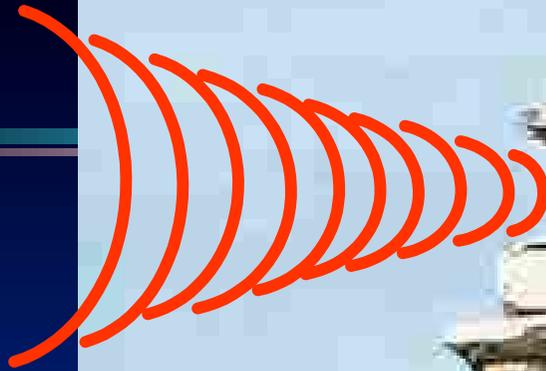


In Vitro Neurotoxicology: Potential
Applications to Space Radiation
Health Risks

E. Tiffany-Castiglioni

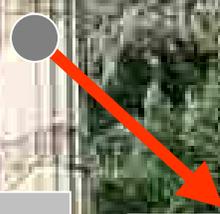
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NEURONS



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**Oligodendroglia
and Schwann
Cells**
• Myelination

**Astroglia and Radial
glia**
• Neurotransmitter
metabolism
• Blood brain barrier
structure
• Stress responses
• Neuronal migration

Microglia
• Inflammatory
responses

Cellular Effects of Ionizing Radiation on Brain Cells

Oligodendroglia

Demyelination

Apoptosis followed by proliferation

Loss of oligodendroglia progenitor cells

Astroglia

Reduced precursor population

Disruption of radial glia

Later gliosis or glial activation

Altered metabolism (Ca, stress responses, glutamate)?

Glial tumor formation?

