Preclinical GWI research; therapeutic targets, plasma biomarkers and clinical translation

Fiona Crawford, Ph.D.
President and CEO
Roskamp Institute
Sarasota, Florida

Our Approach

Develop mouse models of exposure to known Gulf War agents such as Pyridostigmine Bromide and Permethrin

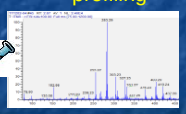
Characterize laboratory models of GWI using Neurobehavioral tests, Neuropathological and Molecular analyses ("omics" technology)

Identify brain molecular pathways, and blood molecular profiles, correlating with GW-agent exposure – dependent behavioral and pathological changes

Translate to human populations with clinical trials of novel therapeutics, and biomarker profiling in patients

Neurobehavior

Molecular profiling



Neuropathology

From mouse brain and blood studies: Identify key molecular functions and blood profiles and validate results in human patients

Evaluate in GWI patients and controls

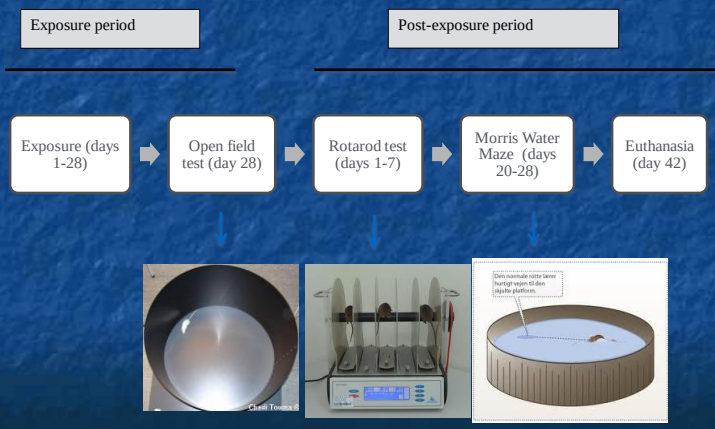
Novel Treatment strategies and **Blood Biomarkers for diagnosis and prognosis of GWI**

PB+PER+DEET+Stress mouse model of GW agent exposure

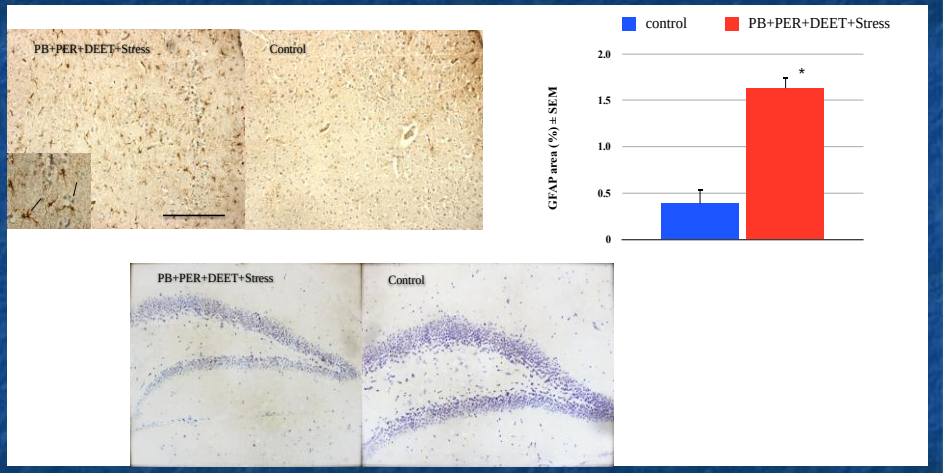
Adapted from the Abdel-Rahman rat exposure paradigm
For 28 days, C57BL6 mice (15-17 weeks old, male and female) were daily exposed to
- 1.3mg/kg PB orally,
- 0.13 mg/kg PER dermally,
- 40 mg/kg DEET dermally, and
- 5 min of restrained stress

Control mice only received vehicle (n = 10 per group)

Behavioral studies and timeline for PB+PER+DEET+Stress mouse model



GFAP neuropathology in PB+PER+DEET+Stress mouse model



Significant increase in GFAP (astrocytosis) in exposed versus controls

PB+PER mouse model of GW agent exposure

Male CD1 mice (9 weeks old) exposed to
- 2mg/kg PB
- 200 mg/kg PER

Daily (i.p.) for 10 days
N = 10-11 per group

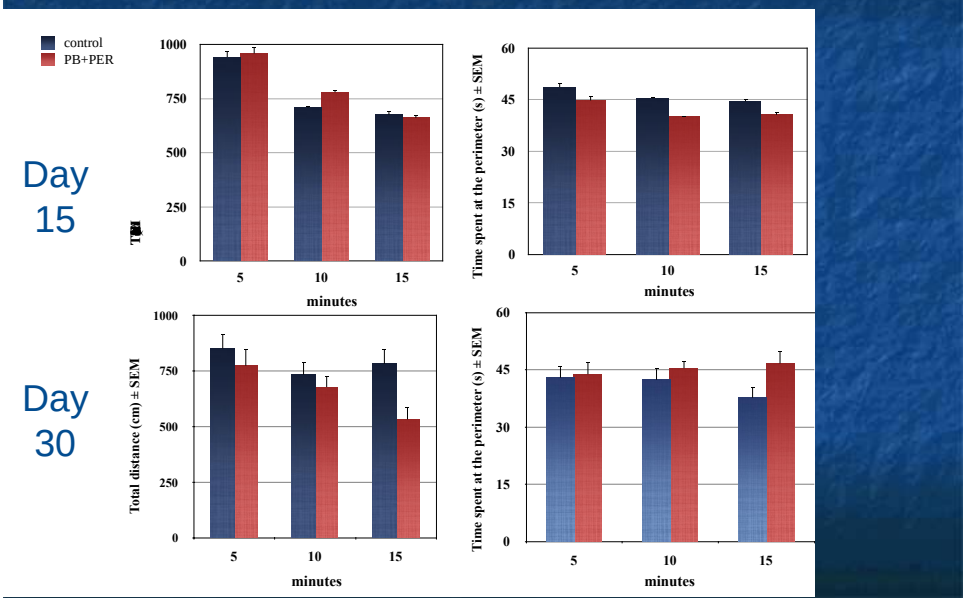
Behavioral studies and timeline for PB+PER mouse model

