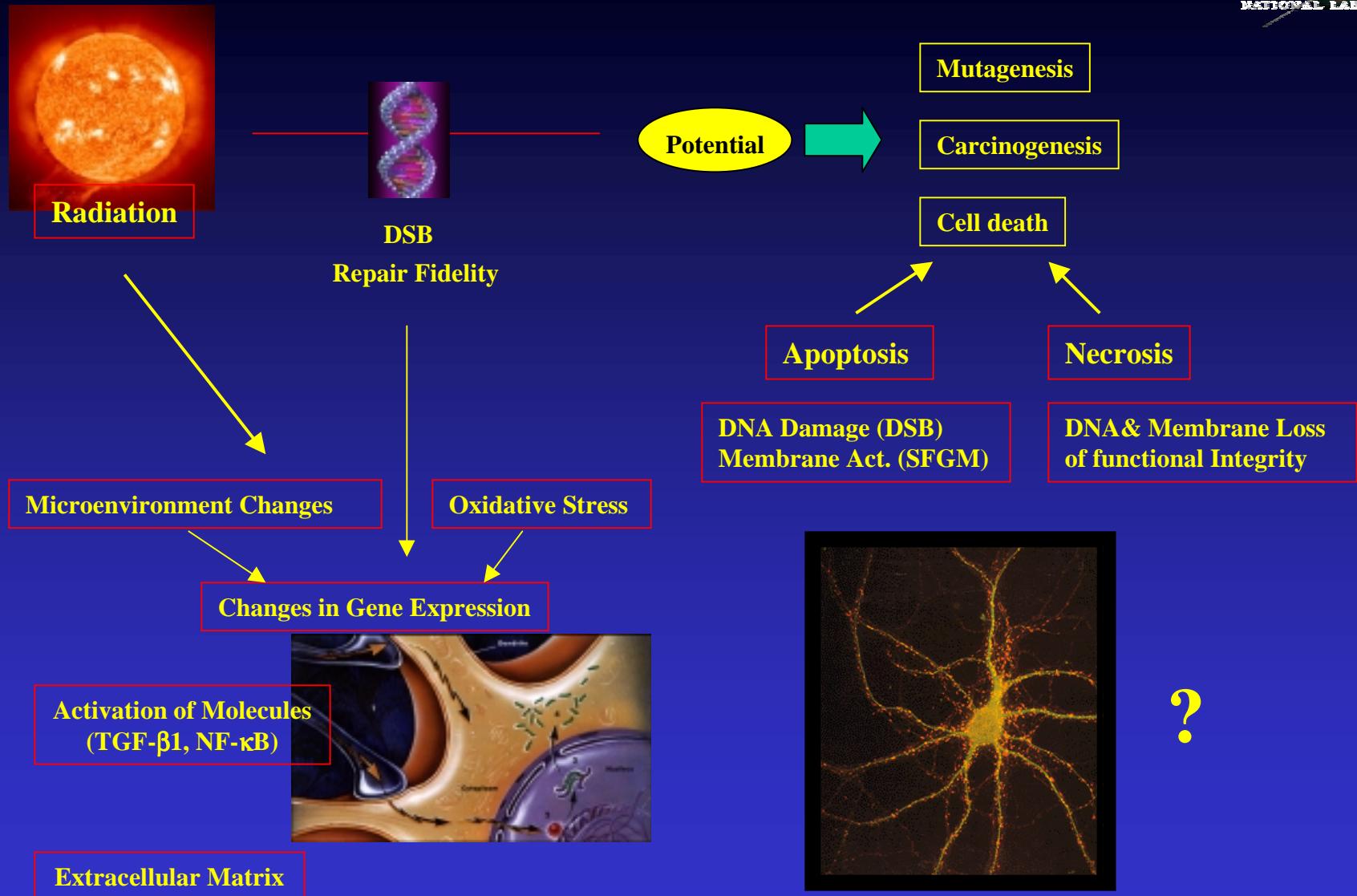


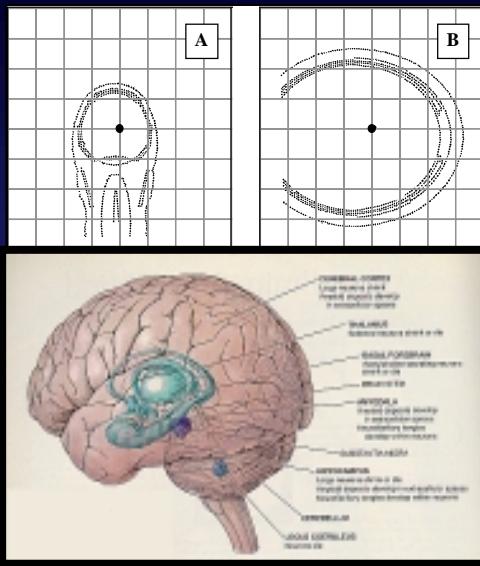


Heavy Ion Effects on Neuronal Cell Populations and Neuroprotection Strategies

**MARCELO E. VAZQUEZ, MD, Ph.D.
Medical Department**



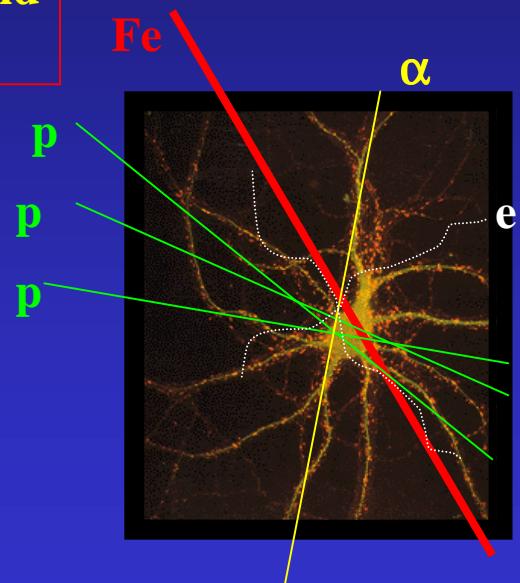
Cosmic ray hit frequencies in CNS critical areas



Three-year mission to Mars:

- Considering cell cross section 60 or $470 \mu\text{m}^2$
- Solar Minimum & Maximum
- $1-5\text{g/ cm}^2$ aluminum shielding
- NASA-CAM-CAMERA, HZETRN

