Presentation 6 – Wilkie Wilson

Neurotoxins and Gulf War Illness: An Overview of VA's Research Enhancement Award Program (REAP)

- Research Enhancement Award Program
  - Promote and support groups of VA investigators in programs of exceptional quality
  - Train new investigators (William Troust, MD -PSY)
  - Support core facilities for multiple investigators
  - Support small innovative pilot projects to generate new and novel approaches to medical problems
  - Durham, NC station (4/1/2003-3/31/2008)

An Overview of VA's Research Enhancement Award Program (REAP)

- Principal Investigator:
  - Roger Madison, Ph.D., Research Career Scientist
- Co-Investigators:
  - Scott Moore, M.D., Ph.D. – Psychiatry
  - Christine Marx, M.D., M.A. – Psychiatry
  - Scott Swartzwelder, Ph.D. – Neuropsychology (Senior RCS)
  - Wilkie Wilson, Ph.D. Pharmacology (Senior RCS)
  - Ashok Shetty, Ph.D. Anatomy (Research Scientist)

Neurotoxins, Hyperexcitability and Gulf War Illness

- Persian Gulf War Syndrome; Haley et al., JAMA, 1997
  - Impaired cognition
  - Confusion; ataxia
  - Arthromyo-neuropathy; muscle & joint pain
  - Phobias, apraxia,
  - Fever, adenopathy
  - Weakness and incontinence
  - Increased incidence of ALS?; Haley, Neurol., 2003

Neurotoxins, Hyperexcitability and Gulf War Illness

- Evidence for neuronal injury/loss in Gulf War Illness
  - Proton magnetic resonance spectroscopy shows decreased functional neuronal mass in basal ganglia of GWI patients compared to normal controls; *Haley et al., Arch. Neurol., 2000*
  - Rat model of GWI involving exposure to low-doses of: pyridostigmine bromide; N,N-diethyl m-toluamide (DEET); or permethrin demonstrates neuronal cell death in numerous brain regions; *Abdel-Rahman, Shetty, and Abou-Donia, Neurobiol. Disease, 2002, 10(3), 306-326; Exp. Neurol., 2001, 172(1), 153-171*

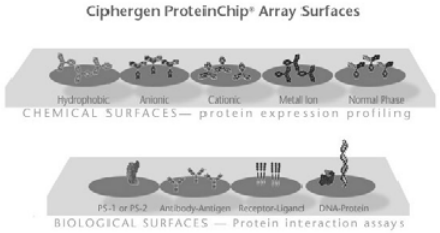
Neurotoxins, Hyperexcitability and Gulf War Illness

- Neurotoxins can lead to neuronal death due to hyperexcitability
  - Neurotoxins can cause local epileptiform discharges
  - Epileptiform activity can cause neuronal death
  - Even "neuroprotective" drugs may produce local hyperexcitability and be neurotoxic---memantine
- Therefore there may be a link between neurotoxins, neuronal hyperexcitability, and Gulf War Illness as well as other neurodegenerative diseases
  - Nerve injury, seizures, neuropsychiatric disorders

Durham VA REAP

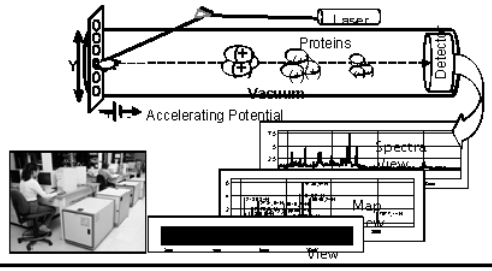
- Gene and protein expression in animal models of neuronal hyperexcitability and neurotoxin exposure
- Interested in brain sub-regions; e.g. hippocampus, basal ganglia, amygdala
  - Increase signal to noise if just the sub-region of interest can be analyzed separately from the rest of the brain
  - Requires technology that can work with small samples
- REAP support of the developing Proteomics Core Resource for the Durham, NC VAMC utilizing the Ciphergen Protein Chip instrument

Neurotoxins, Hyperexcitability and Gulf War Illness: Protein Chip Instrument



Neurotoxins, Hyperexcitability and Gulf War Illness: Protein Chip Instrument

TOF-MS Detection of Proteins Captured on ProteinChip®



Neurotoxins, Hyperexcitability and Gulf War Illness: Protein Chip Instrument

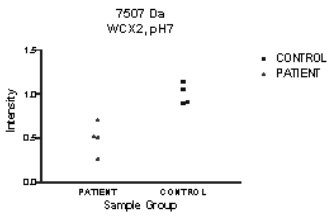
Serum Biomarkers in Schizophrenia

- Objective
  - To demonstrate the utility of ProteinChip®-SELDI-TOF technology for rapid screen of serum biomarkers in Schizophrenia patients
    - Differential biomarkers between patients and control;
    - Differential protein expression within patient or control population
- Materials
  - (small!)~100 ul frozen serum samples: 4 patient, 4 control
  - WCX2, SAX2, IMAC-Cu, H4 ProteinChip® arrays.