Exposure period

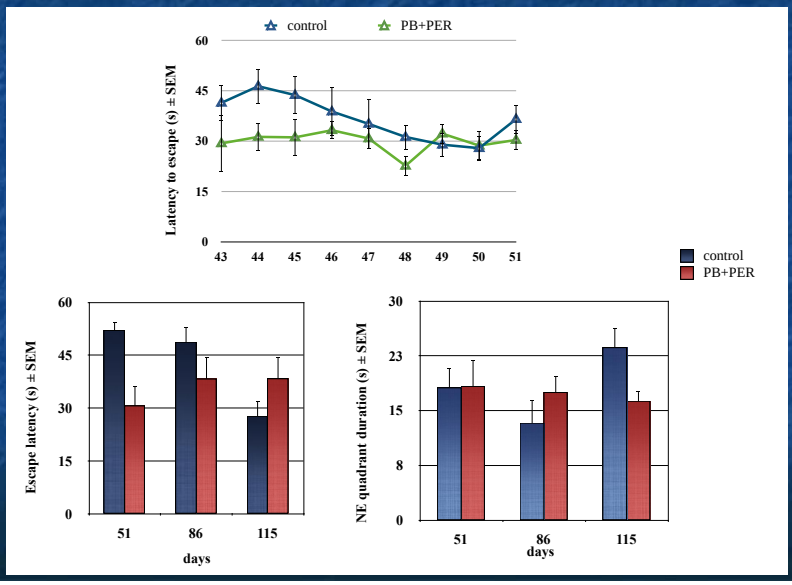
Post-exposure period

Exposure (days 1-10) → Rotarod (days 5-9) → Rotarod test (days 1-7) → Open field test (day 15) → Open field test (day 30) → Morris Water Maze (days 43-115) → Euthanasia (day 150)

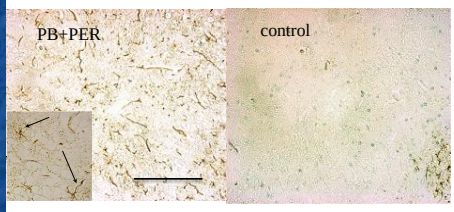
Open field test results from PB+PER model

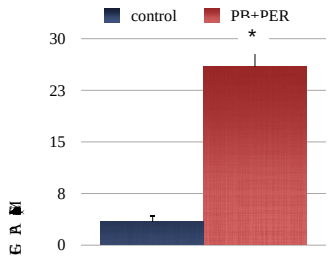


Morris water maze test results from PB+PER mouse model



GFAP neuropathology in PB+PER mouse model

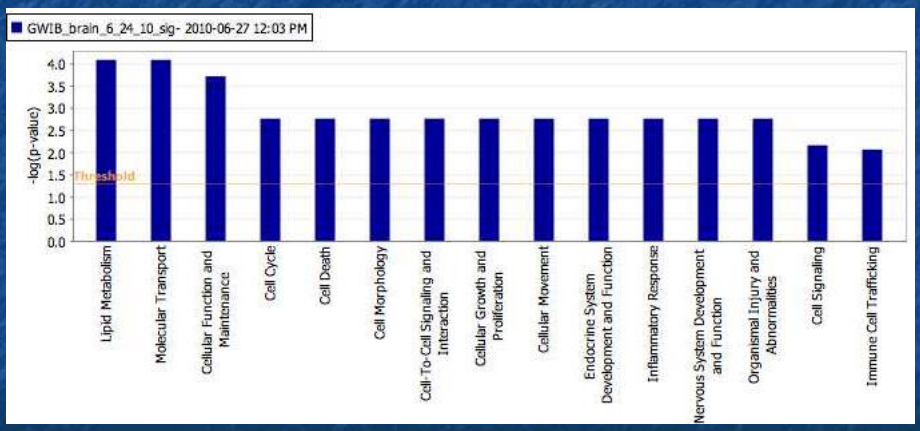




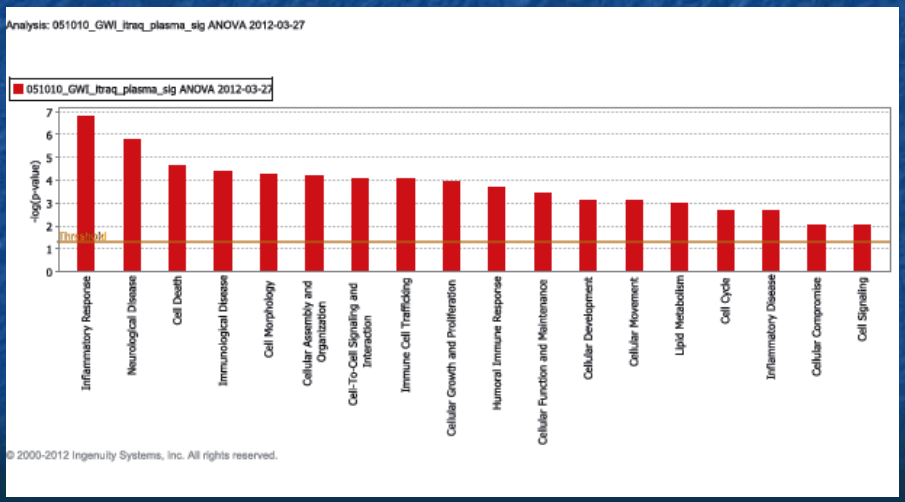
Analysis of proteomic data

- lists of significantly regulated proteins
- upload to Ingenuity Pathway Analysis knowledgebase to identify
  - Canonical pathways
  - Biological functions
- That are significantly modulated in response to GW-agent exposure

Brain biofunctions modulated in response to PB+PER exposure



Plasma biofunctions modulated in response to PB+PER exposure



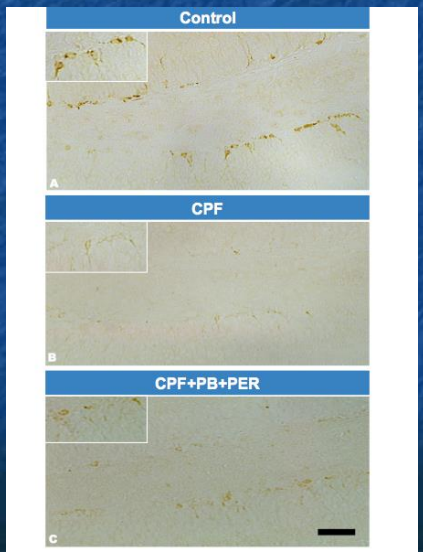
PB+PER+CPF mouse model of GW agent exposure

Pilot study (N=4 per group) on effects of OP exposure
Male C57BL/6 mice aged 24 weeks