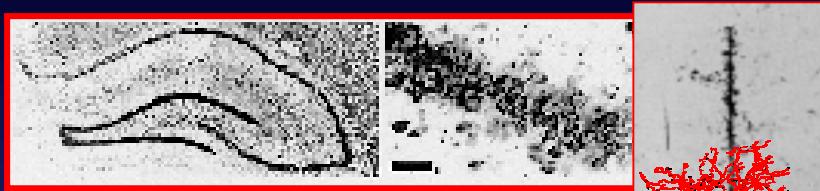
Mixed Field Multi-hit



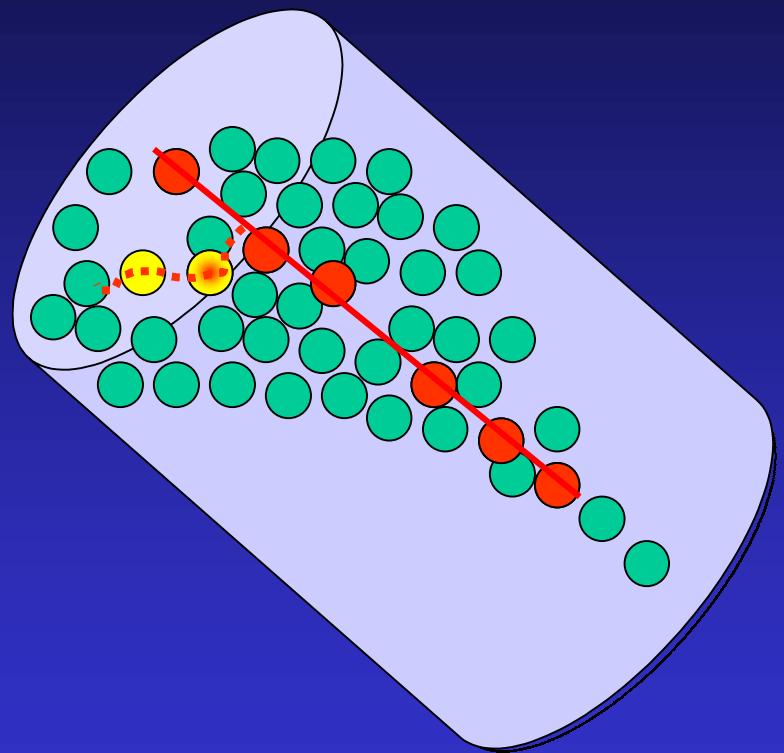
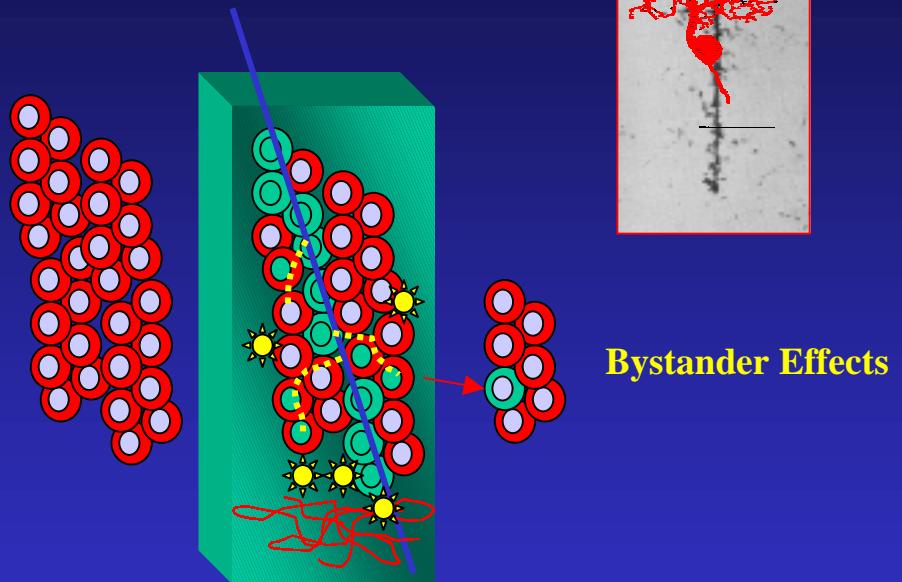
CNS in General

- 2 or 13% cells will be hit at least one Fe particle
- 8 or 46% would be hit by at least one particle with $Z \geq 15$
- Every nucleus will be traversed by a proton once every 3 days and a alpha particle once every 30 days.

High-LET HZE Effects



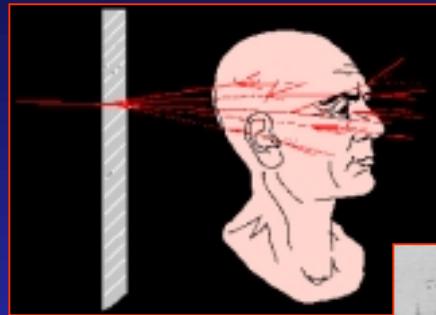
TRACK STRUCTURE - MICROLESION



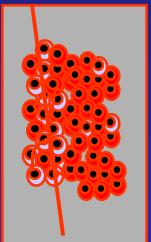
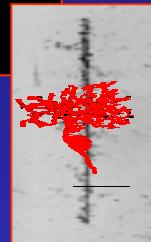
No comparable to Low-LET Effect

Dose or Fluence ?

Space Radiation Effects on CNS



- Heavy Ions
- Protons
- Neutrons
- Photons



• Stress

• Microgravity

- In Vitro Models
- In Vivo Models

• Neurotoxicity



Neurons

Oligodendrocytes

Endothelia

In Vitro Studies

ω Ar (570 MeV/n)

Cerebellum cultures (5 - 60 krad) (Mamoon et al.) 1969

ω He (365 and 54 MeV/n)

Cerebellum and Midbrain explants (1 - 40 Gy) (Mamoon et al.) 1969

ω Fe (600-1000 MeV/n)

Chick Retina explants, NT-2 cells (0.2 - 4 Gy) (Vazquez et al.) 1997-1999

ω C (290 MeV/n)

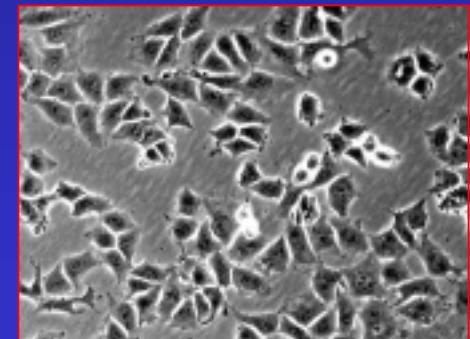
Mouse Brain Cells (2 - 16 Gy) (Nojima et al.) 1998

Chick Retina explants (0.1 - 4 Gy) (Vazquez, Furusawa, Saito) 1998

NT-2, NHNP cells (0.0017 - 1.7 Gy) (Vazquez, Majima) 2000

ω Ne (600 MeV/n)

