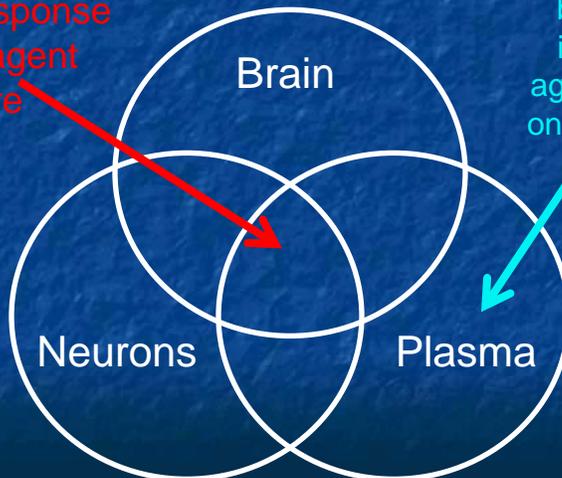


Genomic and Proteomic analysis of laboratory models of exposure to Gulf War agents

Roskamp Institute

Overview of approach

Biomarkers of
CNS response
to GW agent
exposure



Plasma
biomarkers of
impact of GW
agent exposure
on other organs

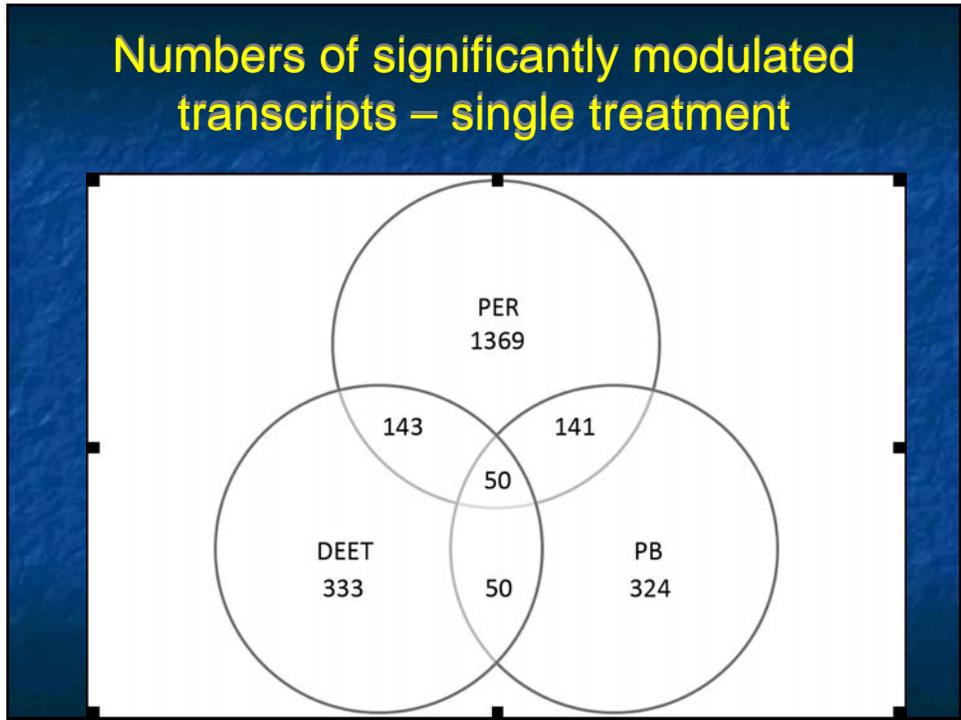
GWI program

- “Gulf War Agents” permethrin, pyridostigmine bromide, DEET
- Cell models
 - – genomic analyses
 - – proteomic analyses
- Animal models
 - – cognitive assessment
 - – proteomic analyses