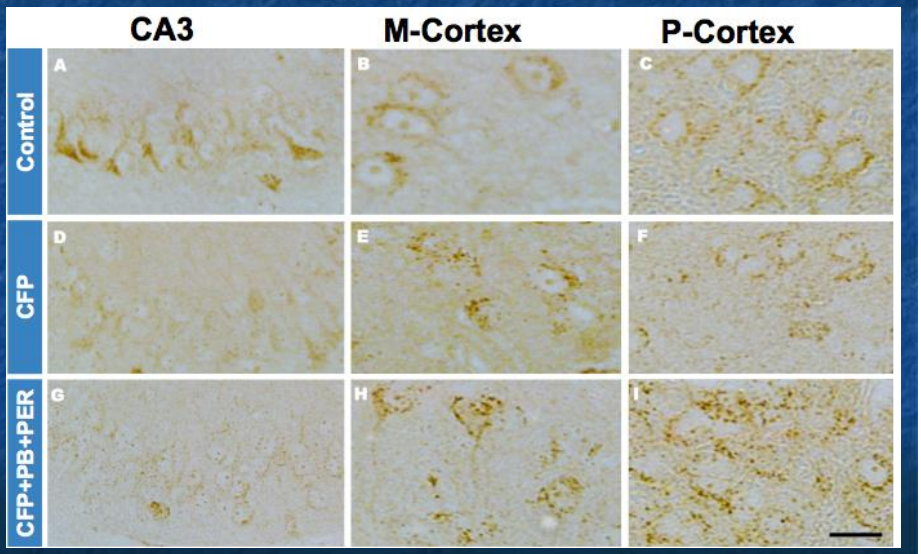
Mice were administered
chlorpyrifos (CPF) 5mg/kg or
5mg/kg CPF + 0.7mg/kg PB + 200mg/kg PER
Daily (i.p.) for 10 days.

Mice were euthanized at 3 days post exposure for neuropathological analyses

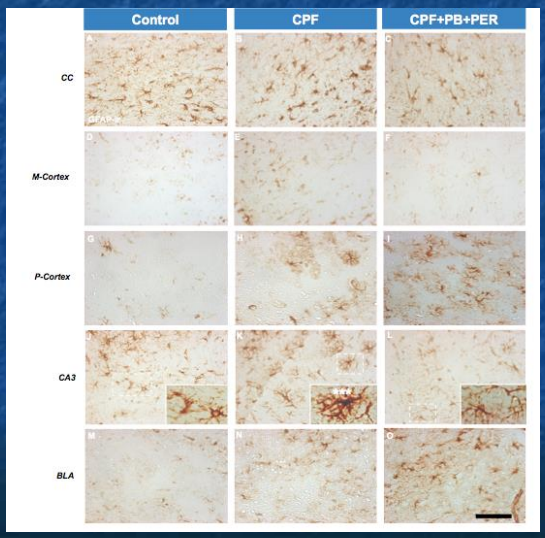
Doublecortin staining in the dentate gyrus after exposure to CPF or CPF+PB+PER



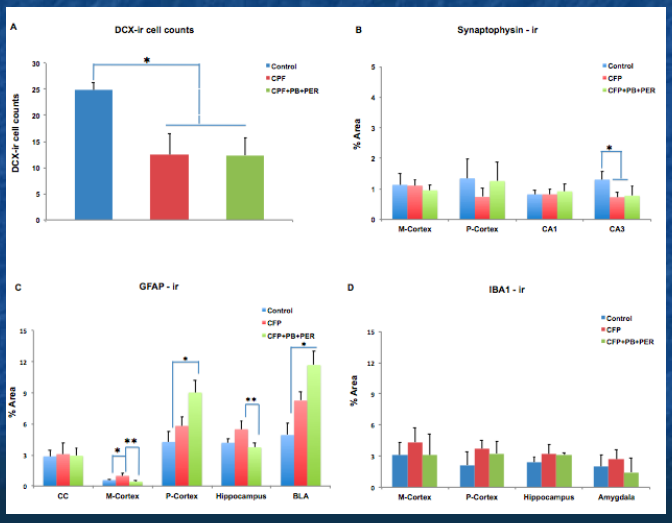
Synaptophysin staining in the hippocampi and cortices after exposure to CPF or CPF+PB+PER



Astrocytic staining in the hippocampi and cortices after exposure to CPF or CPF+PB+PER



Quantification of pathological findings after exposure to CPF or CPF+PB+PER



Characterization of the PB+PER Mouse Model of GWI

Pyridostigmine Bromide (PB) – prophylactic against nerve gas attack
Permethrin (PER) – pesticide extensively applied by troops
Translation to the more common C57BL6 mouse strain
Mice exposed for 10 days daily to 0.7mg/kg of PB + 200 mg/kg of PER

To mimic the experience of GW veterans, acute exposure at a young age to GW agents and longitudinal evaluation over the mouse lifespan

EXPOSURE

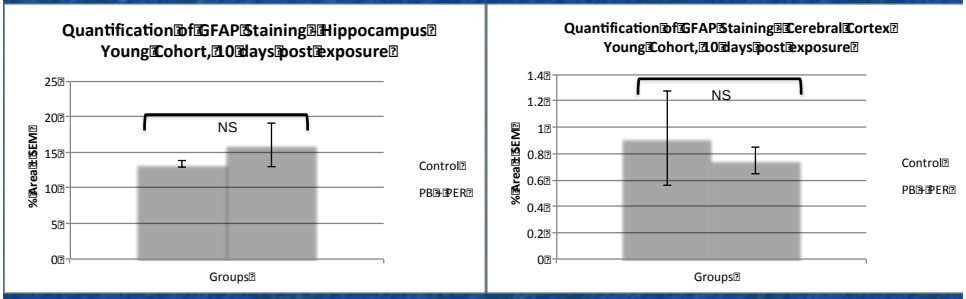
10 days exposure to PB+PER

EVALUATION

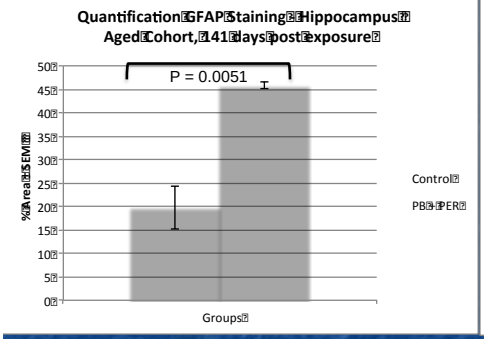
Cognitive Testing ; Neuropathological analyses ; "Omic" analyses

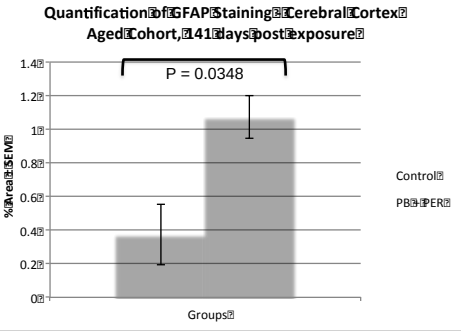
Time post-exposure: acute | 5 months | 15/16 months | 22 months