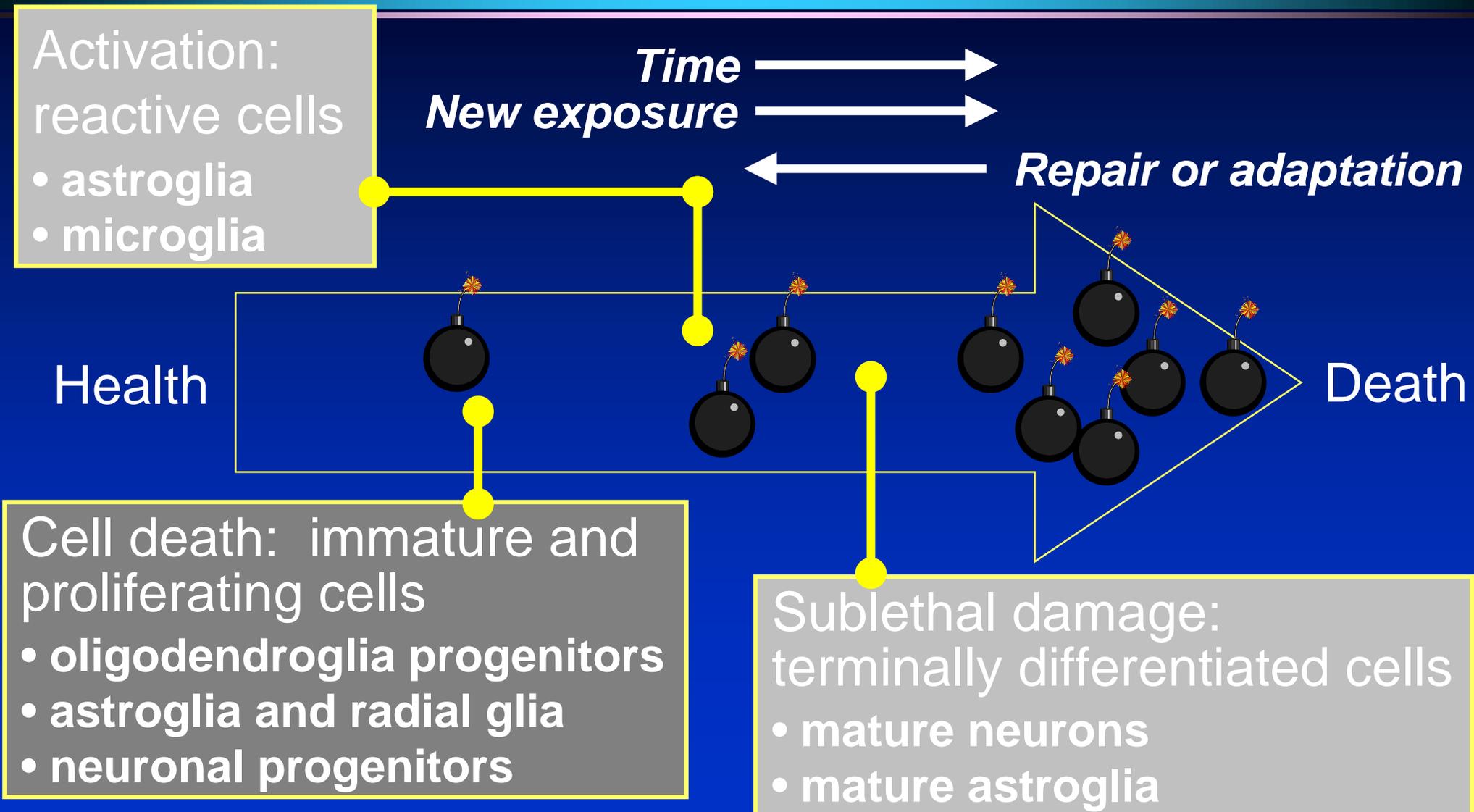
Neurons

Apoptosis of neuronal stem cells

Impaired neuronal migration (secondary effect)

Late onset cognitive dysfunction

Cumulative Radiation Damage in Brain



In Vitro and In Vivo Neurotoxicology

- Features of in vitro models:
 - ◆ individual cell types
 - ◆ ease of direct observation
 - ◆ defined extracellular environment
 - ◆ direct interactions of toxicant with test cells
 - ◆ reduced requirement for test chemicals
- Features of in vivo models:
 - ◆ behavioral endpoints
 - ◆ heterogeneous cell-cell interactions
 - ◆ systemic endocrine and nervous control
 - ◆ metabolism and distribution of toxicants
 - ◆ appropriate age and developmental stage

(Reviews: Harry et al., 1998; Tiffany-Castiglioni et al., 1999)

The Dichotomy

- “Anything a cell is seen to do in culture must be counted among its potentialities.”
-- *Margaret Murray*
- “But, from a fundamental viewpoint, it is only when cells in vitro express their normal functions that any attempt can be made to relate them to their tissue of origin.”
-- *R. Ian Freshney*



