Structural MRI and Cognitive Correlates in Pest-control Personnel from Gulf War I

Kimberly Sullivan, Ph.D.

Maxine Krengel, Ph.D.

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Collaborators and acknowledgements

- Maxine Krengel, Ph.D. - BUSM
- Ron Killiany, Ph.D. – BUSM
- Timothy Heeren, Ph.D. - BUSPH
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Introduction

Many Gulf War (GW) veterans have reported lasting health symptom complaints since their return from the war in 1991. Reported symptoms include:

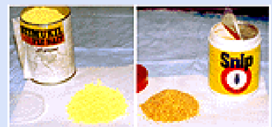
- Fatigue
- Memory disturbance
- Concentration difficulties
- Joint and muscle pain
- Sleep disturbance
- Headache
- Respiratory problems
- Gastrointestinal complaints

Introduction- Pesticides

- Acetylcholinesterase inhibitors such as organophosphate (OP) pesticides, anti-nerve gas pills (PB) and nerve agents are known to produce chronic neurological symptoms at sufficient exposures.
- Combinations of exposures to similarly acting pesticides and PB has been suggested as a likely cause of lasting health complaints in GW veterans and some military pest control applicator's exposures likely reached levels of concern for toxicity. Their exposures and unique knowledge of pesticides made them an ideal group to study.

How were pesticides used in Gulf War Theatre?

- Troops used pesticides for personal use on skin and uniforms and as:
  - Insect repellants
  - As area sprays and fogs
  - In pest strips and fly baits
  - As delousing agents for POWs



- Those who applied the pesticides were likely exposed to more pesticide products and at higher doses.
- They were also much more knowledgeable about pesticide types and usages therefore making them an ideally suited group to study.

How many pesticides were in Gulf War Theatre?

- Pesticides were used widely in the Gulf War to protect the troops from pests such as sand flies, mosquitoes and fleas that can carry infectious diseases.
- US forces used pesticides in areas where they worked, slept and ate. In fact, on any given day during their deployment GW veterans could have been exposed to at least 15 pesticide products of concern with 12 different active ingredients.
- A Health Risk Assessment conducted by DOD estimated that 41,000 GW veterans could have been overexposed to pesticides during the war.

Pesticides of Potential Concern

Repellents	Pyrethroids	Organophosphates	Carbamates	Organochlorines
DEET	Permethrin	Azamethiphos*	Methomyl	Lindane*
	D-Phenothrin	Chlorpyrifos*	Propoxur	
		Diazinon*	Bendiocarb*	
		Dichlorvos*		
		Malathion*		

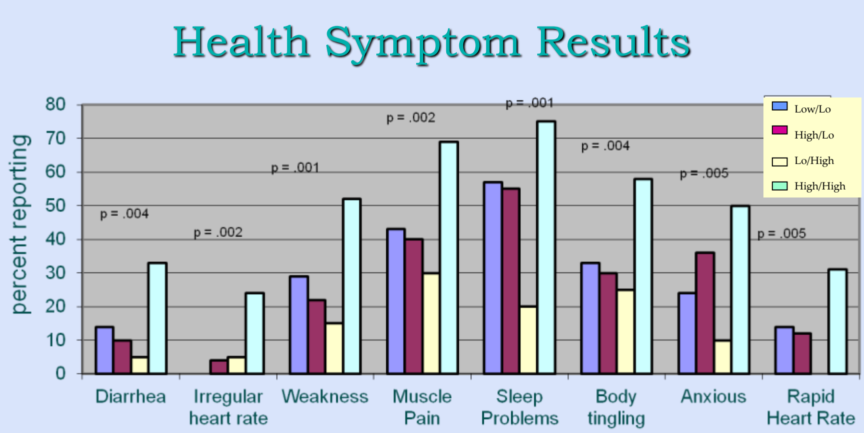
*Current use restricted or banned by EPA as part of the Food Quality Protection Act pesticides review.
Source: DOD Environmental Exposure Report - pesticides