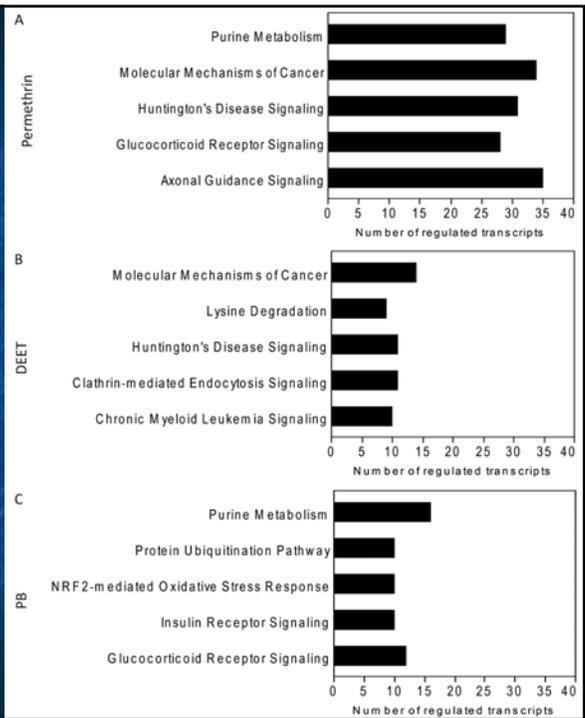
Genomic analyses

- Using SHSY-5Y human neuronal cells as a model of cellular response to agent exposure
- Permethrin, DEET and PB individually or in combination for 10 days
- Mimicking high-dose exposure as estimated from published reports
- Affymetrix GeneChip Arrays
- ANOVA
- Ingenuity Pathway Analysis

Numbers of significantly modulated transcripts – single treatment



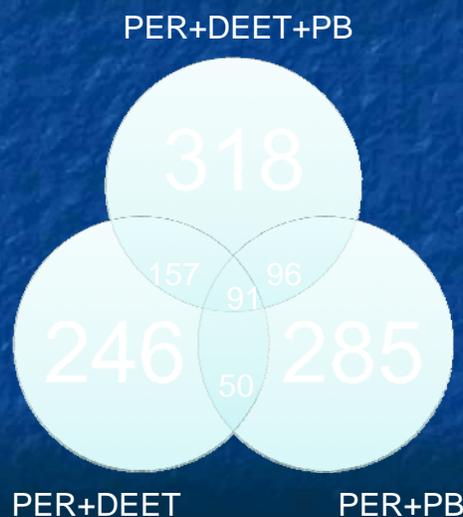
Most significantly modulated canonical pathways responding to each individual treatment



Genomic results from exposure to single agent

- Number of significantly modulated transcripts was greater for PER than for DEET or PB (1703 versus 576 or 565)
- Number of significantly modulated canonical pathways was similarly greater for PER (80) as compared to DEET (28) or PB (57)

Number of transcripts responding to combination exposures

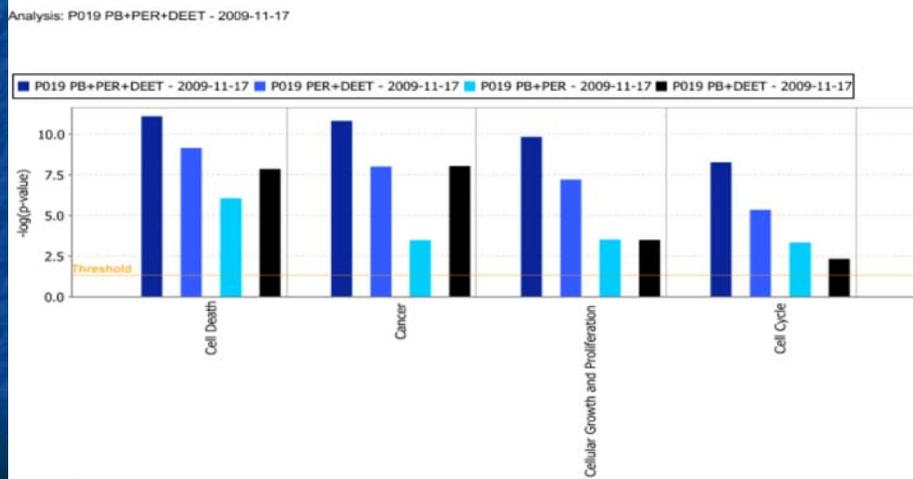


Numbers of significantly modulated transcripts in response to each treatment

	PB	DEET	PER	PB+PER	PB+DEET	PER+DEET	PB+DEET+PER
PB	565	93	176	87	74	82	102
DEET		576	171	67	48	44	74
PER			1703	210	204	235	272
PB+PER				522	126	141	187
PB+DEET					481	238	246
PER+DEET						544	248
PB+DEET+PER							662