No acute differences in GFAP staining after exposure



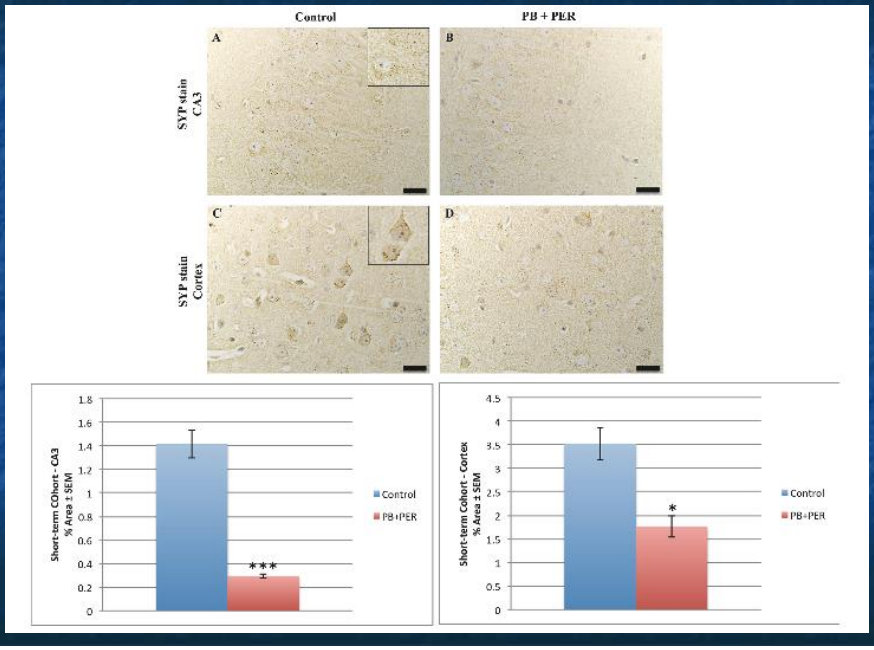
Elevation of GFAP at 5 months post-exposure



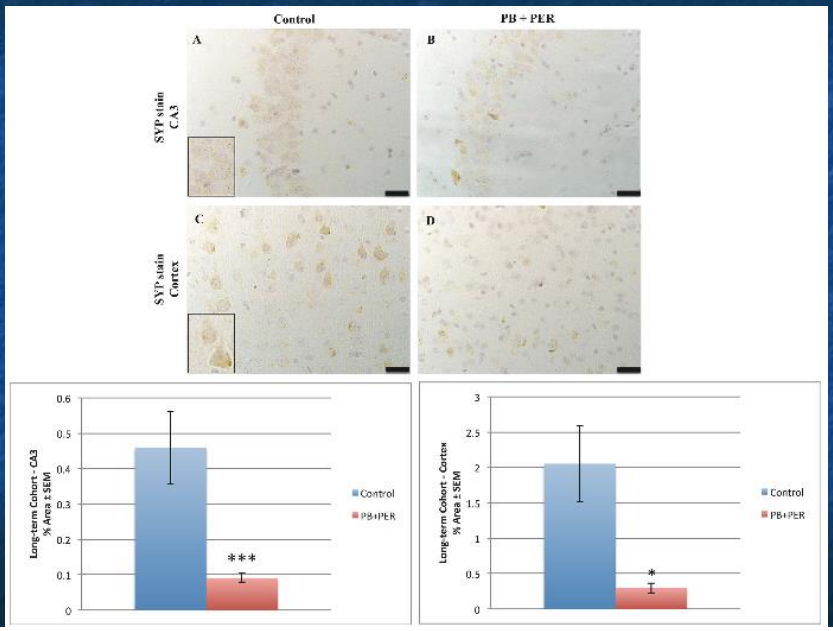


Aged cohort showed significant increase in astrocytosis, and reduction in synaptic markers and measures of neurogenesis
Inflammatory dysregulation – CNS v PNS; pro- v anti-inflammatory markers

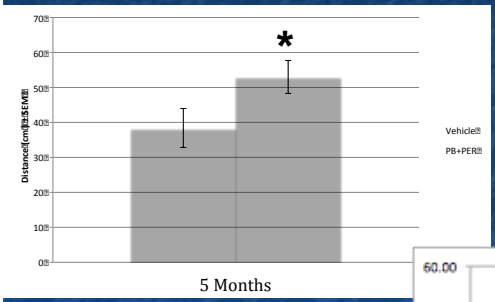
Acute reduction in synaptophysin after exposure

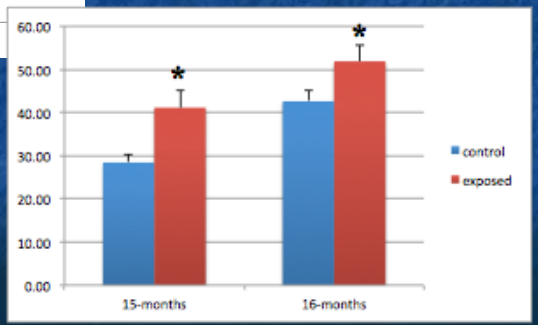


Reduction in synaptophysin 5 months after exposure

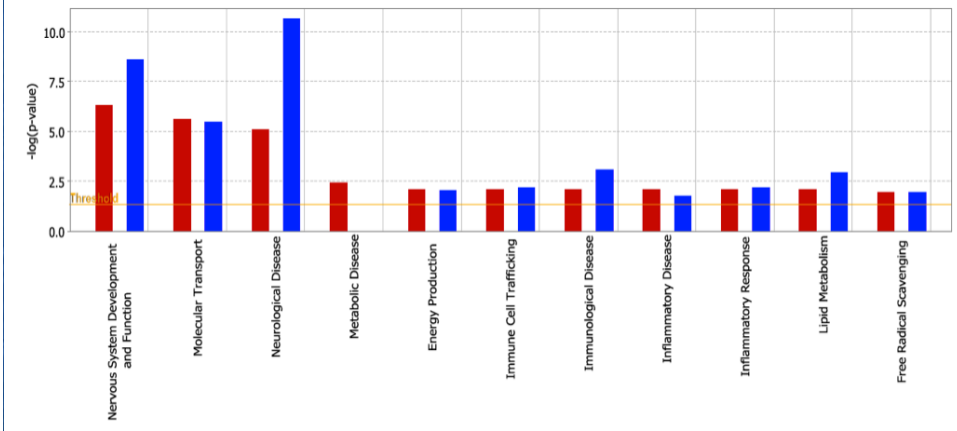


Cognitive performance deteriorates over time in mice exposed to the Gulf War agents PB+PER