Pesticide Use and Application Overview

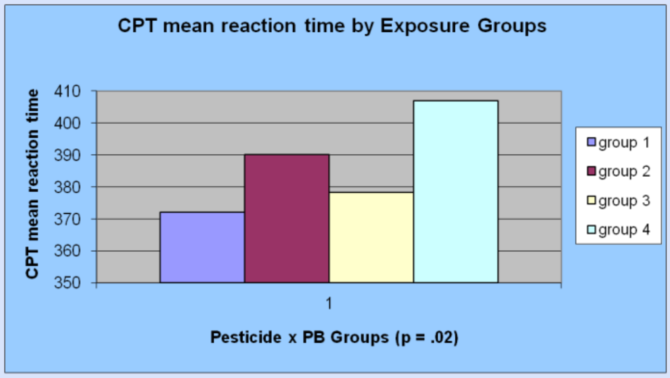
Use	Designation	Purpose	POPCs, Active Ingredient	Application Method	User or Applicator
General Use Pesticides	Repellents	Repel flies and mosquitoes	DEET 33% cream/stick	By hand to skin	Individuals
			DEET 75% Liquid	By hand to skin, uniforms or netting	
			Permethrin 0.5% (P) Spray	Sprayed on uniforms	
	Area Spray	Knock down spray, kill files and mosquitoes	d-Phenothrin 0.2% (P) Aerosol	Sprayed in area	
	Fly Baits	Attract and kill flies	Methomyl 1% (C) Crystals	Placed in pans outside of latrines, sleeping tents	Individuals, Field Sanitation Teams, Certified Applicators
			Azamethiphos 1% (OP) Crystals		
	Pest Strip	Attract and kill mosquitoes	Dichlorvos 20% (OP) Pest Strip	Hung in sleeping tents, working areas, dumpsters	
Field Use Pesticides	Sprayed Liquids (emulsifiable concentrates, ECs)	Kill flies, mosquitoes, crawling insects	Chlorpyrifos 45% (OP) Liquid	Sprayed in corners, cracks, crevices	Field Sanitation Teams or Certified Applicators
			Diazinon 48% (OP) Liquid	Sprayed in corners, cracks, crevices	Certified Applicators
			Malathion 57% (OP) Liquid		
			Propoxur 14.7% (C) Liquid		
	Sprayed Powder (wettable powder, WP)	Kill flies, mosquitoes, crawling insects	Bendiocarb 76% (C) Solid		
	Fogs (Ultra-Low Volume Fogs, ULVs)	Kill flies, mosquitoes	Chlorpyrifos 19% (OP) Liquid	Large area fogging	Certified Applicators
			Malathion 91% (OP) Liquid		
Delousing Pesticide	Delousing Pesticide	Kill lice	Lindane 1% (OC) Powder	Dusted on EPWs, also available for personal use	Certified Applicators, Military Police, Medical Personnel

Pesticide Cognition study

- In a prior study, the Pesticide Cognition Study (PCS), a group of 159 pesticide controllers from the GW were assessed for cognitive functioning.
- Those in the high pesticide and high anti-nerve gas (PB) group reported significantly more health symptoms and performed less well on cognitive functioning measures.



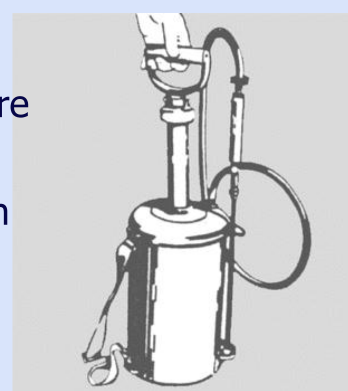
Continuous Performance Test by Pesticide Exposure Groups



Individual comparisons among the groups showed a significant difference between exposure Group 1 (low/low) and Group 4 (high/high) at p=.007.

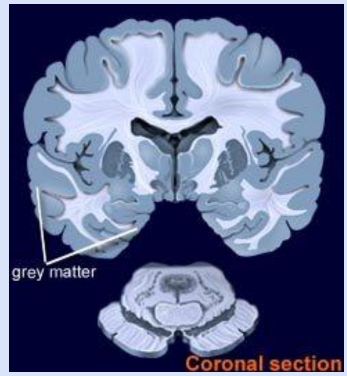
Pesticide Cognition Study

Individual pesticides including pest-strips, delousers, flybait were also found to be independently related to mood and information processing speed.