Red – total for the treatment
 Black – overlapping between treatments

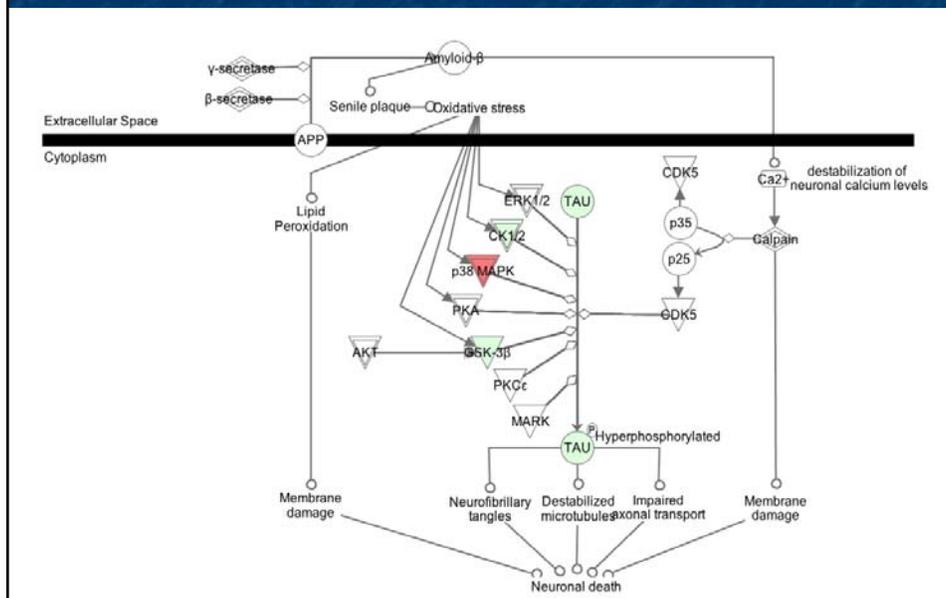
Impact on Four key molecular functions was common to all combination treatments



Observed significant modulation of canonical pathways both common and unique to the different exposures e.g.

- PER+DEET: *p53 Signaling, Biosynthesis of Steroids, IL8 Signaling, mTOR Signaling, Regulation of Actin-based Motility by Rho and sphingosine 1 phosphate signaling*
- PER+PB: *Molecular Mechanisms of Cancer, Androgen Signaling, Glucocorticoid Receptor Signaling, Estrogen Receptor Signaling, AMPK Signaling and Integrin Signaling*
- PB+DEET: *Protein Ubiquitination Pathway, Hypoxia Signaling in the Cardiovascular System, Arginine and Proline Metabolism, TR/RXR Activation, Androgen Signaling, Glutamate Metabolism and wnt/b-catenin signaling*
- PER+PB+DEET: *p53 Signaling, Oxidative Phosphorylation, Hypoxia Signaling in the Cardiovascular System, Amyloid Processing and Protein Ubiquitination Pathway.*

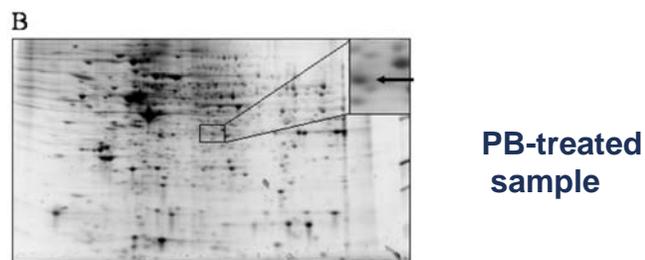
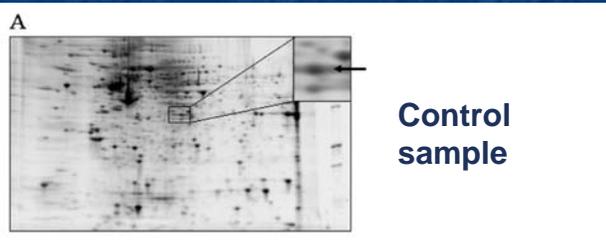
Amyloid Processing Pathway – uniquely regulated in the PB+DEET+PER dataset



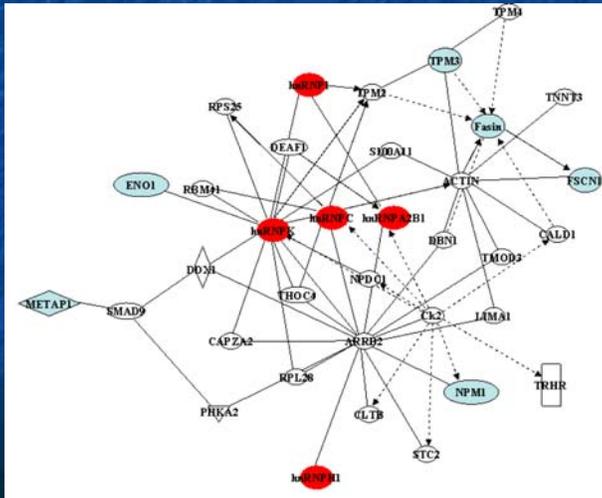
Proteomic analyses of human neuronal cells following PB exposure