NB41A3 Neuroblastoma (12 Gy) (Gauger et al.) 1986

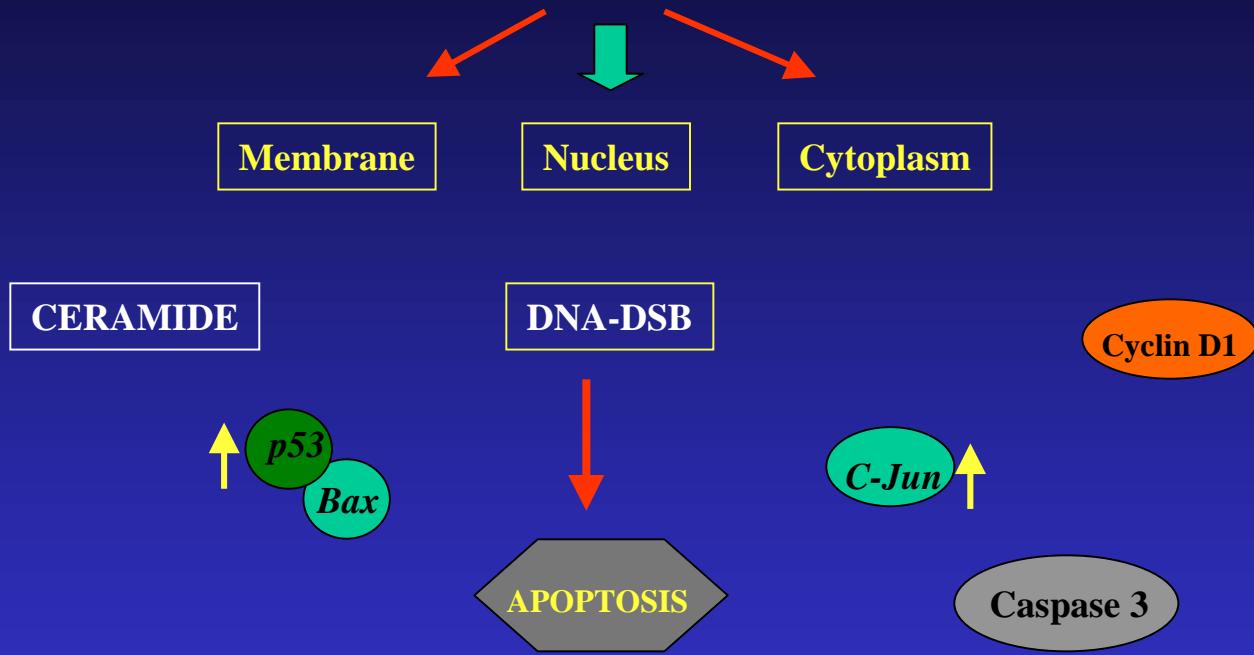


Low-LET In Vitro Studies



Min Dose (Gy)	Source	Model	Endpoint	Author
10	¹³⁷ Cs	Hippocampal Mice wt, p53-/-, Bax-/-	Survival, Apoptosis	Johnson et al., (1998)
2	x-ray	Rat Cortical	Survival, Apoptosis, DNA-DSB	Gobbel et al., (1998)
2	x-ray	Chick Retinal Explants	Apoptosis, Survival, Neuritogenesis	Vazquez et al., (1998)
2	¹³⁷ Cs	NT2 Cell	Apoptosis, Survival	Burger et al., (1998)
2	x-ray	Hippocampal Pyramidal Neurons	Survival, Apoptosis, DNAB Gene expression	Jordan et al., (1997)
1	¹³⁷ Cs	Mouse Prim. Cerebellar Neurons, wt, p53+/-, p53-/-	Survival, Apoptosis	Enokido et al., (1996)
1	x-ray	Cerebellar Granule Cells	Survival	Gelman et al., (1996)
0.5	x-ray	Mouse Brain Aggregates	Survival, NGF, DNA, proteins	Dimberg et al., (1992)
1	⁶⁰ Co	Rat Hippocampus Organotypic cultures	Morphology (EM)	Hamdorf et al., (1992)
7	¹³⁷ Cs	Rat Cerebral Cortical Neurons	DNA topoisomerrase I	Ivanov (1988)

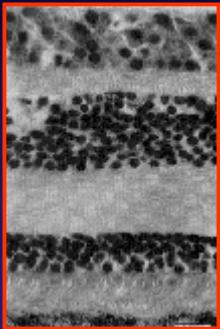
Low-LET Radiation Induced Apoptosis



High-LET Radiation Induced Apoptosis in Neurons ?

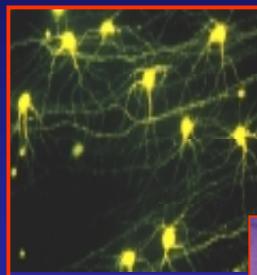


ORGANOTYPIC CULTURE SYSTEMS



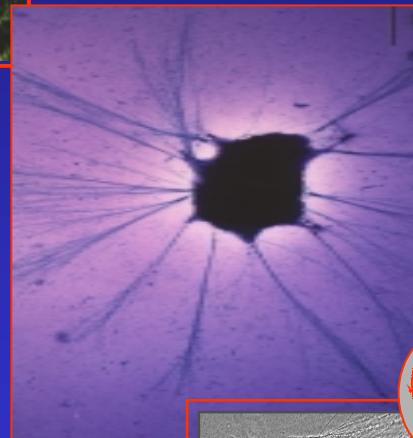
CNS MODEL:

- NEURORETINA



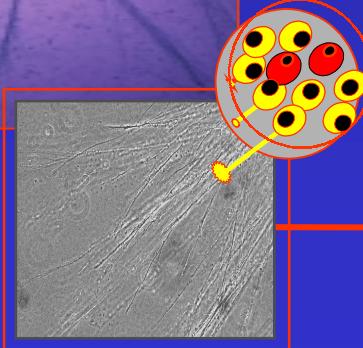
TARGET CELL POPULATION:

- POST-MITOTIC RETINAL GANGLION CELLS



IN VITRO MODEL:

- RETINAL EXPLANT

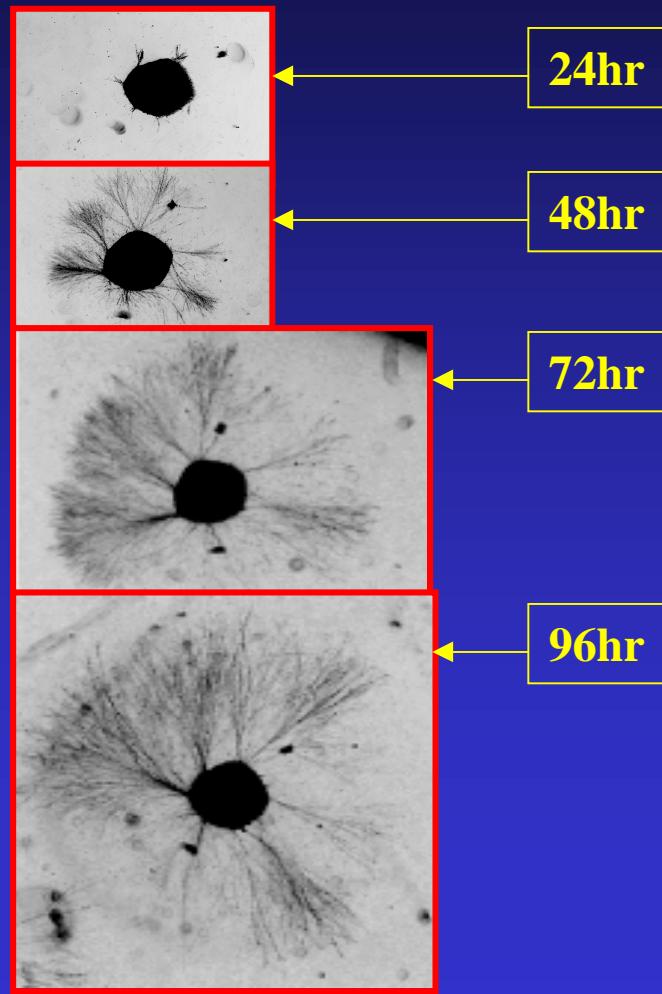


CELLULAR ENDPOINTS:

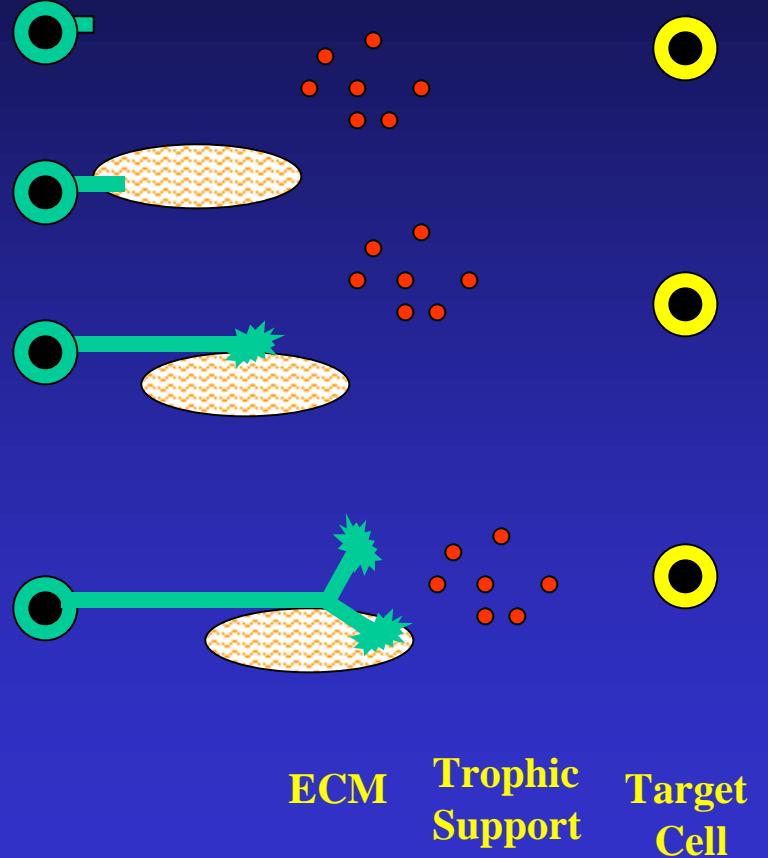
- NEURITE OUTGROWTH
- NEURONAL VIABILITY



