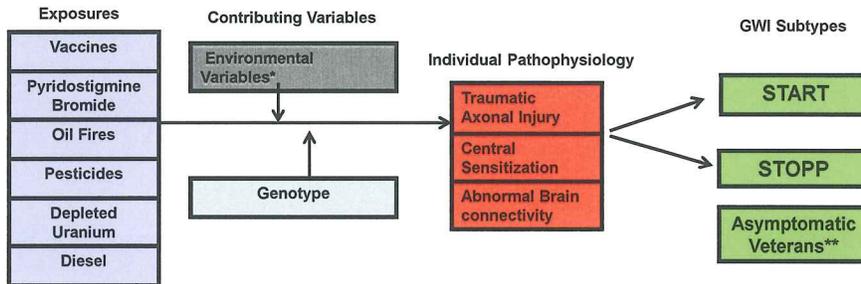
Phenotype brain volume differences



(A) START subjects (in contrast to controls) had less gray matter volume in the left lingual gyrus extending into the left cuneus ($P < 0.025$) and (B) right pons and right medulla ($P < 0.02$) (C) STOPP subjects (in contrast to controls) demonstrated a trend of less gray matter in the right superior parietal lobule extending into the right precuneus ($P < 0.07$). (D) START subjects had reduced white matter volume (in contrast to STOPP) in the left pons ($P < 0.004$) and (E) left cerebellar tonsil and left pyramis ($P < 0.012$) (F) Analyses also demonstrated START subjects (in contrast to STOPP) had decreased gray matter in the right culmen extending into the right fastigial and left dentate nucleus of the cerebellum ($P < 0.035$). All P values are corrected for age, gender and multiple comparisons using non-stationary cluster correction.



Summary and Future Implications



• GWI subtypes may be the end result of a variety of exposures with contributing factors inducing an individual/combinations of pathophysiological mechanisms leading to phenotypes.

• **Exercise can be used as a potential model to study the effects of physically perturbing the system.**

*Specific Occupation during duty may have caused differential exposure concentrations.

**Veterans who had no exposures, minimal exposures, or show no phenotypes of symptoms.