- SHSY-5Y cells exposed to PB or vehicle control for 10 days
- 2DGE
- Image analyses – ≥ 1.5 fold change
- 21 protein spots showing differential regulation in response to treatment

2DGE analyses



Network analysis of significantly modulated proteins



Conclusions from *in vitro* work

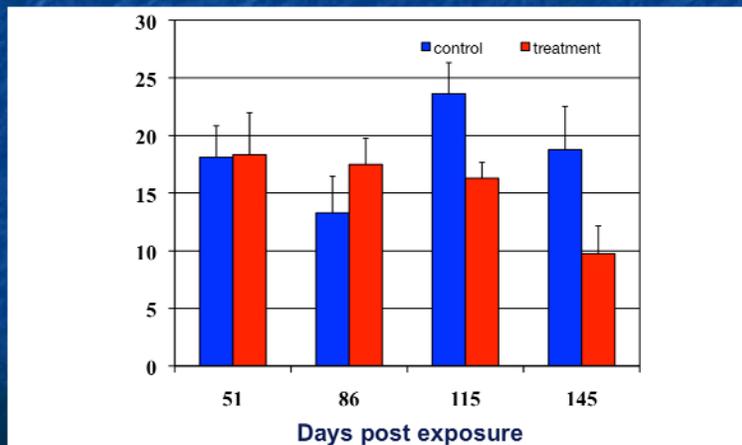
- Genomic and proteomic analysis in a highly controlled neuronal model demonstrates the activation of specific functional and canonical pathways following exposure to these GW agents.
- The cell model is insufficient to model the complexity and heterogeneity of GWI
- Need for animal models of specific clinical presentations of GWI in order to apply proteomic technology to the identification of therapeutic targets and plasma biomarkers

GWV mouse model development

- Existing animal models for GWV
- Model development:
 - Translate Abdel-Rahman model to mice
 - Novel PB-PER treatment (2mg/kg PB; 200mg/kg PER) for 10 days
- Abdel-Rahman model demonstrated motor deficits
- PB-PER model demonstrated cognitive deficits