ORGANOTYPIC CULTURE SYSTEMS

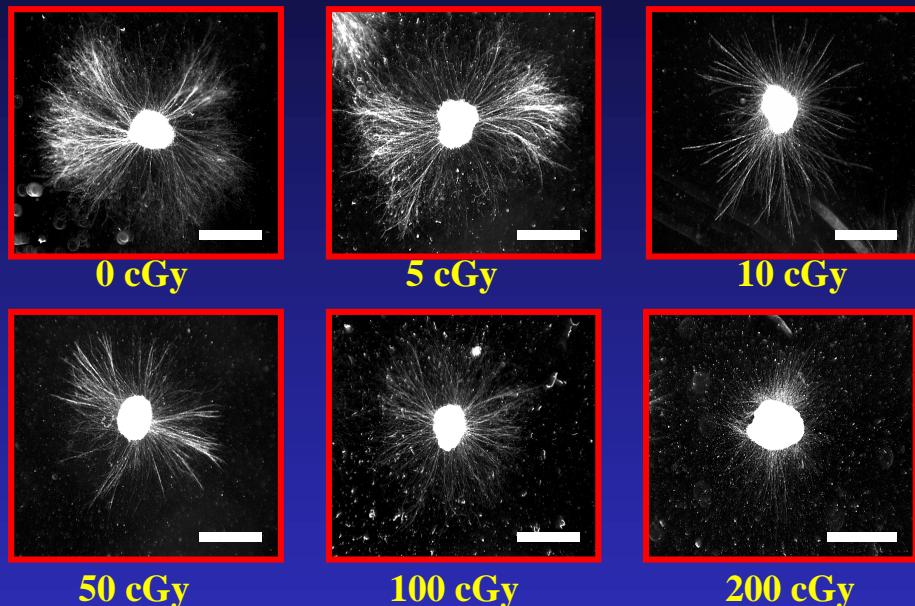
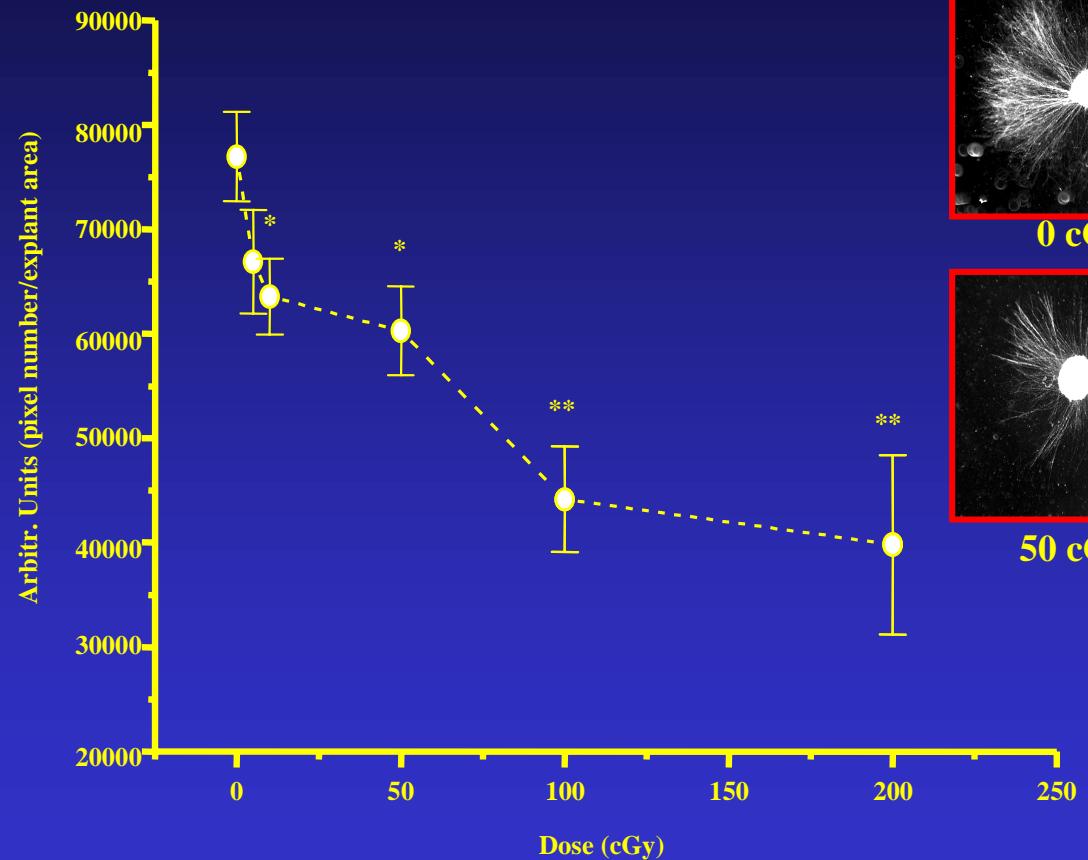


NEURITOGENESIS



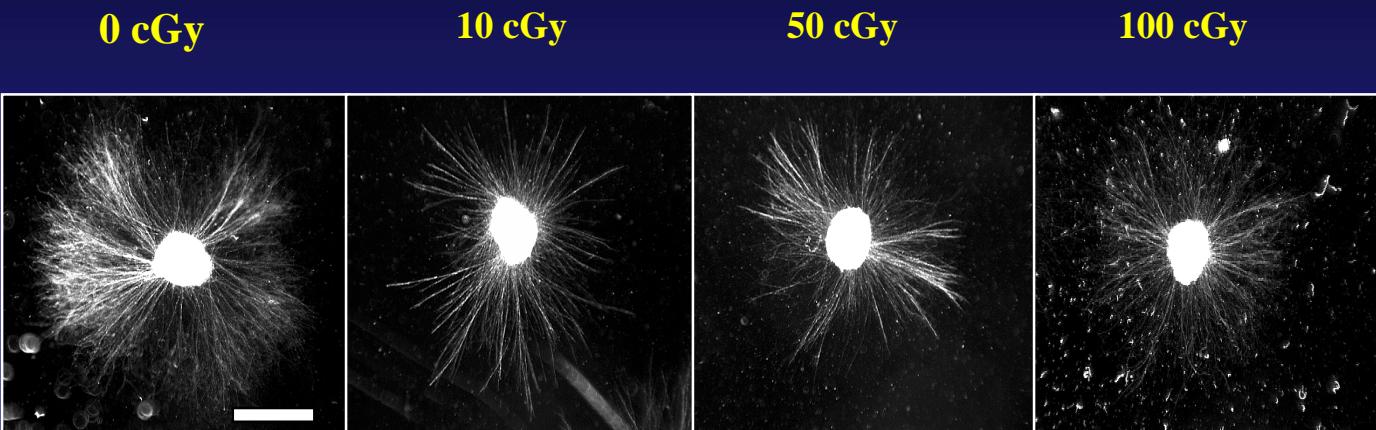
In Vitro Neurotoxic Effects of ^{56}Fe Ions on Retinal Explants