Pesticide MRI Study

The current study utilized structural MRI and neuropsychological testing to investigate brain-behavior patterns in pest-control personnel from the Gulf War.



These GW veterans had known high or low pesticide exposures based on their military occupational specialty. This sample included physicians, environmental science officers, entomologists, preventive medicine specialists, military police, field sanitation members and other pest controllers.

Hypothesis

- It was hypothesized that the pattern of neuropsychological function between the exposure groups would correlate with structural brain volumes and with reported health symptoms.
- Specifically, it was hypothesized that GW veterans with higher levels and more exposures to pesticides and low level nerve agents would show lower white matter volumes, report more health symptoms, and perform less well on cognitive testing.

Study Participants

- Study participants included a uniquely knowledgeable group of 24 GW veterans drawn from a larger group of 159 pest-control personnel who have been well characterized in terms of demographics and pesticide and PB exposure histories by a previous study.
- Subjects were 87% male with a mean age of 54 years and a mean education of 16 years.

Study Procedures

- Structural brain MRI
- Neuropsychological Assessment
- Health Symptom report

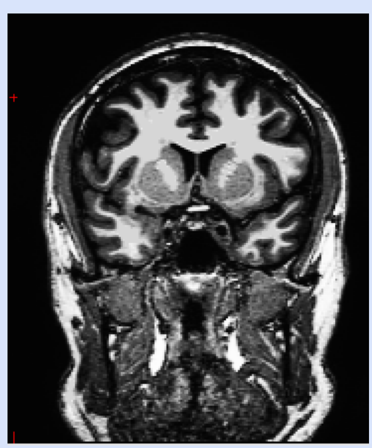
Structural MRI Methods

Neuroimaging

- Each imaging session acquired a MPRAGE sequence which is a T1 weighted image used as a standard for structural brain investigation.
- The MPRAGE acquisition had a FOV of 256 with a matrix of 256, 170 slices with a thickness of 1.2mm, and a TR of 3000ms for each subject.
- Each of the MPRAGE images were post processed using FreeSurfer software.
- Each brain was processed through an automated Talaraich based analysis, with skull removed, then checked for errors of grey and white matter borders, segmented, and statistically corrected for intracranial cavity volume.

MRI Post-Processing Methods

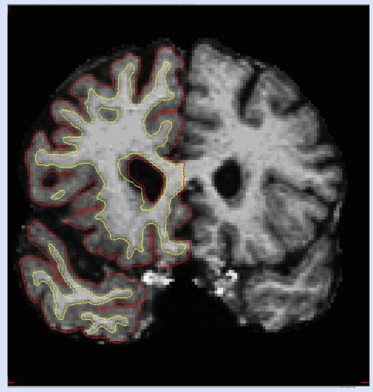
The first step in post-processing involved motion correction, intensity normalization and skull and neck removal so that only the brain remained for further analysis.



MRI Post-Processing Methods

The second step was determining white and gray matter borders using pixel intensity.

The white/gray matter border was then used to provide information for brain segmentation.



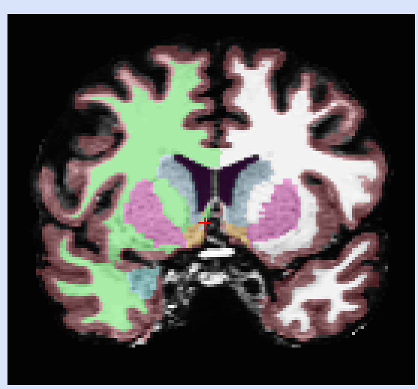
White Matter = yellow border
Gray Matter = red border