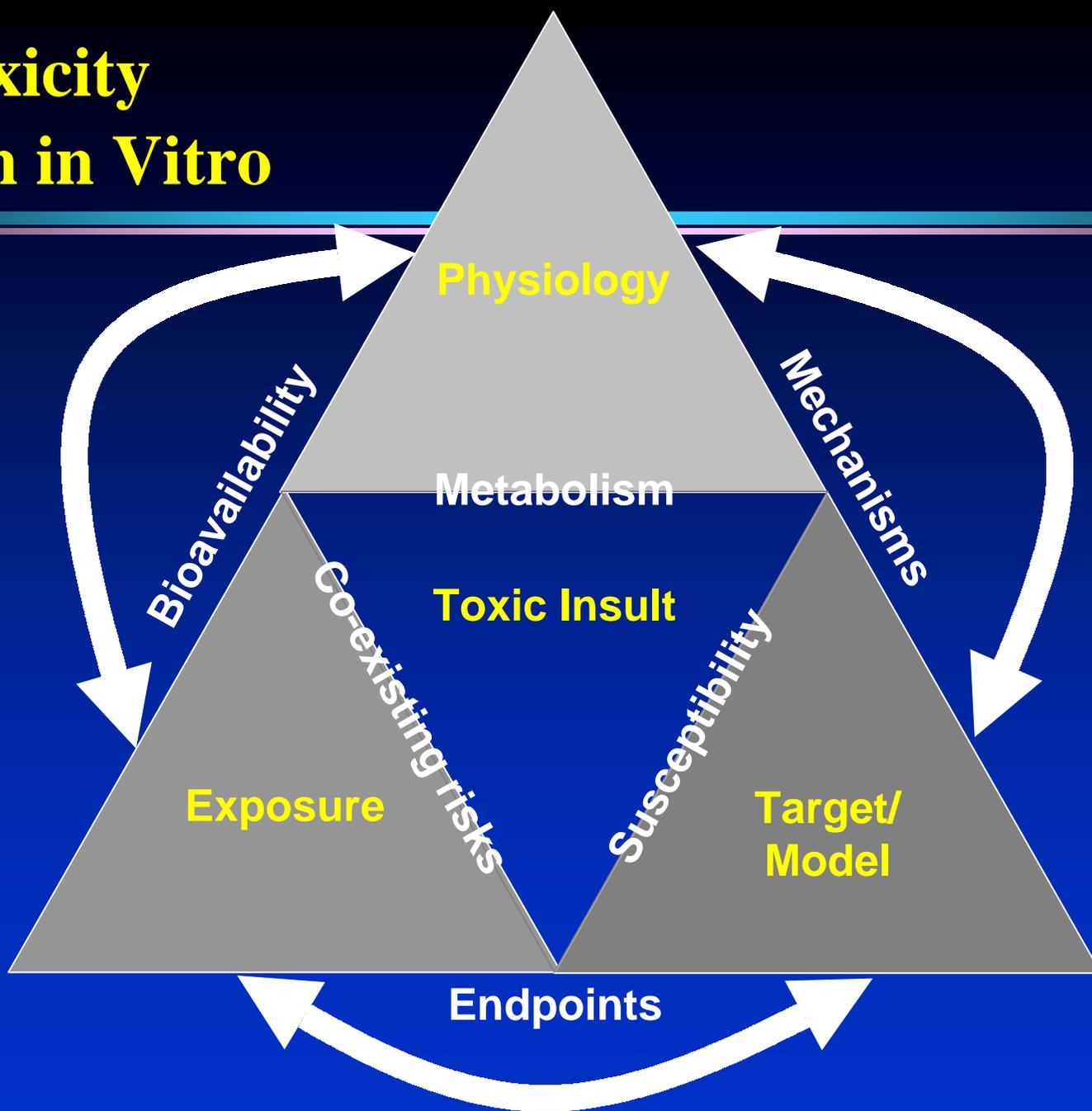


Trends in In Vitro Assessment of Neurotoxicity

- Refinement of endpoints
 - ◆ from cytotoxicity to molecular targets (fine dissection of biochemical mechanisms)
- Increased complexity of in vitro models
 - ◆ from cell lines to organotypic cultures
 - ◆ from individual cell types to cells in mixed communities
- Emphasis on validation
 - ◆ dosimetry, pharmacodynamics, developmental relevance
- New applications
 - ◆ mechanism-based therapies

(Review: Tiffany-Castiglioni et al., 1999)

Neurotoxicity Research in Vitro



Metabolism

- Toxic by-products of ionizing damage
- Heterogeneous cell-cell interactions
(co-dependent metabolic pathways)

Toxic Insult

Ionizing radiation

Co-existing risks

- Microgravity
- Other oxidants
- Endogenous metals

Susceptibility

- Species
- Cell type
- Age (development and aging)

Potential Neuronal Targets for Damage by Ionizing Radiation

- Perturbation of synaptic overproduction and pruning by developmental exposure
- Perturbation of plasticity and repair
- Synaptic function (presynaptic neurotransmitter release, post-synaptic receptor function, post-synaptic intracellular signaling)

*Digression for tutorial sonnet on
neuritogenesis*

“Sonnet to a Growth Cone”