DOSE vs NEURITE GROWTH INDEX

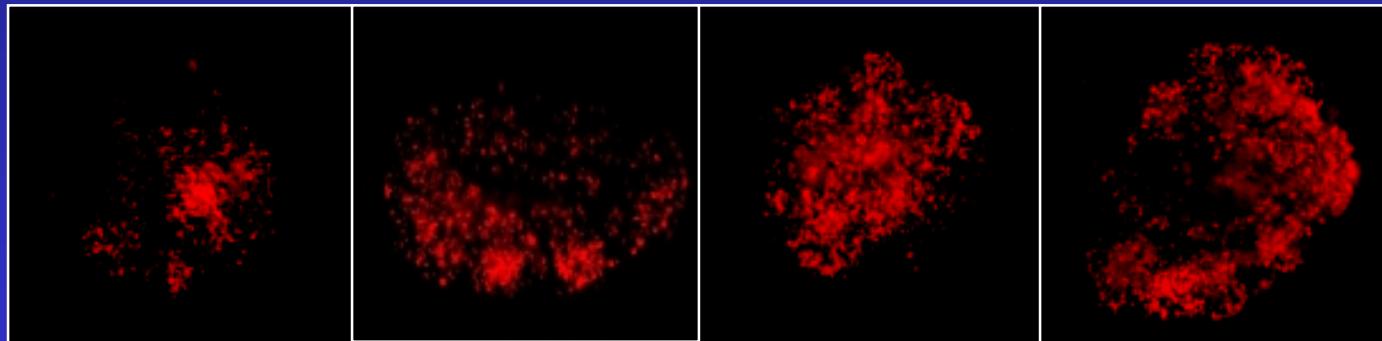


EFFECTS OF 1 GeV/n Fe IONS ON RETINAL EXPLANTS

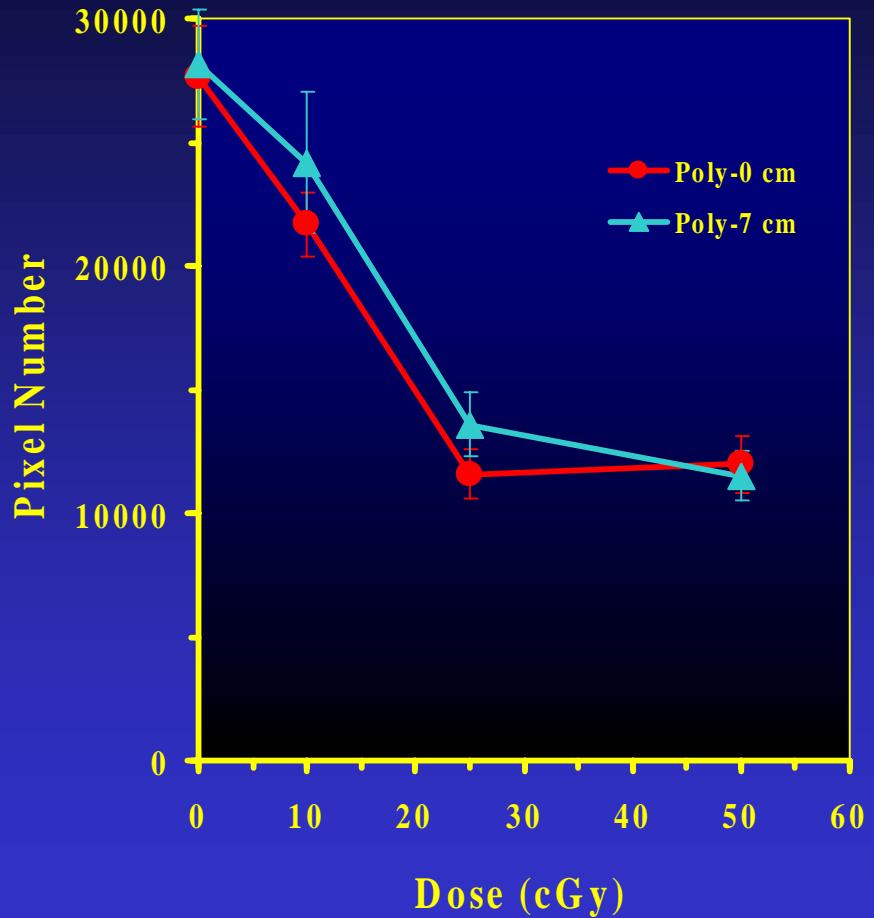
DARK FIELD MORPHOMETRY



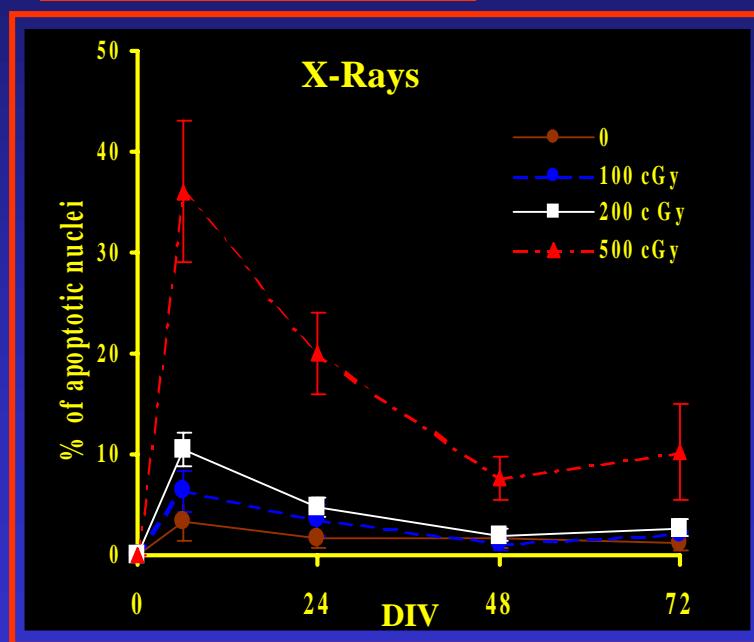
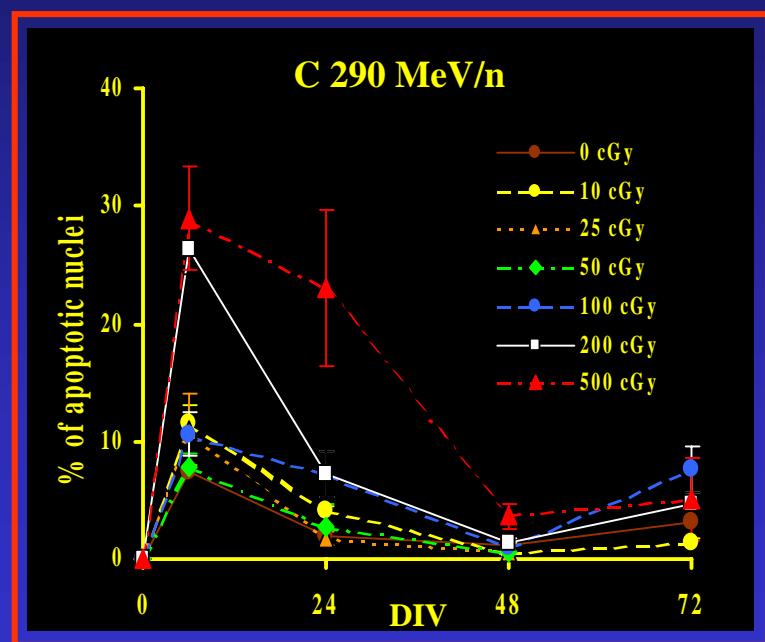
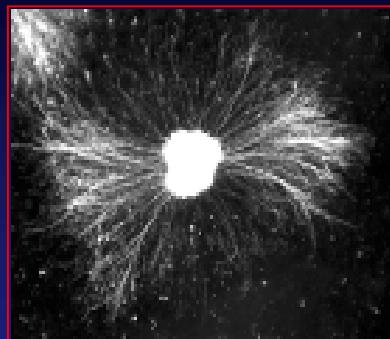
PI TEST



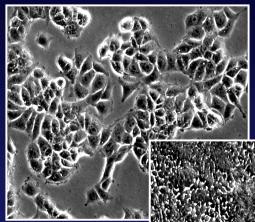
EFFECTS OF FRAGMENTED 1 GeV/n Fe ION BEAM ON RETINAL EXPLANTS



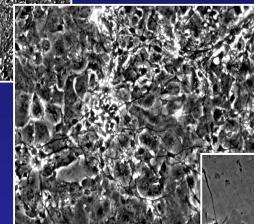
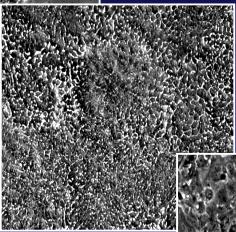
RADIATION-INDUCED APOPTOSIS IN RETINAL EXPLANTS