MRI Post-Processing Methods

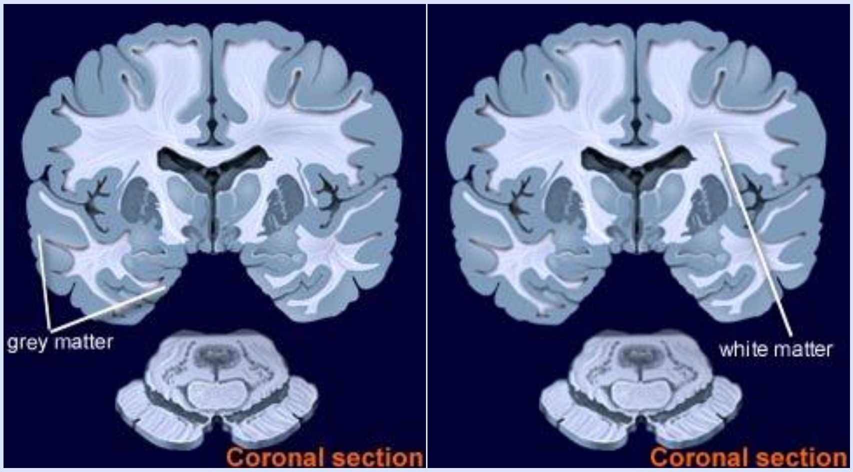
The third step included subcortical segmentation using the FREESURFER program.

This procedure divided the brain into 56 areas per hemisphere including the hippocampus, caudate nucleus and basal ganglia.

FREESURFER was also used to perform cortical parcellation.



Gray and White Matter

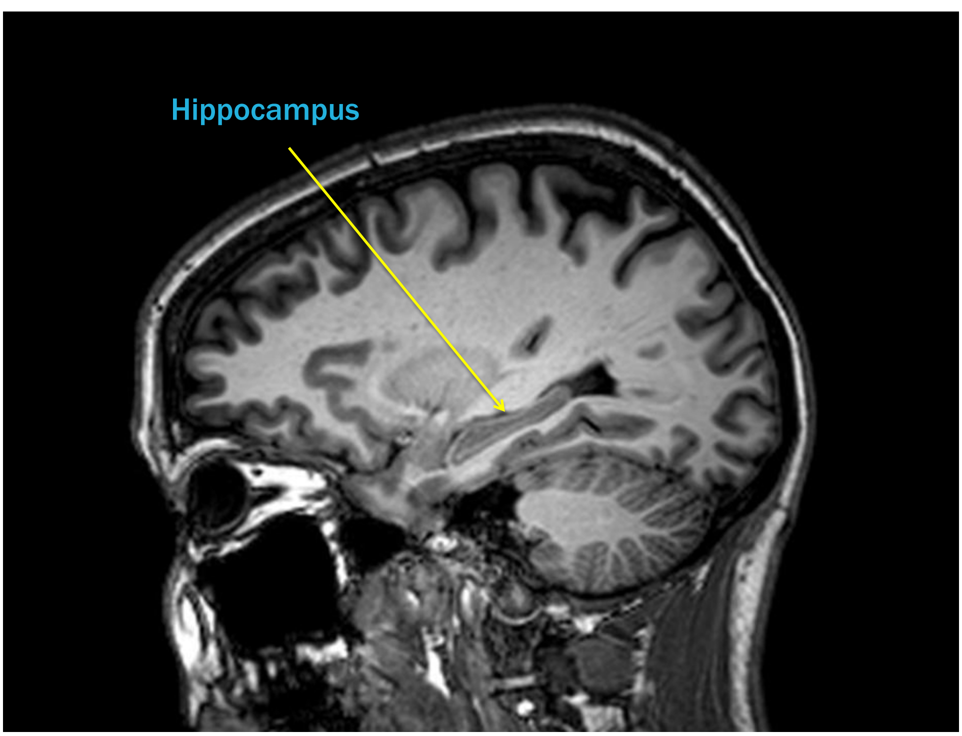


Why focus on the White Matter?

- White matter is highly susceptible to the effects of neurotoxicants.
- GWI symptoms include fatigue, information processing speed and memory retrieval difficulties that are associated with WM disorders.
- Lower white matter volumes were found in two other studies from our group of GW veterans related to exposure to low-level chemical weapons (sarin/cyclosarin) (Heaton et al., 2008) and to higher health symptom report (Powell, 2009; Sullivan, submitted).

Limbic System

- The limbic system is a circuit of highly interconnected midline structures in the brain.
- The major structures in the limbic system are the amygdala, basal forebrain, cingulate gyrus, fornix, hippocampus, mammillary bodies and septum.
- The main functions of the limbic system are to integrate the more primitive survivalistic functions of the brainstem with the higher order cognitive functions of the cerebral cortex.



