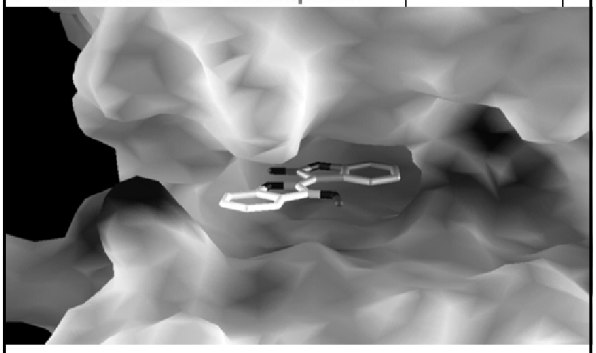
**Presentation 18 – Allen Fienberg**

Intra-Cellular Therapies



Research Advisory Committee on Gulf War Veterans' Illnesses
June 29th 2004

## Neuronal Communication

- Nerve cells communicate by chemical neurotransmission; over 99% of all synapses in the brain use chemical neurotransmission
- There are two categories of neurotransmitters: 'fast neurotransmitters' and 'slow' neurotransmitters'

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## Neuronal Communication (Cont'd)

- 'Fast' neurotransmitters control events occurring in the brain at 1/1,000 of a second
- 'Slow' neurotransmitters control events occurring in the brain over milliseconds to minutes

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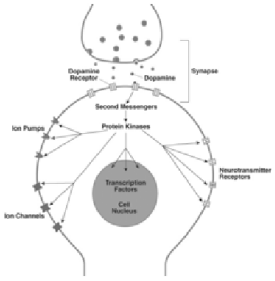
## Chemical Neurotransmission In the Brain




- 'Slow' neurotransmitters, like dopamine, bind to receptor proteins at the surface of post-synaptic neurons.
- Activation of these receptor proteins leads to changes in second messenger molecules and a cascade of chemical effects involving protein kinases, and other cellular factors.
- These cellular cascades, in turn, control the activity of many essential neuronal proteins including ion pumps, ion channels, and neurotransmitter receptors by changing their state of phosphorylation

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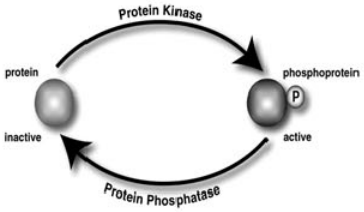
## Protein kinases and protein phosphatases regulate protein phosphorylation




- The state of phosphorylation of proteins is determined by the activity of both protein kinases and protein phosphatases
- *In vitro* and *in vivo* methods have been designed to minimize artifactual post-mortem and post-treatment activity of kinases and phosphatases
- Phosphorylation state can be preserved following pharmacological or physiological treatments to provide a 'snapshot' of the state of phosphorylation at multiple sites

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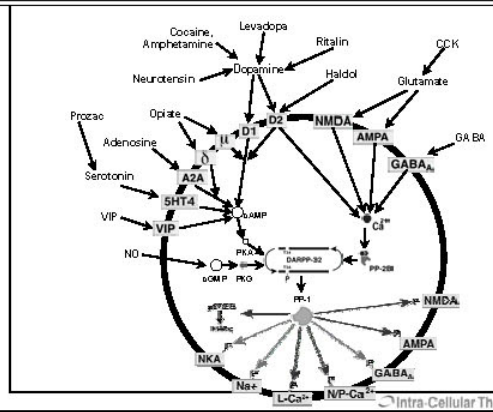
Intra-Cellular Therapies *The Inside Track for CNS Drug Discovery*

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## *In Vitro* and *In Vivo* Methods for Measuring Brain Phosphoproteins