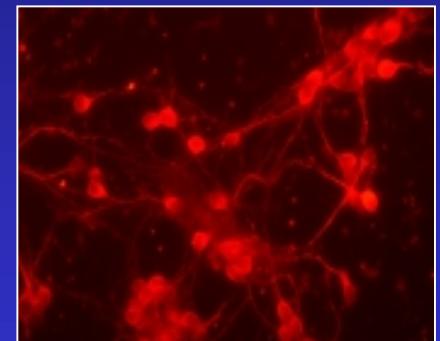
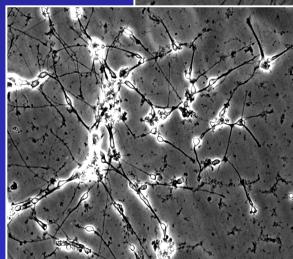
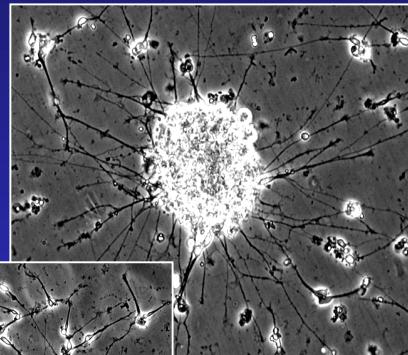
IN VITRO MODELS



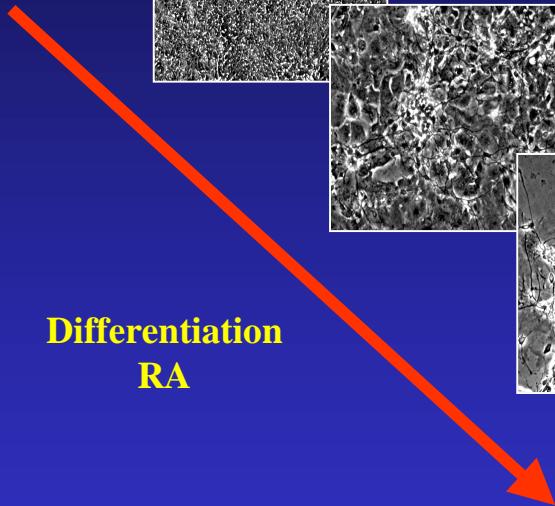
NT2 Human Neural
Stem Cell



Rodent dopaminergic
primary cultures



Differentiation
RA

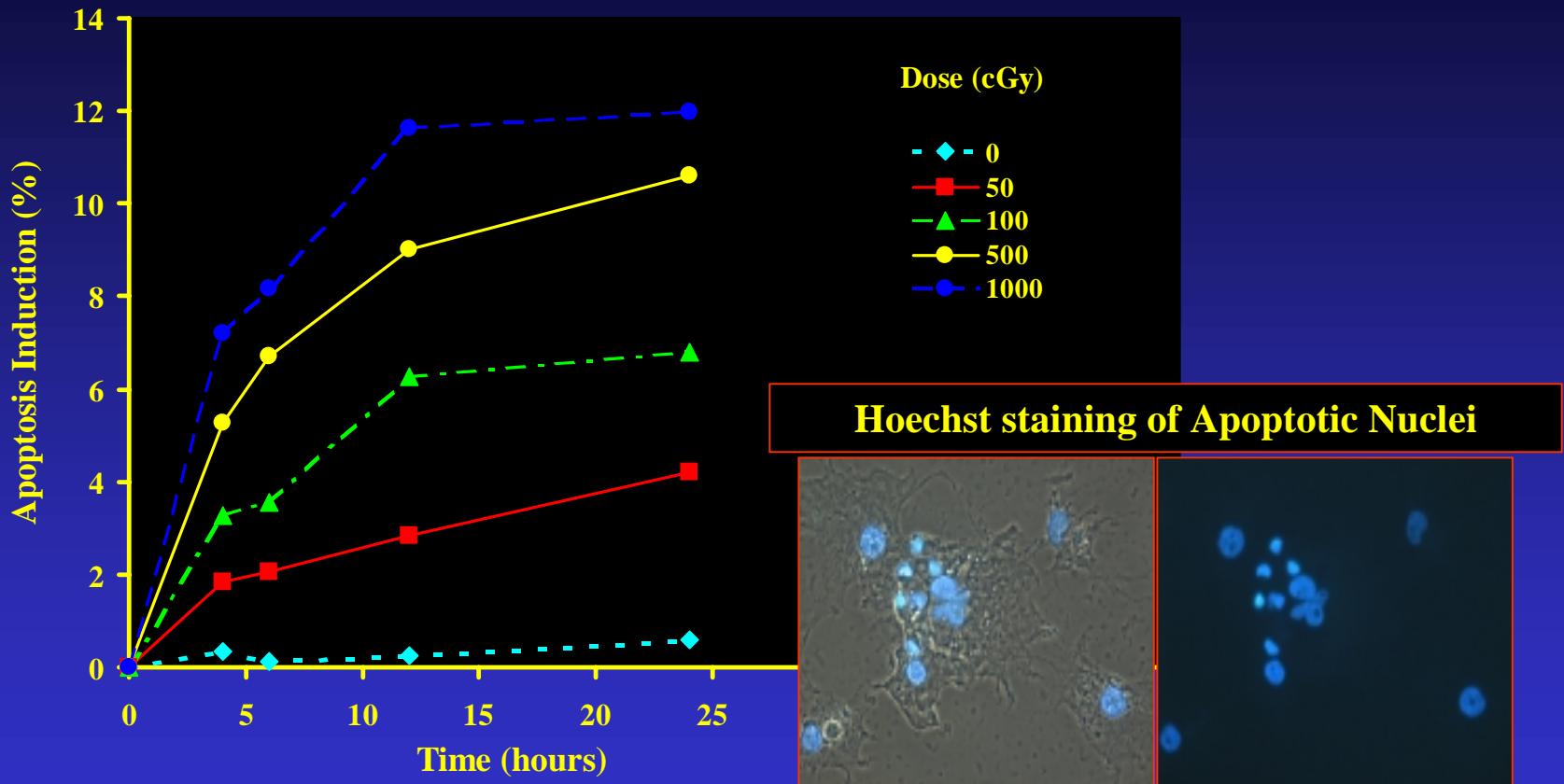


hNT Human
Neuron

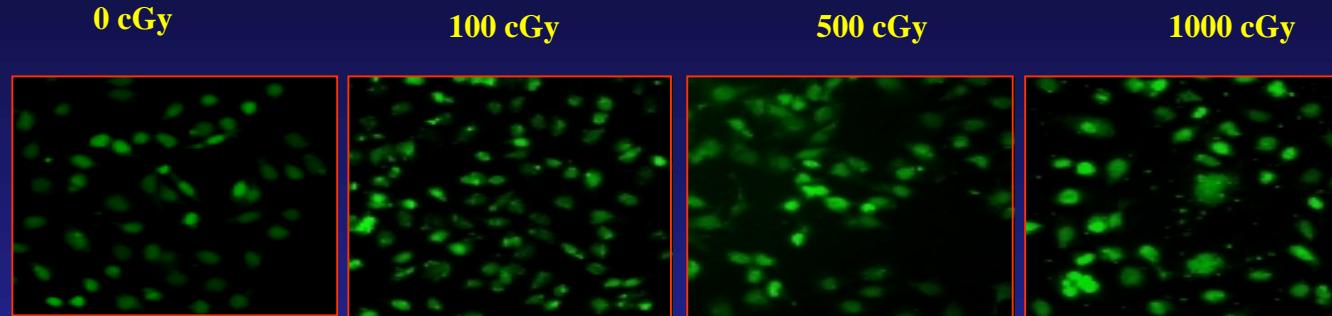


Dopaminergic phenotype

Dose-response and time-course of radiation-induced apoptosis in NT2 cells

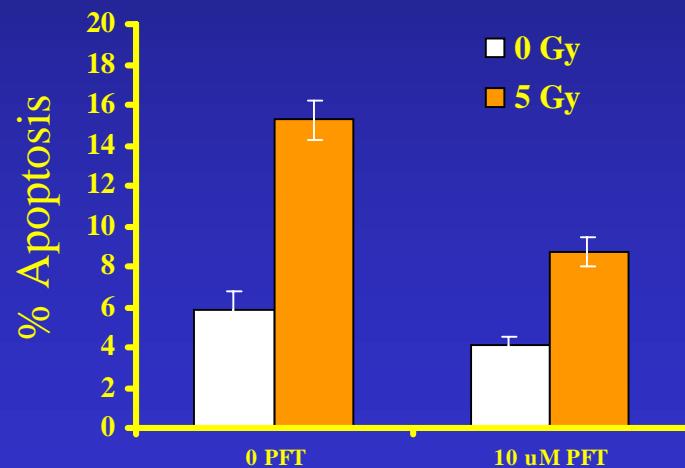


Dose-response and time-course of radiation-induced p53 expression in NT2 cells (x-rays)