Neuropsychological Test Methods

Battery of neuropsychological tests included cognitive domains of:

- **Attention/executive** – Continuous Performance Test (CPT), Trail Making Test, COWAT, multiple loops, recurrent series writing.
- **Memory** – Rey-Osterrieth Complex figure Test (ROCFT), California Verbal Learning Test
- **visuospatial** – Hooper Visual Organization Test, Grooved Pegboard, ROCFT copy
- **Motor** – Grip Strength, Finger Tap Test
- **mood** – Profile of Mood States

California Verbal Learning Test

List A Immediate Free-Recall Trials (number correct)	List B Trial	List A Delayed Recall	
	______	Short-Delay Free Recall	_____
Trial 1 ______		Long-Delay Free Recall	_____
Trial 2 ______		Short-Delay Cued Recall	_____
Trial 3 ______		Long-Delay Cued Recall	_____
Trial 4 ______		Long-Delay Recognition	_____
Trial 5 ______			

Grooved Pegboard

Data Analysis

- Multivariate analyses of Variance were performed to assess group differences between the high and low exposed groups with respect to brain volumetrics, cognitive test performance and health symptoms.
- Regression and correlation analyses were also performed with continuous variables.

Results – Subject Demographics

- Study participants were 87% male
(3 females)
- Mean age for study participants was 54 years.
- Mean education for study participants was 16 years.

Results – White Matter and Health Symptoms

Correlation of White Matter Volume with Health Symptoms in 24 Gulf War Veterans		
	Total white matter volume	**p value (2 tailed)**
Health symptoms	-.505*	0.012
*Pearson correlation coefficient		

Results: Brain Volumes and Combined Exposures (1)

Brain Volume	Unexposed group mean	Pest-strip x delouser exposed mean	Signif.
WM	34	33	.03
GM	33	27	.008
WM cerebellum	1.9	1.75	.03

Mean white matter, gray matter and cerebellar volumes adjusted for age and presented as percent of intracranial volume.

Results: Brain Volumes and Combined Exposures (2)

Brain Volume	DEET Mean	DEET Signif.	PB Mean	PB Signif.	DEET x PB Mean	DEET x PB Signif.
Right hippocampus	.30	Ns	.31	Ns	.25	.004
Left hippocampus	.30	Ns	.30	Ns	.25	.005
Total hippocampus	.60	ns	.61	Ns	.50	.004

Hippocampal volumes were adjusted for age and presented as percent of intracranial volume.

Results: Cognitive Domains and Combined Exposures (3)

Cognitive Outcome	PB Mean	DEET Mean	DEET x PB Mean	DEET x PB p-value
Verbal Memory	84	78	81	.92
Visual memory	49	44	35	.02
Rey-O immed. recall	24.3	23.8	17.7	.01
Rey-O Delay Recall	24.9	20.1	17.4	.04
Visuospatial domain	61.2	57.2	54.6	.03
Rey-O Copy	32.5	30.9	27.5	.04

Overall Results (1)

- Brain white matter volumes were significantly correlated with total health symptoms reported (p=.01).
- Brain white matter volumes were significantly correlated with attention/executive system domain (p=.001)

Overall Results (2)

- Cerebral and cerebellar white matter and gray matter volumes were significantly lower in veterans over-exposed to pest-strips (dichlorvos) and the delouser (lindane).
- Hippocampal volumes were significantly lower in veterans exposed to DEET and PB. This group also performed significantly worse on visual memory tests.

Structure-function Relationships?

- DEET x PB exposed = lower hippocampal volumes and worse visual memory performance.
- Higher number health symptoms = lower white matter volumes.
- Lower attention/executive system scores = Lower white matter volumes.