*In Vitro* Slice Techniques

- Technique can be adapted to analyze drug actions in many brain regions (e.g., striatum, PFC, hippocampus, accumbens)
- Slice technique can be adapted to facilitate higher throughput drug screening (e.g., ED50 estimates)
- Slices offer an intact, anatomically-defined cell system in which to test region-specific drug effects
- Slice techniques enable testing in brain tissue of compounds which do not readily cross the blood-brain barrier

*In Vivo* Microwave Techniques

- Inactivation of brain enzymes by microwave irradiation enables estimates of *in vivo* levels of protein phosphorylation from mice treated systemically with pharmacological or physiological agents
- Microwave irradiation essential for preservation of labile phosphoproteins

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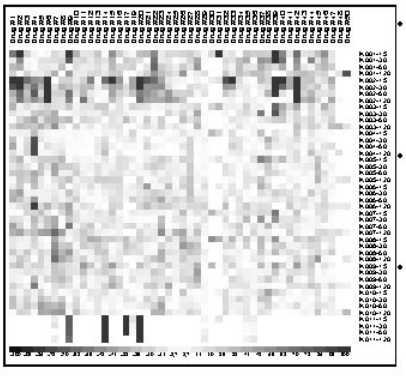
## Measuring Protein Phosphorylation



- The state of phosphorylation is detected using phosphospecific antibodies
- A panel of antibodies is used to evaluate 11 separate phosphosites.
- Spatial and temporal patterns of phosphorylation create a unique signature of drug action.

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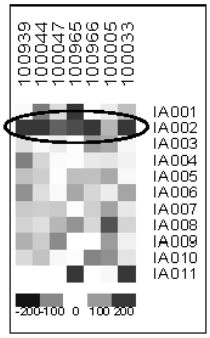
## PhosphoProfile of Antipsychotic Drug Actions

| SITE IA1002 | | |
|---|---|---|
| Antipsychotic Drug | Mean ± SEM | |
| 100939 | *182+14 | p=.0005 |
| 100044 | *270+16 | p<.0001 |
| 100047 | *171+11 | p<.0001 |
| 100965 | *167 + 19 | P<.01 |
| 100966 | *241+10 | p<.0001 |
| 100005 | *137+4 | p=.0096 |
| 100033 | *153+18 | p=.03 |

*significant at p<0.05

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## CNS Profile can be implemented to:

- Generate a molecular signature of a class of compounds to serve as a reference for screening compounds of unknown mechanism of action
- Identify novel targets for future drug discovery efforts

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## Gulf War Illness

- Gulf War Illness (GWI) is characterized by symptoms which include irritability, anxiety, headache, depression, poor concentration, memory impairments
- GWI may be associated with exposure to nerve agents (i.e., sarin), insecticides (i.e., DEET), and other compounds such as pyridostigmine bromide (PB) or a combination of these agents
- Nerve agents and PB inhibit an enzyme (acetylcholinesterase or AChE) that is critical for maintaining normal levels of the neurotransmitter acetylcholine in the brain
- As a result they cause supra-physiological levels of acetylcholine to occur in the brain and periphery

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## GWI (Continued)

- Massive increase in acetylcholine levels in the brain leads to the activation of multiple nicotinic (12) and muscarinic (5) receptors
- Increasing brain acetylcholine has a highly complex secondary response
  - Activation of nicotinic and muscarinic receptors leads to secondary release of multiple neurotransmitters, including Dopamine, Glutamate, and GABA
- Signaling pathways that mediate effects of acetylcholine under these conditions are poorly understood
- An understanding of these pathways would provide targets that would greatly advance the search for therapeutic interventions for GWI

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## ITI's efforts to identify targets for treatment of GWI

- Characterization of signaling pathways for acetylcholine
  - Pathways activated by physiological levels of acetylcholine resulting in nicotinic and muscarinic receptor activation
    - Supported by DAMD 17-03-2-0019
- Preliminary collaboration with USAMRICD to characterize pathways activated by nerve agents resulting in nicotinic and muscarinic receptor activation