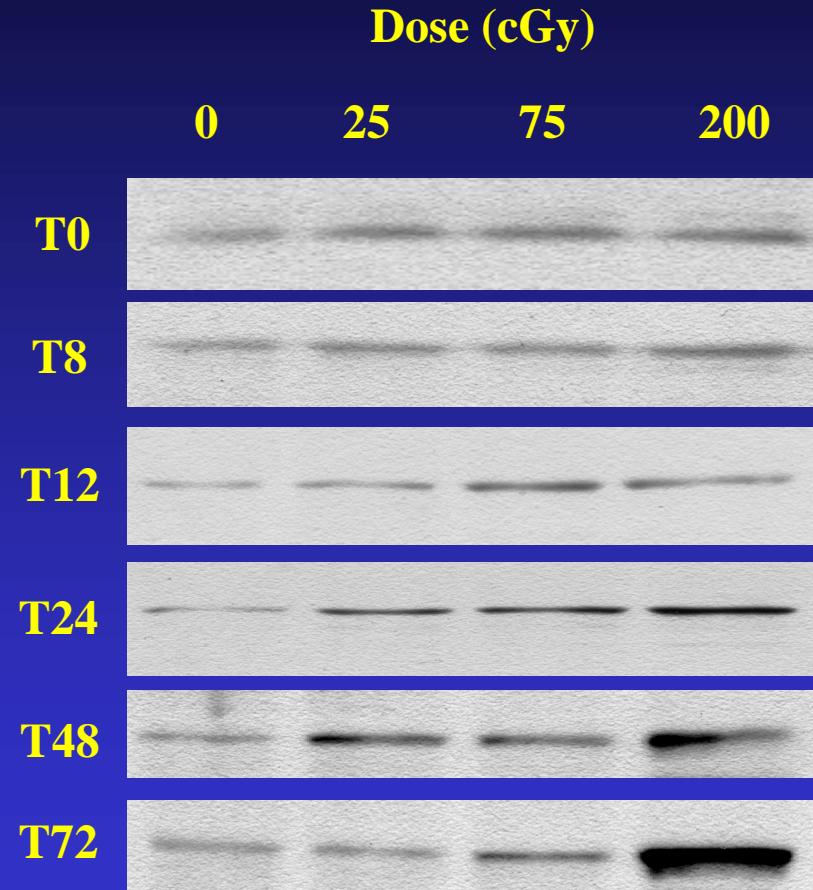
IMMUNOCYTOCHEMISTRY

PFT α (p53 modulator (-))
Reduces Radiation
Sensitivity in NT cells



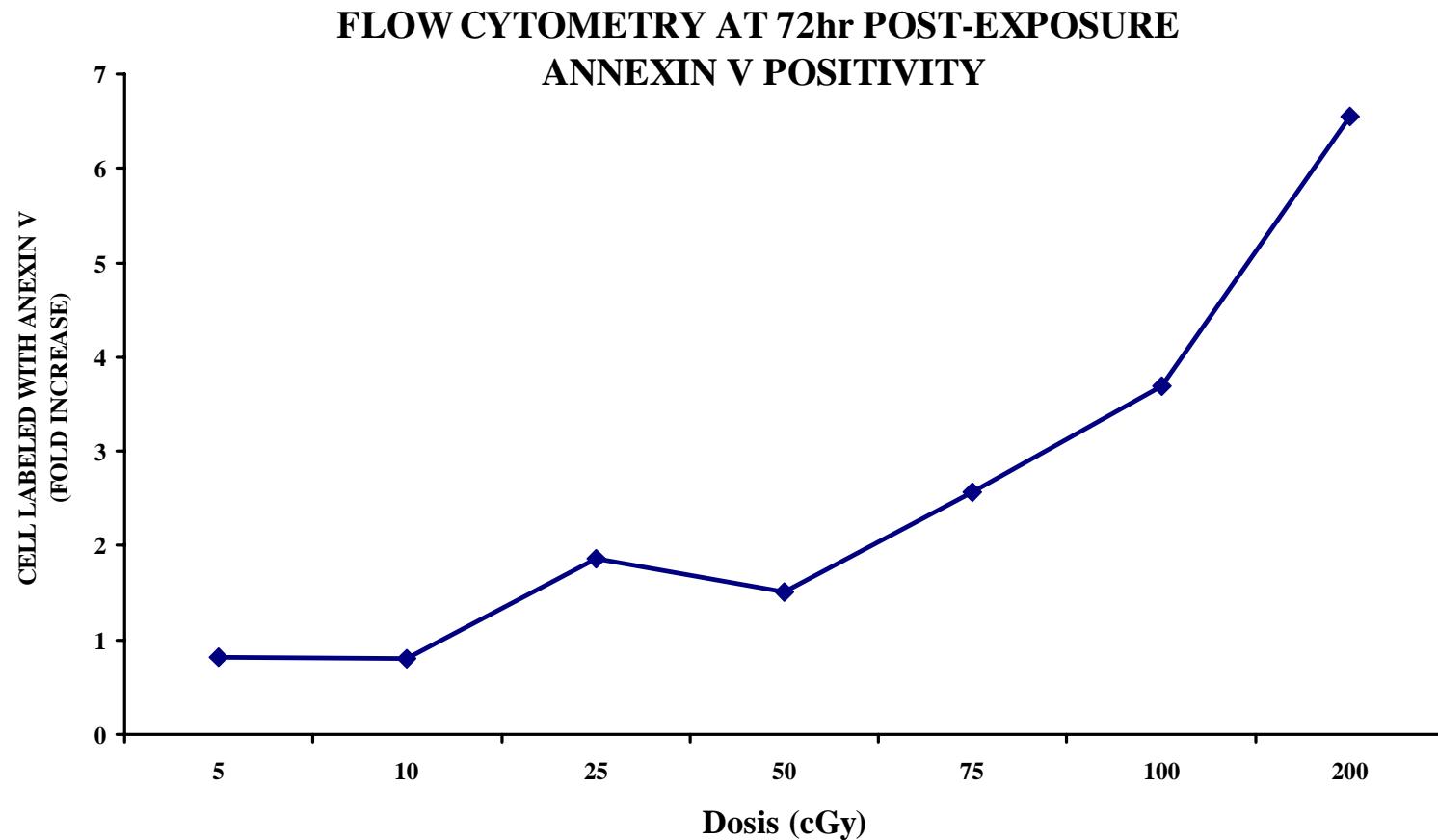


EFFECT OF Fe IONS ON THE EXPRESSION OF p53 IN NT-2 CELLS





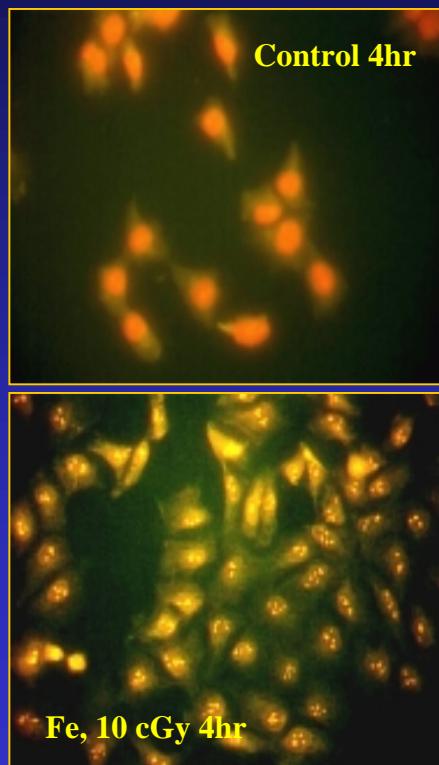
Dose-response of iron-induced apoptosis in NT-2 cells at 72 hr.