Performance of PB-PER mouse model in Morris Water Maze

Time spent in goal quadrant

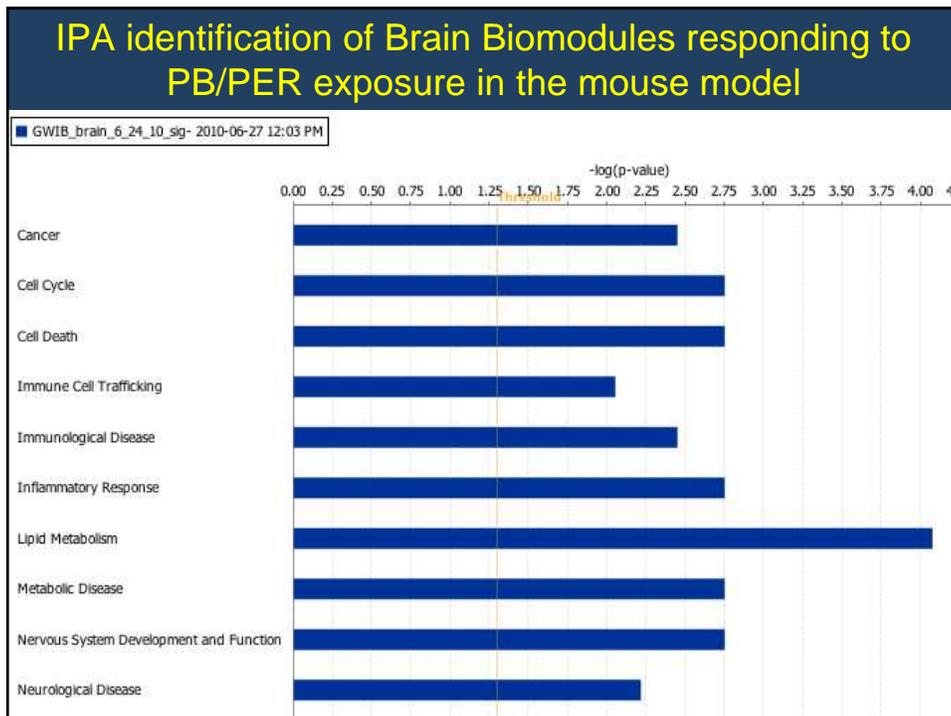
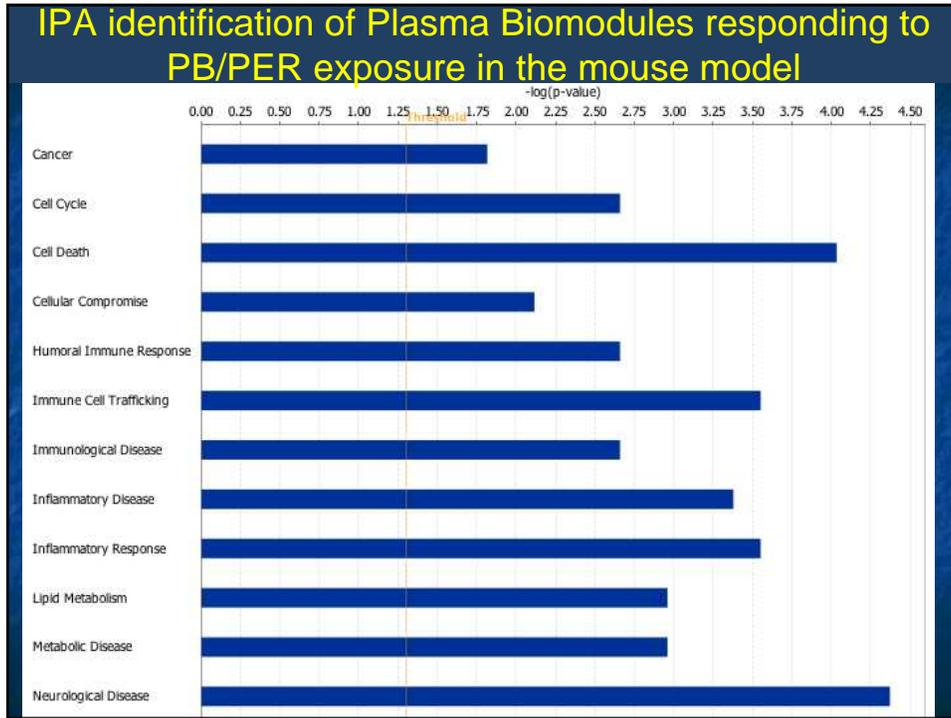


Hippocampal biomodules responding to TBI

Biomodules	24 hours		1 month		3 months	
	Injury	Inter	Injury	Inter	Injury	Inter
Neurotransmitters and Other Nervous System Signaling						
Cellular Immune Response						
Disease-Specific Pathways						
Organismal Growth and Development						
Cytokine Signaling						
Cardiovascular Signaling						
Cancer						
Carbohydrate Metabolism						
Pathogen-Influenced Signaling						
Ingenuity Toxicity List Pathways						
Nuclear Receptor Signaling						
Apoptosis						
Cell Cycle Regulation						
Xenobiotic Metabolism						
Intracellular and Second Messenger Signaling						
Amino Acid Metabolism						
Cellular Growth, Proliferation and Development						
Growth Factor Signaling						
Humoral Immune Response						
Cellular Stress and Injury						
Metabolism of Complex Lipids						
Lipid Metabolism						
Metabolism of Complex Carbohydrates						

Preliminary proteomic data from mouse model of PB/PER induced cognitive dysfunction

- Proteomic analyses of plasma and brains of mice euthanized at 145 days following a 10 day exposure to PB+PER



Summary

- Genomic and Proteomic analyses support the feasibility of the identification of molecular targets that could be modulated to mitigate the effects of GW agent exposure
- Plasma proteomic analyses demonstrate significantly modulated plasma proteins at 145 days post-exposure in a mouse model and thus support the pursuit of GWI biomarkers specific to particular clinical presentations

Future Directions

- Further characterization of the mouse model of PB-PER exposure
 - Proteomic analyses of plasma and brain at different timepoints following exposure
 - Identification of responding proteins that are common to plasma and brain
 - Other “-omic” approaches
- Investigation of the effects of genotype on cognitive dysfunction following GW agent exposure

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