



Neurotoxin Exposure Treatment Research Program (NETRP)

Background

Parkinson's Disease (PD) affects nearly one million Americans. Recent evidence that genetic transmission is a minor component of this disorder confirms the importance of environmental factors, some of which are known, such as exposures to specific toxins. Military occupational exposures may be risks for neurodegenerative disease, including some Parkinson's symptoms. Fundamental mechanisms underlying the development of PD may also be pertinent to other neurodegenerative diseases. In PD there is a loss of brain cells in the substantia nigra that contain the neurotransmitter dopamine. Insufficient dopamine neurotransmission underlies many of the symptoms of PD. New technologies such as neuroimaging and genetic knockout models, and recent advances in neurobiology such as new drugs and growth factors (e.g., GDNF) are being exploited to advance this research. This program directly contributes to common objectives in psychological stress and toxicology research in the core-funded basic research program and Gulf War Illnesses/Force Health Protection research program.

Funded Research

Early Detection

Valid and reliable biological markers of neurodegeneration (e.g., cognitive testing, neuroimaging, specific biochemical markers) are an important technological hurdle. One study tests changes in the ability to manipulate aspects of language to determine if these precede motor dysfunction, as a test for early PD-associated degeneration in the cortical dopaminergic system. A separate study will develop imaging markers diagnostic for early PD and clarify the relationship between striatal dopamine integrity and cortical activation during motor and memory tasks. Several other projects study biomarkers to detect and monitor neuronal degeneration: a marker of dopamine terminal integrity which may directly evaluate the rate of degeneration and will also be used to study the neurotoxicity of L-dopa. Another study creates a genomic database to identify unique molecular markers associated with progressive decrease in striatal dopamine integrity and consequent impaired motor and cognitive performance in inbred and knockout mouse models of PD.

Soldiers may be at risk for neurodegenerative diseases from occupational exposures to psychological stress, toxic industrial and agricultural chemicals, chemical threat agents, head injury, and even radiofrequency radiation. Parkinson's Disease (PD), as a particularly relevant disorder induced by a variety of environmental exposures, is a central focus of the research program. Basic research on mechanisms of neurodegeneration will lead to better diagnosis, treatment, and prevention.

The NETRP was funded by a total of \$70M in FY97, FY98, and FY99 special congressional appropriations to study neurodegenerative mechanisms and treatments, with a special emphasis on Parkinson's Disease. There are currently 63 discrete research projects in the program.

Pathogenesis

Oxidative Damage: A large number of studies focus on oxidative damage as a common mechanism of neurotoxicity. The brain is particularly susceptible to oxidative damage for several reasons: in addition to receiving a high percentage of the body's total oxygen, the brain is relatively deficient in catalase which protects against reactive oxygen species (ROS) damage; it's most important defense mechanism against ROS damage, the glutathione system, is easily saturated; it has a high concentration of polyunsaturated lipids, easily peroxidized by free-radicals; and regions such as the substantia nigra have high concentrations of iron which catalyzes the conversion of H_2O_2 to $OH\cdot$, allowing production of excess quantities of ROS.

Excitotoxic Damage: Increased action of excitatory amino acid transmitters such as glutamate (Glu) and aspartate may initiate or prolong depolarization of neurons, with subsequent clinical signs (e.g. seizures) or neuronal exhaustion and cellular degeneration. The ionotropic glutamate receptors (iGluR) have a primary role in fast neuronal excitation, including the excitotoxic actions of Glu. The prominent role of the NMDA-preferring class of iGluR in the pathophysiology of excitotoxic neurodegeneration is being studied to develop potentially neuroprotective agents.

Apoptosis: Many studies in the program specifically investigate aspects of apoptosis, a process in which developmental cues and environmental stimuli initiate a genetically established cascade that results in cellular degeneration and cell death. Several studies concentrate on interactions of apoptotic cascade events while others focus on mitochondrial intervention points.

Accumulation of Intracellular Aggregates and Other Cell Damage: Mechanisms of cellular disruption are being studied in four cellular protein systems (alpha-synuclein, tissue plasminogen activator, p38 protein kinase family, and neurotoxic esterase). Studies of the alpha-synuclein gene family will investigate expression/cell distribution, mutations, and over-expression on neurodegeneration. Another study tests the hypothesis that gene expression and functional up-regulation of specific neural sodium channels are contributors to neuronal cell death after cerebral ischemia.