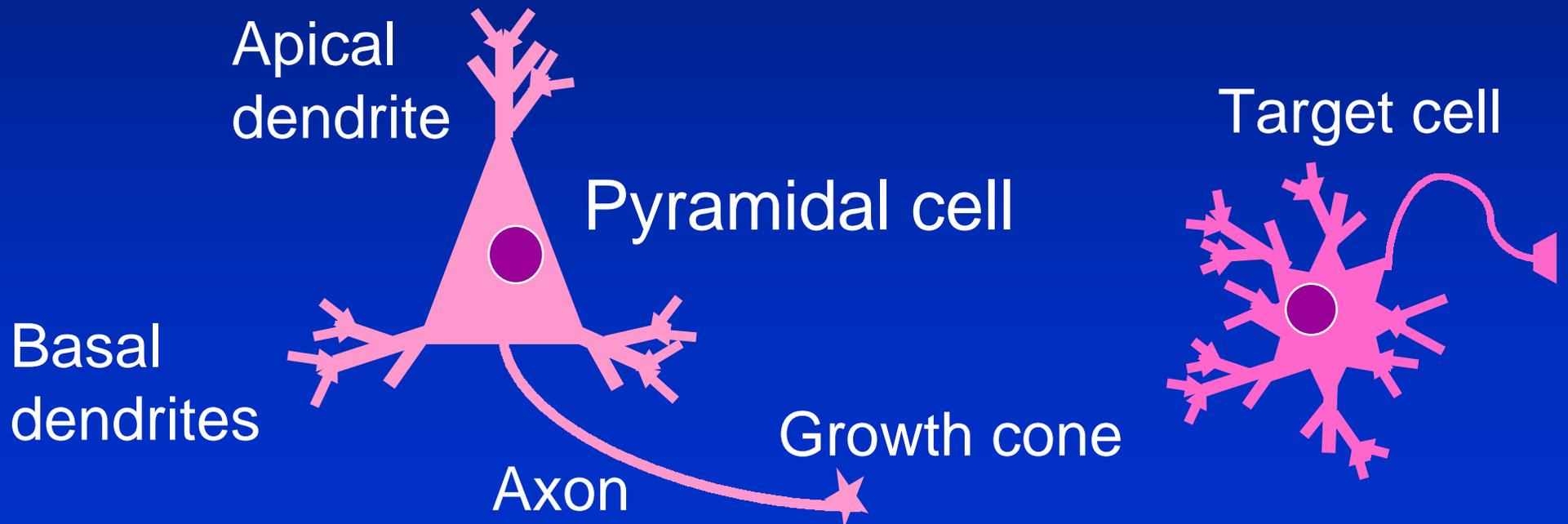
by

Evelyn Tiffany-Castiglioni

(with apologies to Elizabeth Barrett Browning)

Perspectives in Biology and Medicine 1990; 34:525

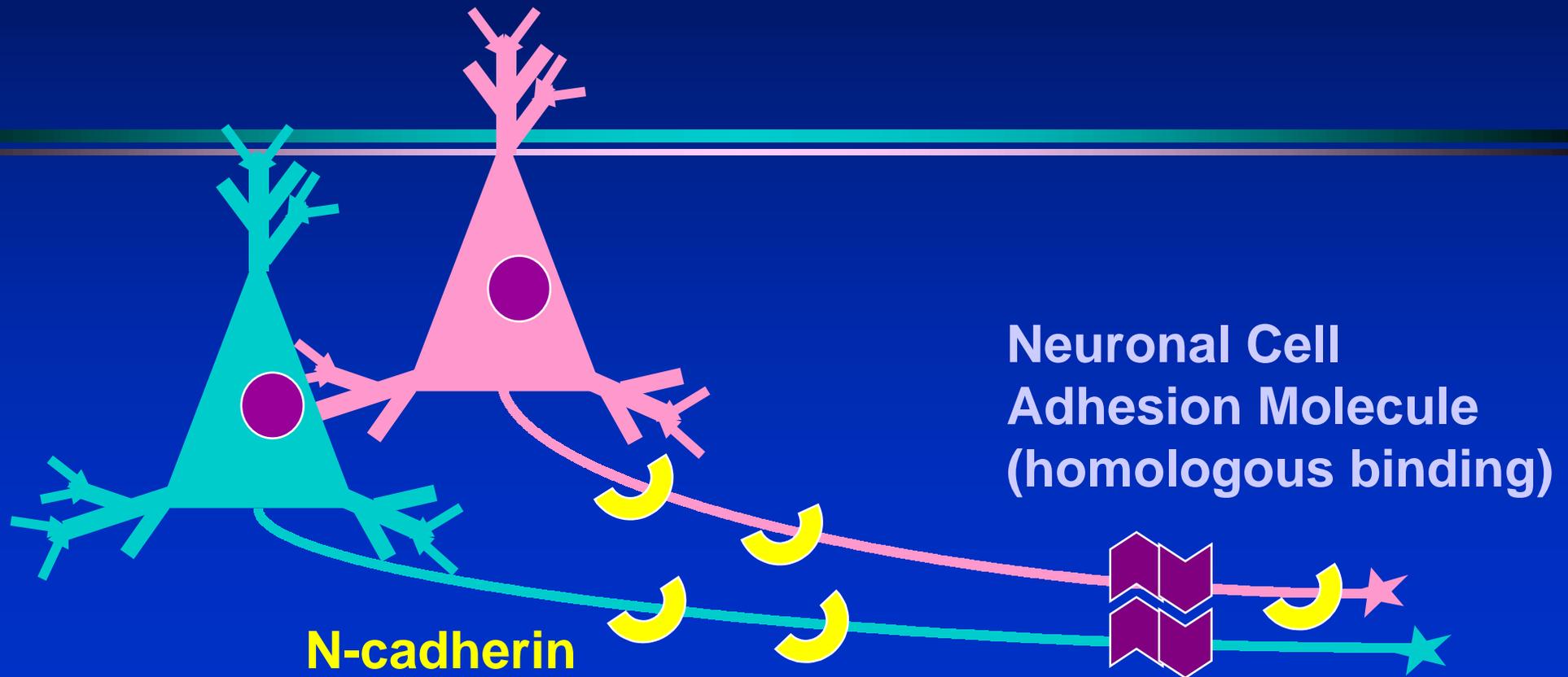
*How do neurites grow? Let me count the ways.
They grow profusely, bi- and tri-partite;
They grow diffusely, feeling out of sight
For dendritic branchlets in unpruned arrays.*