Effects of 1 GeV/n Fe Ions on NT2 Stem Cells

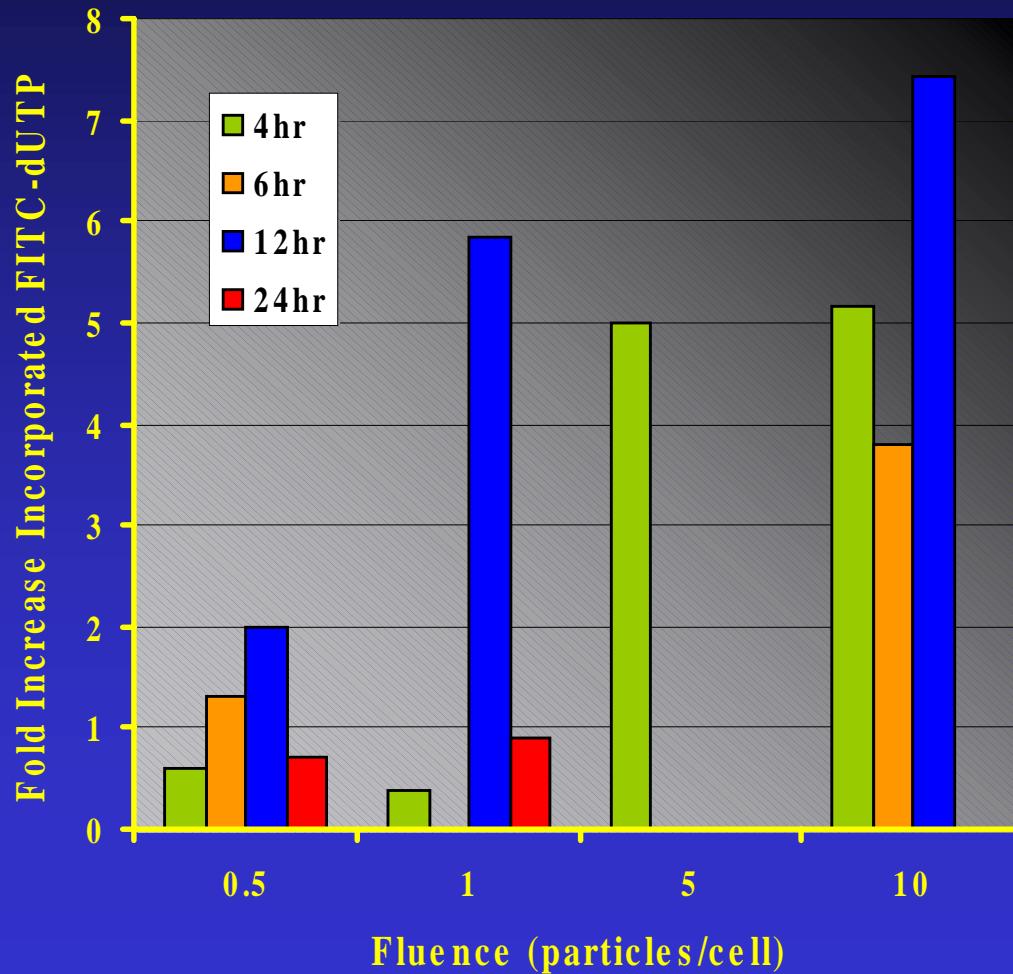
Apoptosis Induction

APO-DIRECT-LSC



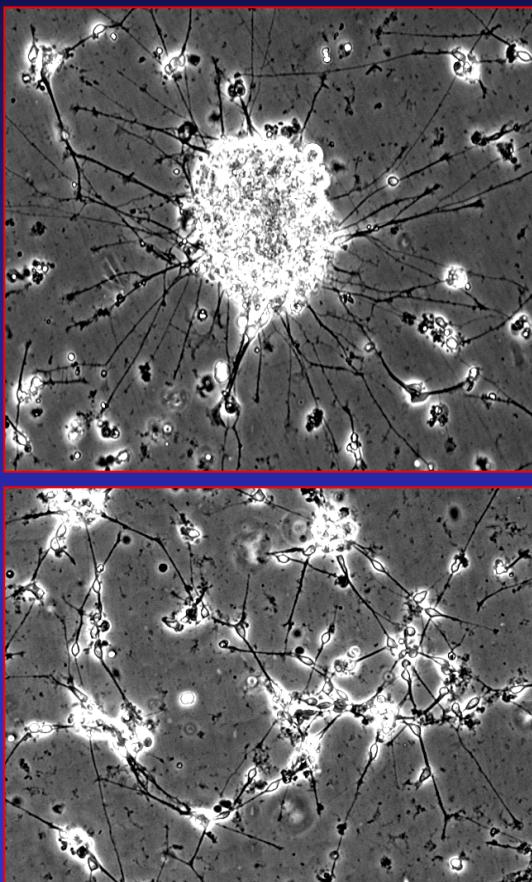
Method: The assay is based on the ability of TdT to incorporate FITC-conjugated dUTP into free 3' ends of nucleic acids.

Quantification: Laser Scanning Cytometer (LSC)

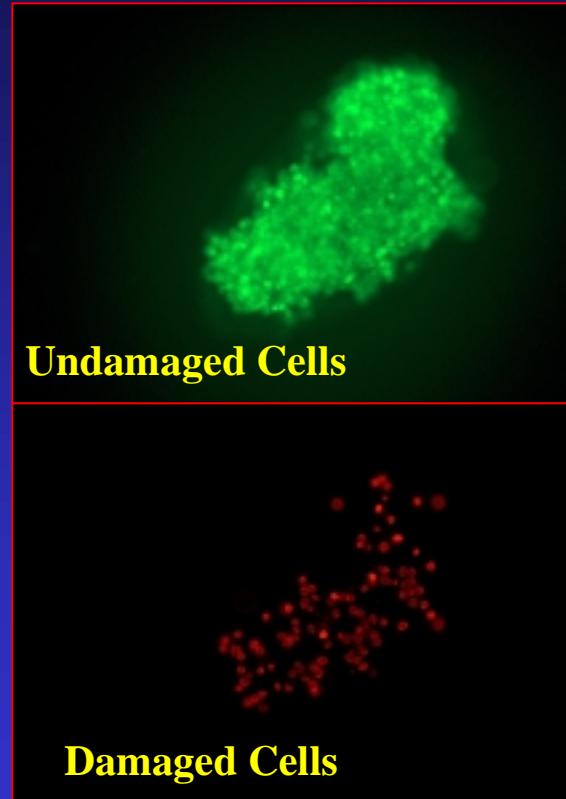


Effects of X-Rays on hNT Human Neuronal Cells

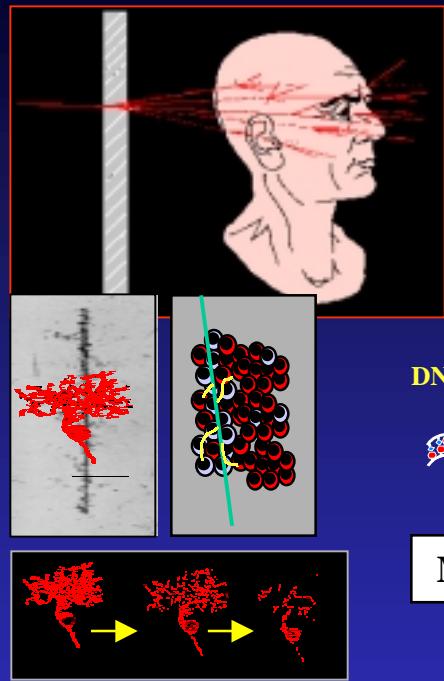
Cell Damage



100 cGy
Live and Dead Differential Staining



Research Strategies



- Heavy Ions
- Protons
- Neutrons
- Photons

• Microgravity



Validation

Testing

• Countermeasures

Risk
Prediction

• Markers

System

Cellular

Molecular

• Neurotoxicity

In Vivo Models

In Vitro Models

Behavior

Neuroimaging

Shielding