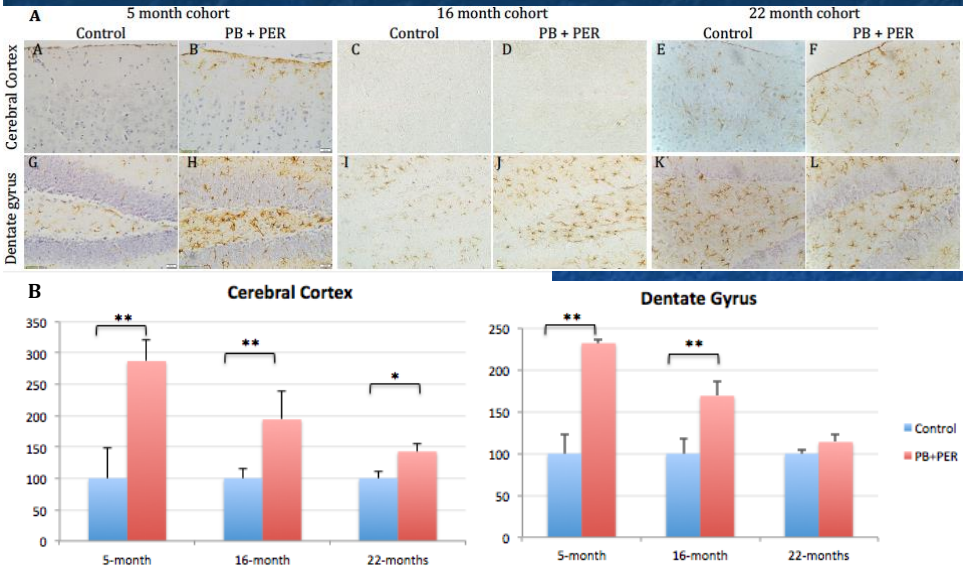


Proteomic profiling shows significant changes in expression of brain proteins in the GW mouse model compared to control at 5 (red) and 16 (blue) months post-exposure



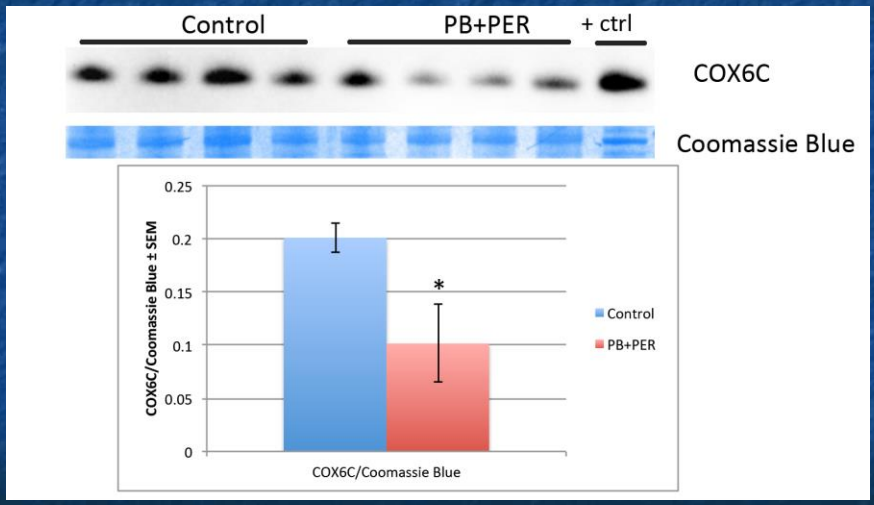
Highlighting : Immune/inflammatory functions
Mitochondrial function
Lipid metabolism

Persistence of inflammatory (astroglial) pathology over the mouse lifespan after exposure to GW agents



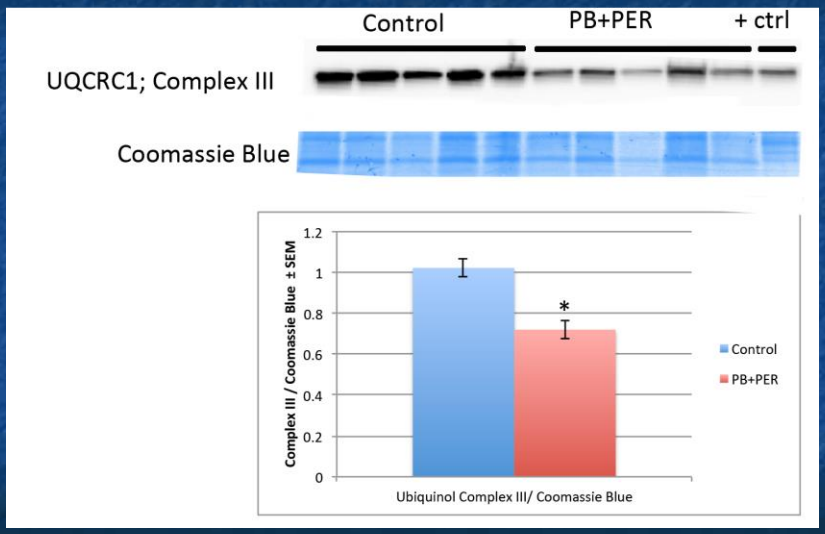
GFAP staining as a % of control

Disruption of mitochondrial proteins in mice exposed to the Gulf War agents PB+PER (COX6C)



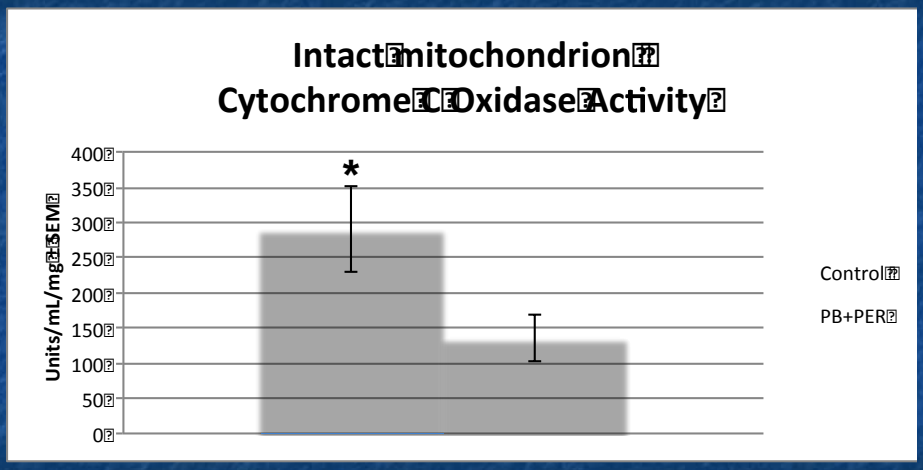
Cytochrome C Oxidase subunit VIc

Disruption of mitochondrial proteins in mice exposed to the Gulf War agents PB+PER (UQCRC1)