Neurotoxins, Hyperexcitability and Gulf War Illness: Protein Chip Instrument

Serum Biomarkers in Schizophrenia vs. Controls

M/Z	p
7507	0.0288
7958	0.0288
10288	0.0288
5228	0.0288
4648	0.0288
4253	0.0288
3884	0.0288
4121	0.0288



Neurotoxins, Hyperexcitability and Gulf War Illness: Amygdala and psychiatric disorders

- The amygdala is critical for conditioned fear (a model of PTSD and phobias)
- Amygdala lesions impair acquisition and expression of conditioned fear
- Partial amygdala kindling produces exaggerated fear and aggression
- Amygdala hyperexcitability has been proposed as a model of anxiety disorders

Neurotoxins, Hyperexcitability and Gulf War Illness: Amygdala and psychiatric disorders

- The amygdala has one of the lowest thresholds of any brain region for kindling and epileptiform activity
- Amygdala hyperexcitability may be produced by electrical stimulation, toxic agents, or stress.
- Maintenance of these forms of aberrant synaptic function ultimately depend on gene induction and new protein synthesis

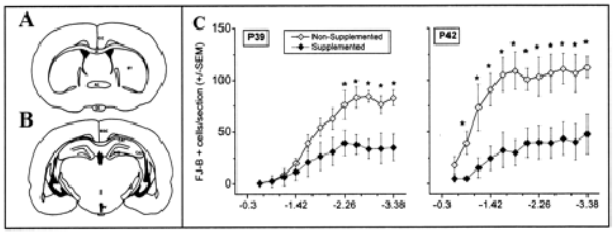
Neurotoxins, Hyperexcitability and Gulf War Illness: Amygdala and psychiatric disorders

- Proposed Pilot Study
  - Develop an *in vitro* model of psychopathology in a rodent brain slice preparation
  - Using multiple approaches, induce altered excitability in the amygdala
  - Perform microarray analysis of gene expression and SELDI/MS protein characterization to characterize specific biomarkers associated with the hyperexcitable amygdala network

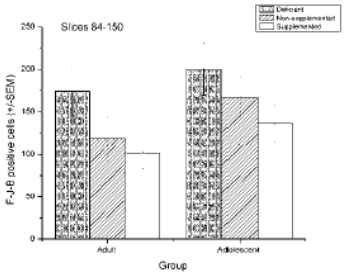
Neurotoxins, Hyperexcitability and Gulf War Illness: Neuroprotection by dietary choline

- Choline is a required dietary nutrient
- Required for neuronal integrity and synaptic transmission (acetylcholine)
- Agonist at nicotinic alpha-7 receptors
- Prenatal supplementation with choline provides neuroprotection
- Some evidence that adults are protected by choline supplementation

Prenatal choline supplementation is neuroprotective



Is postnatal choline supplementation neuroprotective?



Neurotoxins, Hyperexcitability and Gulf War Illness: Neuroprotection by dietary choline

Goals:

- Effects of dietary choline levels on neuronal excitability
- Effects on alpha-7 receptor function (alpha-7 desensitization?)
- Does adult supplementation provide neuroprotection?

Neurotoxins, Hyperexcitability and Gulf War Illness: Alcohol Exposure

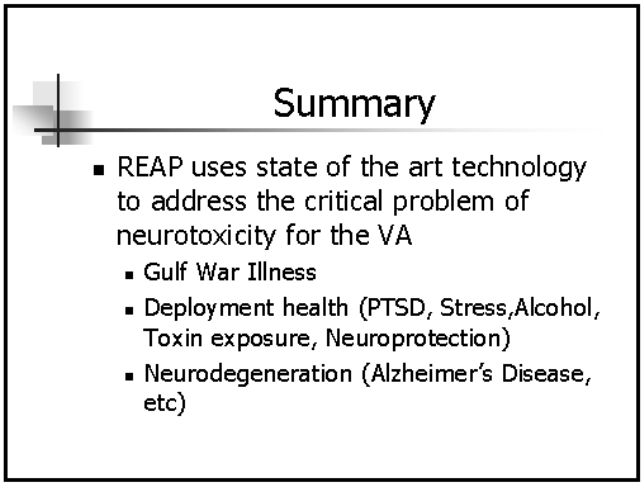
- The hippocampus is critical for learning and memory
- The hippocampus is damaged by repeated heavy exposures to alcohol
- One mechanism of this vulnerability is neuronal hyperexcitability

Neurotoxins, Hyperexcitability and Gulf War Illness: Alcohol Exposure

- Cholinesterase Inhibitors May Decrease Brain Choline Availability
  - AChE activity promotes brain choline availability
  - Blocking AChE action may decrease brain choline levels resulting in increased vulnerability to excitotoxicty in the brain.

Neurotoxins, Hyperexcitability and Gulf War Illness: Alcohol Exposure

- Proposed Pilot Study
  - Assess alcohol-induced neurotoxicity in animals undergoing pharmacological exposure to anticholinesterase drugs.
  - Determine if dietary choline supplementation attenuates alcohol-induced neurotoxicity under these circumstances


Summary
REAP uses state of the art technology to address the critical problem of neurotoxicity for the VA
Gulf War Illness
Deployment health (PTSD, Stress,Alcohol, Toxin exposure, Neuroprotection)
Neurodegeneration (Alzheimer's Disease, etc)