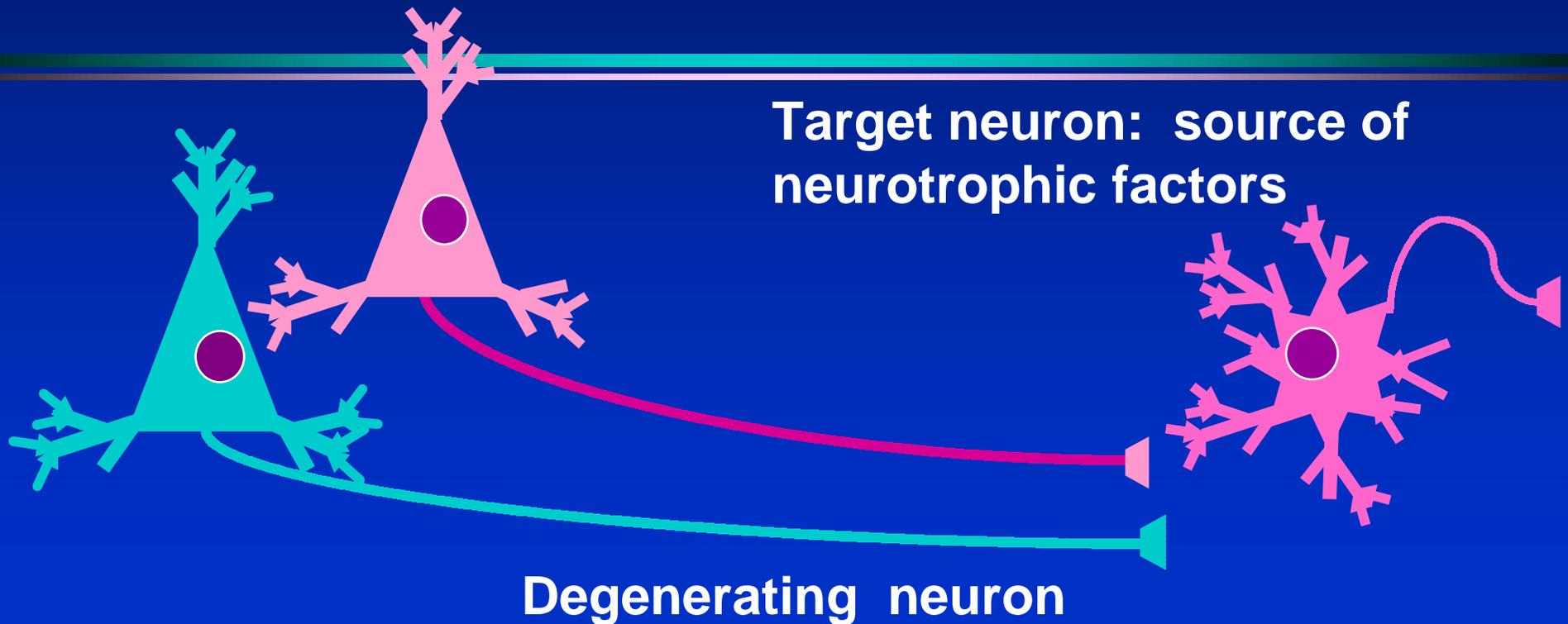
*They send forth growth cones gingerly to graze
New clearings with judicious appetite;
Alluring scents of NGF incite
Receptive neurites swifter trails to blaze.*



*Toe-holds of N-cadherin help confine
Growth cones to proper paths. Should neurites meet,
NCAM seeking NCAM they align.*



*Both neurons for one target might compete,
(Too many neurons is the Grand Design)
And one will synapse, one be obsolete.*