Etiologies

Stress: Early markers of neurodegeneration, such as hippocampal changes and memory impairment, are being studied in a social mouse model of combat stress. Regional susceptibility of the nigrostriatal system to damage after prolonged periods of stress is being studied in 6-OHDA lesioned rats. Another study investigates beneficial short-term and deleterious long-term actions of acetylcholinesterase (AChE) in animals subjected to acute psychological stress or anticholinesterases. Stress, AChE inhibitors (chlorpyrifos), and fuel additives (which may synergistically promote degeneration in structures such as the hippocampus) are being investigated for changes in behavior and CNS excitability. Other studies include the construction of a tetracycline-inducible vector system in which either an anti-sense message for the AChE or a ribozyme directed against the AChE will be expressed via a plasmid injected into the hippocampus.

Toxins: Insecticides (permethrin and chlorpyrifos), in combination with MPTP, will be studied to clarify whether insecticide exposure can intensify development of PD and will provide a full dose-effect curve useful in extrapolating animal data to man for risk assessment. Mechanisms by which mustard chemical warfare agents induce neuronal cell death (a model for neurotoxic DNA damage) are being studied using mice deficient in DNA repair mechanisms. Another study examines synergistic effects of excitotoxicity, free radicals, and a depleted bioenergetic state in causing dopaminergic cell death. A sample of men who have been well characterized by serial neurologic examinations over a 26-year period, will be used to correlate environmental and occupational risk (including pesticide residues in brain tissue) for development of PD and related neurodegenerative disease.



New technology is increasing soldier exposures to radiofrequency radiation. Nonthermal effects on neural tissue are being actively investigated for militarily relevant frequency and power mixes.

Trauma: Mechanisms of cell death due to traumatic brain injury will be studied and may lead to identification of optimal brain region targets for calpain inhibitors to prevent further neurodegeneration in already damaged brain tissue.

Viral: Animals infected with Venezuelan equine encephalitis virus (VEE) variants will be studied for gradations of neurotoxicity resulting from glial cell activation, including assessment of alterations in production of cytokines and reactive nitrogen intermediates that influence neuronal degeneration in VEE infection.

Therapeutic Strategies

Interventions Targeting Specific Mechanisms of Damage: Several studies test treatments for excitotoxic damage (including neuroprotective effects provided by a metabotropic GluR (mGluR)



Chronic psychological stress may arise from multiple sources including conflicting new mission roles, isolation, family separation, anxiety, and trauma.

agonist); neuroprotectant combinations of vitamin E, Co-enzyme Q, melatonin, FK506, and benzamide; Huperzine A analogs to shield glutamate ionotropic receptors in cultured neuronal cells; and efficacy of NMDA receptor agonist drugs to mitigate excitotoxic neuronal damage (from retinal laser irradiation). The mechanisms by which transforming growth factors protect neurons against excitotoxic damage and induce motor axon growth using gene transfer of GDNF or Bcl-2 into nigral neurons in animal models of PD are also being studied in a comparison of gene delivery methods:

encapsulated BHK cells genetically modified to secrete GDNF and intrastriatal infusion of an adenovirus GDNF transgene vector in aged monkeys. One project investigates mechanisms by which some neuronal cell lines resist ROS damage following exposure to the neurotoxins nitrogen mustard and arsenite.

Neuroprotectants: Potential neuroprotective effects of progesterone, based on recent evidence that progesterone decreases excitotoxicity leading to neuronal cell loss, will be explored to determine if the effects are direct or involve neurosteroid metabolites acting on GABA receptors. Another project investigates the effects of glycosphingolipids (GSL) using two unique insect model systems that permit structure-function studies of GSLs on growth and repair of nerve cells. A study investigating liposome delivery systems for a recombinant enzyme (OPAA-2) may greatly increase neuroprotection against alkyl-phosphate chemicals, and the protective actions of various anti-inflammatory drugs, anticonvulsants, and glutaminergic potentiator agents (ampakines) will be studied to identify neuroprotectant drugs which will not block other important receptors or signal transduction systems.

Restoration of Dopaminergic Secretory Capabilities: Replacement graft strategies have suffered from poor survival, insufficient axonal outgrowth from surviving neurons, poor functional recovery provided by the procedure, and disabling side effects such as dyskinesia; however, functional recovery associated with surviving grafts encourages further work. Several studies attempt new approaches to improve transplant survival including use of caspase inhibitors, regulation of Bcl-2 and GDNF to improve survival of transplanted neural cells, and identification of neurotrophic factors in other types of brain cells (O2-A cells). A study of differentiation of mesencephalic progenitor cells will attempt to create new dopaminergic neurons induced by hematopoietic cytokines such as IL-1.