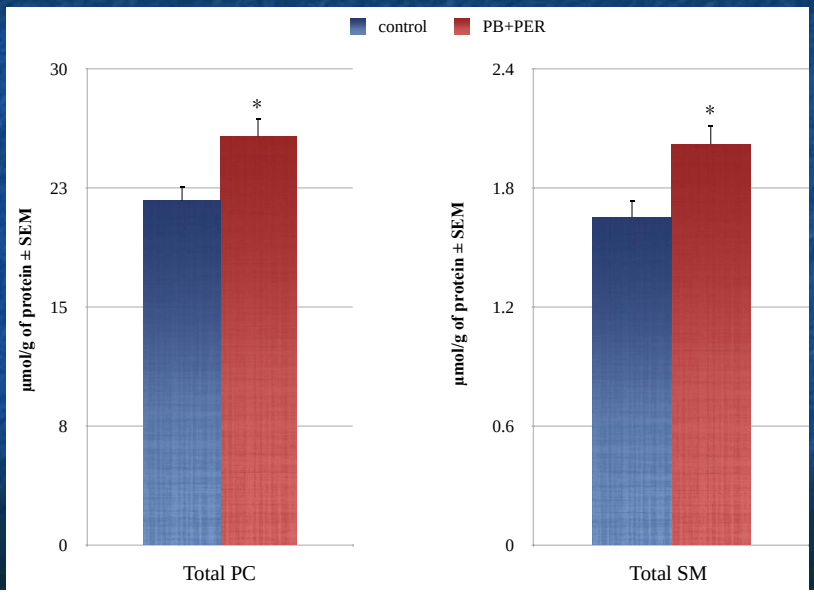
Ubiquinol-Cytochrome C Reductase Core Protein I

Mitochondrial function is compromised in mice exposed to the Gulf War agents PB+PER

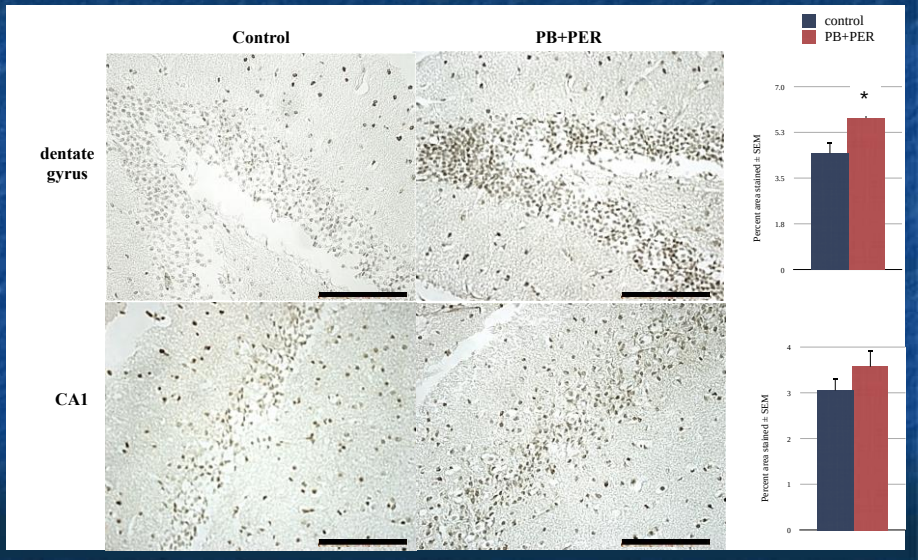


Cytochrome C oxidase is the final step in the electron transport chain – its activity is thought to be a good indicator of metabolic capacity required for neuronal function in neurons.

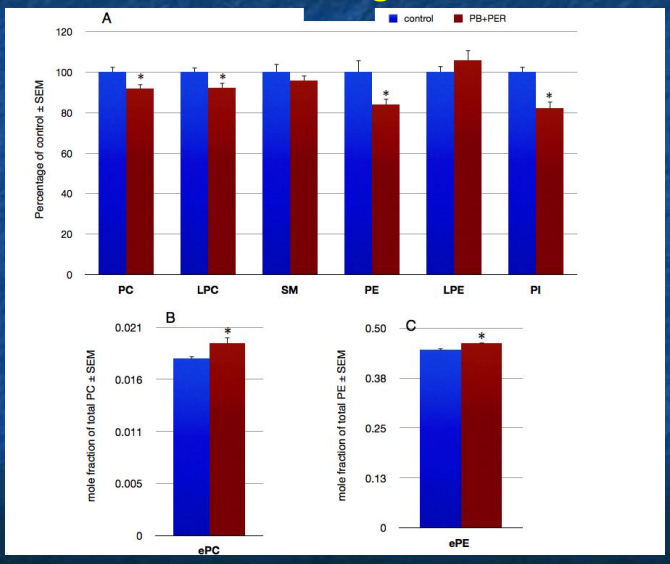
Increased Total Brain PC and SM at 5 months post-exposure



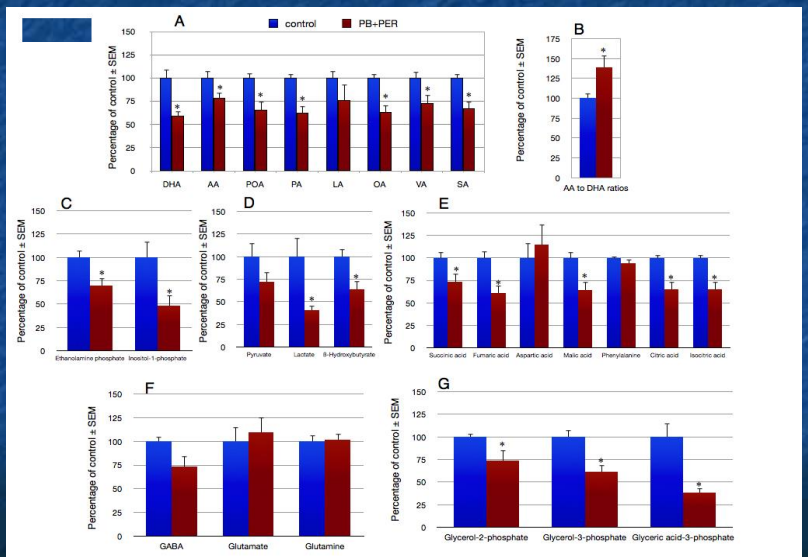
Catalase staining at 5 months post-exposure



Lipidomic profile at 16-months post-exposure to GW agents



Metabolomics profile at 16-months post-exposure to GW agents



Role of omega-3 and omega-6 fatty acid-containing lipids in human health

AA (an ω-6 fatty acid [FA]) and DHA (an ω-3 FA) - essential fatty acids primarily acquired through diet owing to the low capacity of the body to synthesize these lipids.

AA metabolism initiates a **pro-inflammatory** cascade whereas DHA metabolism produces **anti-inflammatory** metabolites.

Dietary intake of DHA is particularly important in aging adults to maintain cognitive function and for optimal neurotransmission.