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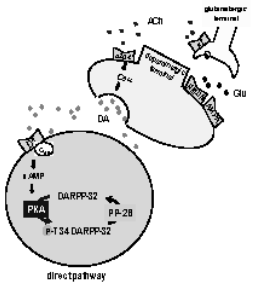
## Elucidation of Nicotinic receptor signaling pathways




A scheme for the response pathway downstream of high levels of nicotine:

- Nicotine binding to α7 nicotinic receptors stimulates release of glutamate from glutamatergic terminals,
- Glutamate binding to NMDA and AMPA receptors on dopaminergic terminals drives bursts of dopamine release,
- This high-level dopamine signal stimulates D1 dopamine receptors on direct pathway neurons, leading to elevated phosphorylation of T34.

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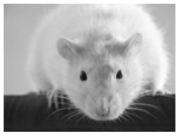
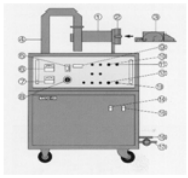
## Elucidation of Signaling Pathways Activated by Sarin Exposure

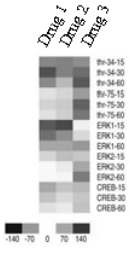


*In Vivo* Phosphoprotein Measurements
•Systemic administration of Sarin to rats in collaboration with Drs. T.M. Shih and John McDonough, USAMRICD

Focused microwave irradiation
Used to preserve phosphoproteins



PhosphoProfile of Sarin
•Monitor effect of Sarin at multiple phosphorylation sites
•Time-dependent responses (15-30 min)
•Dose responses (LD50, 0.5 LD50)
•Several brain regions including striatum

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## A convulsant dose of Sarin (LD50) dephosphorylated several phosphoproteins

*These include:*

- *Spinophilin, a protein phosphatase 1 (PP1) targeting protein*
- *DARPP-32, at T34, the site controlling PP1 inhibition*
- *Glutamate receptors (NMDA-type)*

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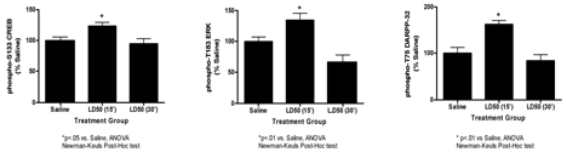
## Sarin (LD50) increases phosphorylation of several phosphoproteins

- *These include:*
  - *CREB, a gene transcription factor*
  - *MAP kinase, a growth factor-associated protein kinase*
  - *DARPP-32 at T75, a substrate for CDK5, a kinase involved in neuronal migration/development*



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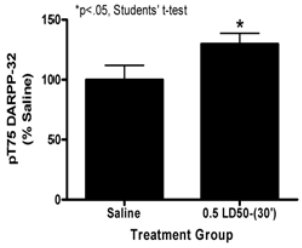
## DARPP-32 T75, a marker for Sarin exposure

- ***A sub-convulsant dose of Sarin increases <u>only</u> phosphorylation of DARPP-32 at T75, the CDK5 site***
  - *T75 may be a marker for Sarin exposure*

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## Ongoing and Future Studies

- **Continue to delineate nicotinic and muscarinic receptor pathways**
  - Continue to identify additional targets for acetylcholine receptor activation
  - Create gene 'knockouts' for each muscarinic receptor
- **Use molecular biological techniques to discover molecules that interact with nicotinic and muscarinic receptors and modify their activity**
  - 'Two-hybrid' screens

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## Strategy for Therapeutic Intervention in GWI

- **Generate complete map of cellular effects of sarin and other nerve agents**
  - Low-dose and high-dose exposure
  - Acute and chronic exposure
- **Identify targets common to these exposure conditions**
- **Screen chemical libraries to discover small molecules capable of modulating/reversing toxic effects of these agents**
  - Acute effects: anti-convulsant
  - Chronic effects: neural protectant/prophylactic
  - Test in animal models for GWI

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