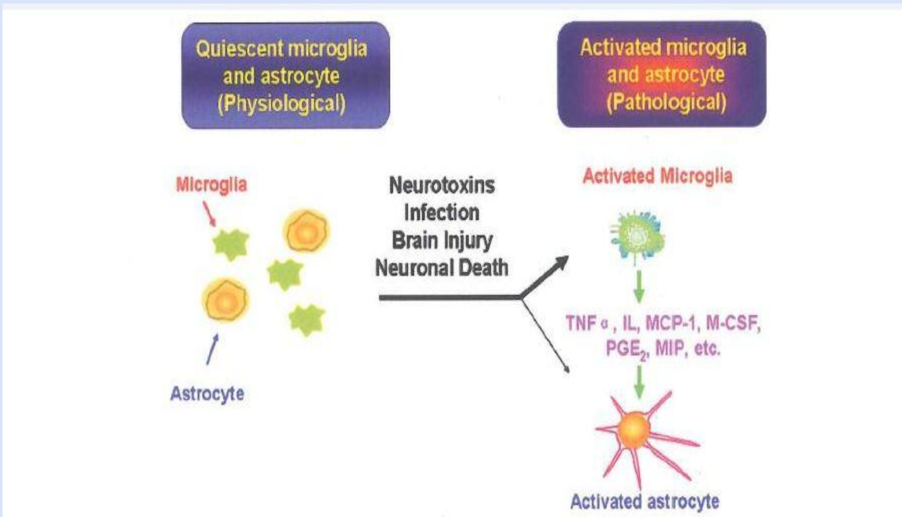
Conclusion

- Although this was a small pilot study and needs to be replicated in a larger study sample, brain-behavior relationships appeared present in this study that correlated with our prior studies (white matter and health symptoms) and with animal models of exposures (hippocampal volumes and DEET x PB interactions).
- These emerging brain-behavior relationships among brain imaging, neuropsychological functioning, health symptoms and environmental exposures suggest biomarkers may be present for GWI that can be targeted for future therapeutics.

Conclusion

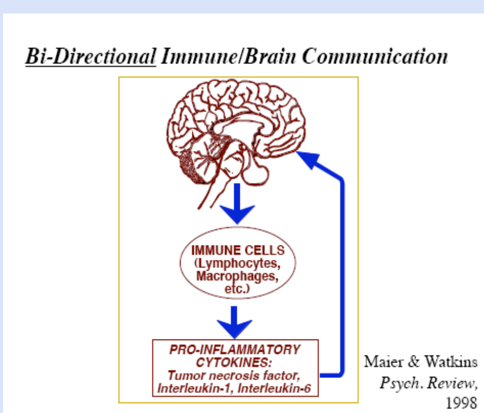
- GW veterans with known pesticide exposures and high numbers of health symptoms showed structural (MRI) differences in lower white matter volumes.
- Correspondingly, glial overactivation (including microglia and astrocytes) has recently been found to be associated with chronic pain syndromes suggesting a potential mechanism for increased health symptom report and altered white matter or glial functioning in exposed groups through chronic neuroinflammation.

Glial Activation and Priming



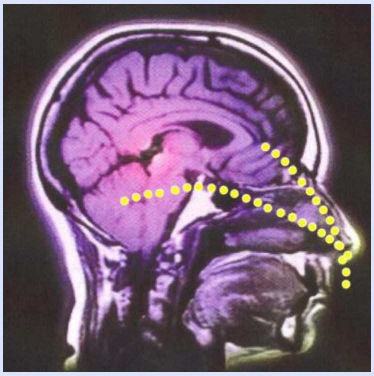
Zhang, O'Callaghan , 2011

Future Directions –Treatments and Mechanisms



Glial modulators, immune modulators, intranasal insulin and other cognitive enhancers

Treatments – Intranasal Insulin



Insulin - important modulator of brain function.

Brain insulin receptors are located in the hippocampus and frontal cortex. Thought to enhance synapse formation and long-term potentiation (LTP) to improve memory functioning in AD and others (Craft, 2012).

Intranasal insulin also increases levels of neurotransmitters including acetylcholine, dopamine and neuroepinephrine (Figlewicz et al., 1993) and is thought to decrease inflammation by altering proinflammatory cytokines (IL-1, IL-6, TNF) (Fishel et al., 2005).

Intranasal insulin does not alter peripheral glucose levels (Reger et al., 2007; Craft et al., 2009) suggesting that it is safe, can be self-administered and does not change plasma glucose or insulin levels (Benedict et al., 2004).

Thank You