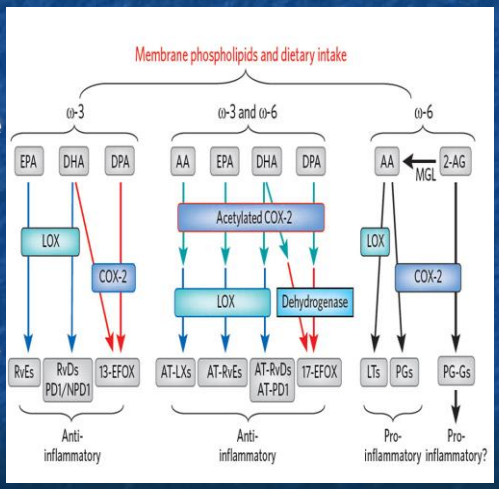
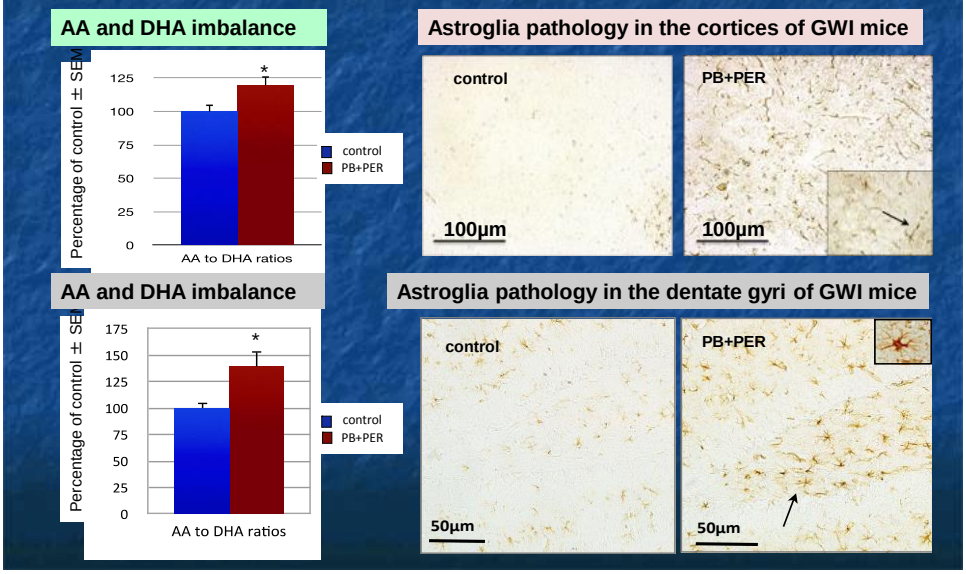
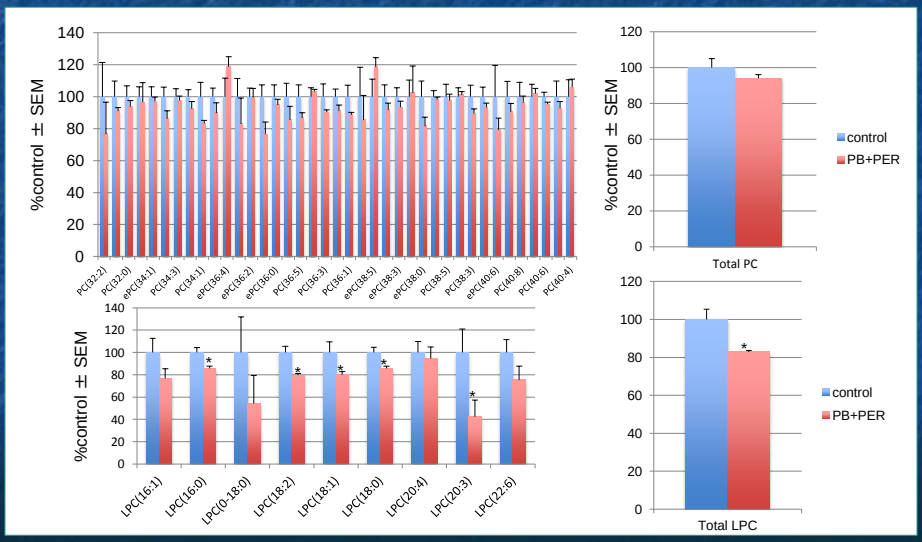


Figure from: *Nature Chemical Biology* **6**, 401–402 (2010)

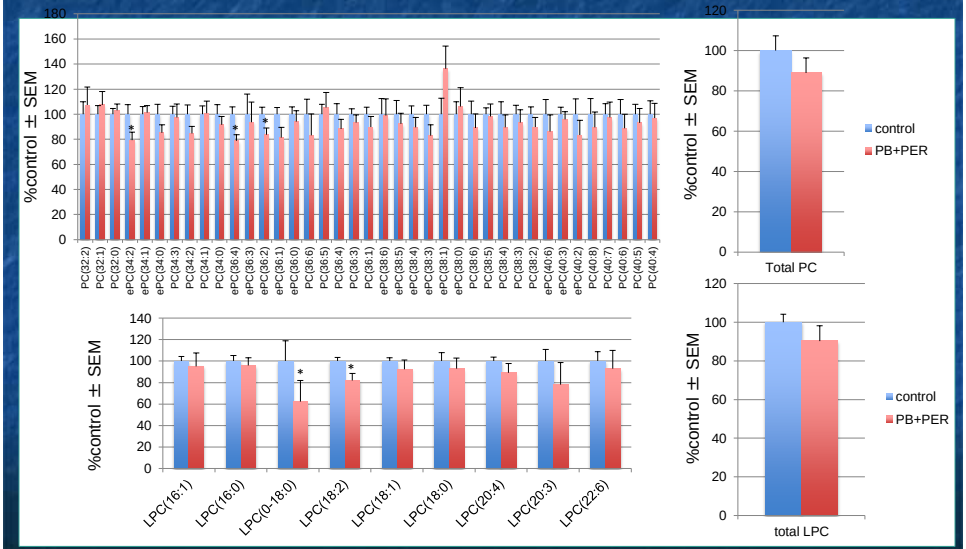
A chronic imbalance of AA and DHA correlates with astroglial pathology in mice exposed to PB+PER



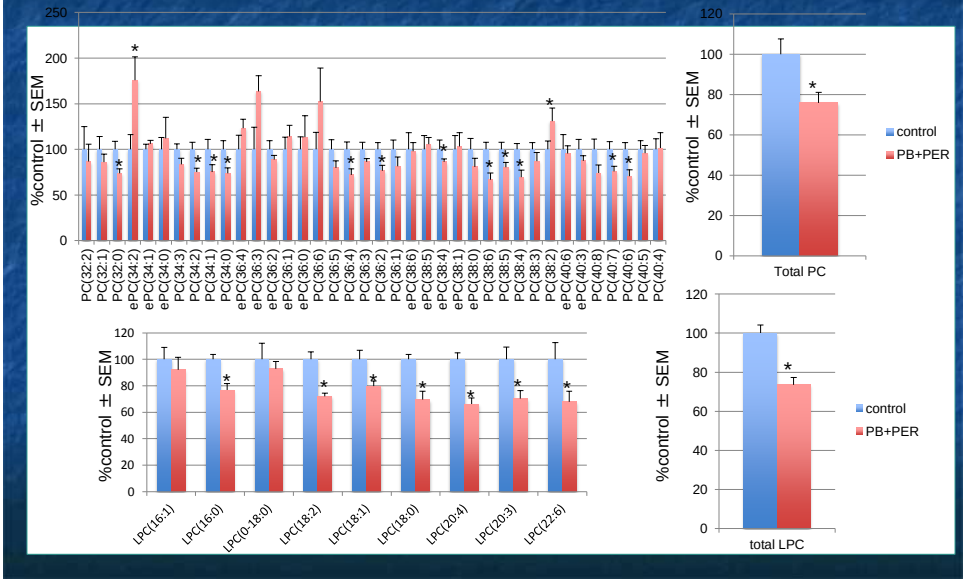
Plasma PC and LPC profiles 18 days following GW agent exposure



Plasma PC and LPC profiles 5-months post-exposure to GW agents



Plasma PC and LPC profiles at 16-months post-exposure to GW agents



Summary of results

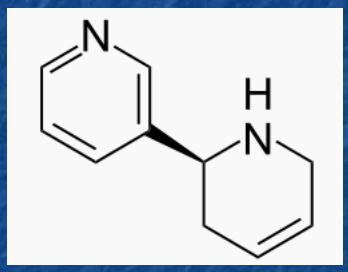
Plasma biomarkers of GW-agent exposure are evident at chronic timepoints post-exposure

Characterization of these mouse models of GWI with neurobehavior, neuropathology and proteomic and lipidomic profiling have identified a number of potential therapeutic targets for further evaluation.

- Inflammatory responses
- Lipid dysmetabolism
- Mitochondrial dysfunction

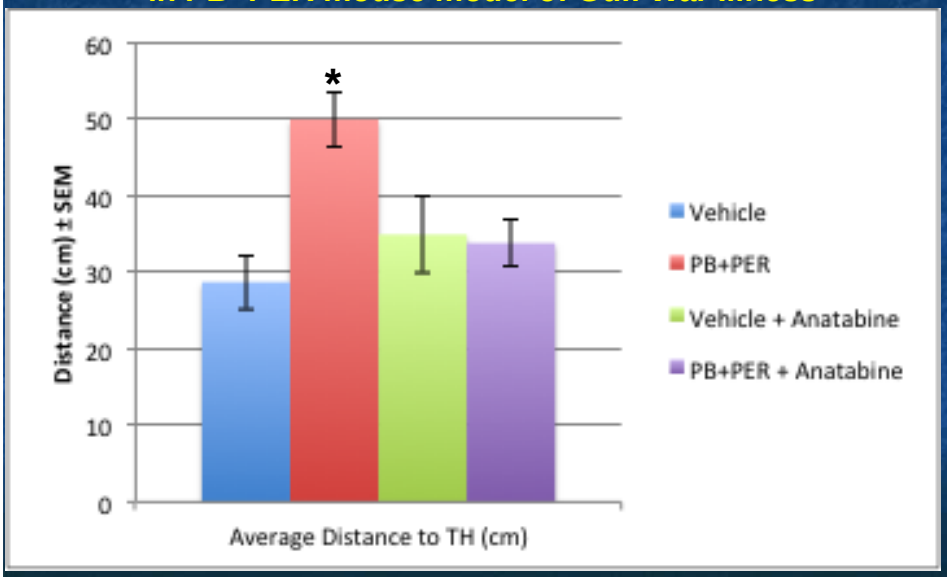
Targeting Inflammation

Anatabine

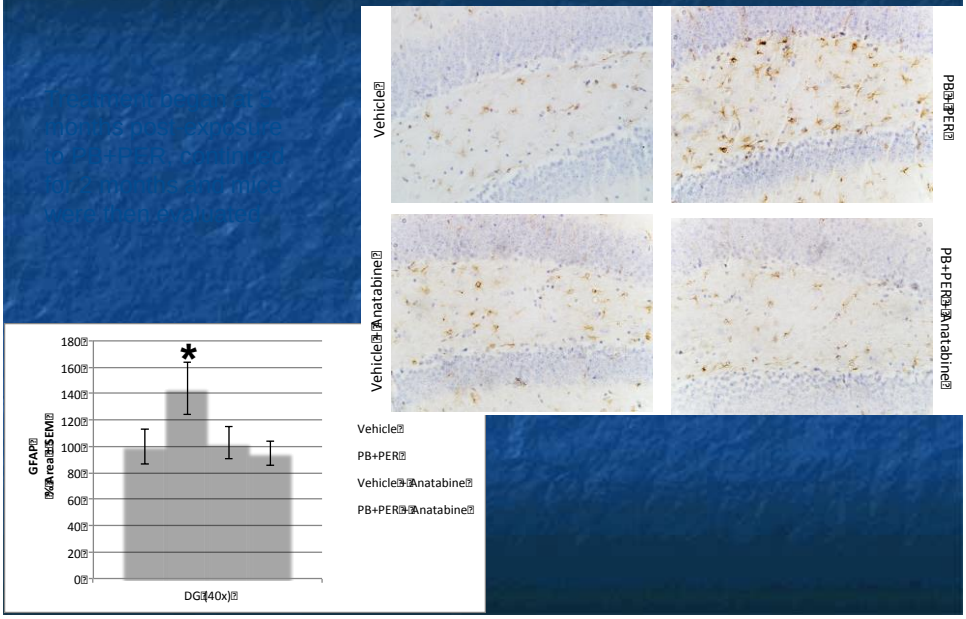


- Alkaloid derived from tobacco and plants of solenaceae family
- 3 year history of safe use as a dietary supplement
- Potent anti-inflammatory
- Efficacy in mouse models of AD, TBI, EAE, Tauopathy

Treatment with the anti-inflammatory compound Anatabine significantly improves cognitive function in PB+PER mouse model of Gulf War Illness



Anatabine treatment significantly reduces astrogliosis in a mouse model of Gulf War Illness



Clinical studies of plasma biomarkers of GWI

Recruitment of patients through the Boston and Bronx VA hospitals (Drs. Krengel, Sullivan and Golier)

160 GWI veterans, 120 healthy GW veterans and 40 GW-era veterans

Additional recruitment of Ft. Devens cohort

Plasma samples for proteomic/lipidomic profiling and targeted analyses

Correlation with clinical presentation (and response to treatment)

Translational Research from preclinical models

Biomarker studies

- **Generation of blood protein and lipid profiles from human GWI patients**
- **Correlation of human blood profiles with mouse blood and brain profiles**

Therapeutics

- **Ongoing preclinical validation of therapeutic targets in laboratory models of GWI**
- **Facilitated by Collaborations between Gulf War Illness clinical and basic science research teams**

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