Proposed In Vitro Study

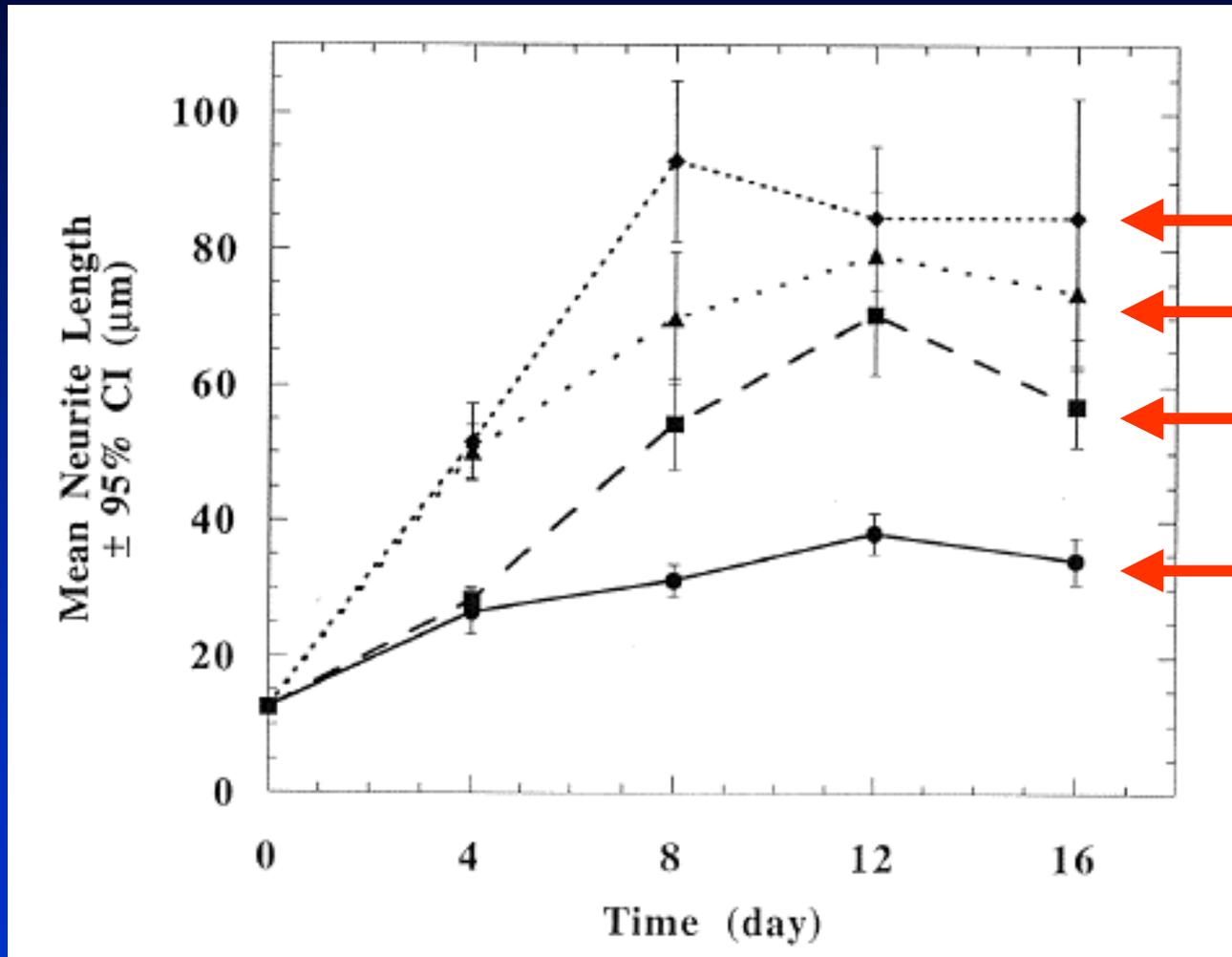
Hypotheses: sub-acute ionizing radiation alters gene expression profiles of proteins involved the maintenance of neurite extension and connectivity.

Approach:

- identify a panel of genes and proteins that show altered expression as a result of neurite extension in a neuronal cell culture model
- identify signature genes and proteins from the above panel whose expression is altered by sub-acute radiation
- design a specific gene expression array with which to detect alterations in neuronal differentiation and the maintenance of neurite extension as a result of exposure to radiation

Future applications: identify developmental windows of neuronal vulnerability; test the ability of prophylactics (glutathione, astrocyte-conditioned medium, etc.) to protect neurons from radiation

Effect of NGF Dose and Treatment Time on Neurite Extension in SY5Y Cells



20 ng NGF/ml

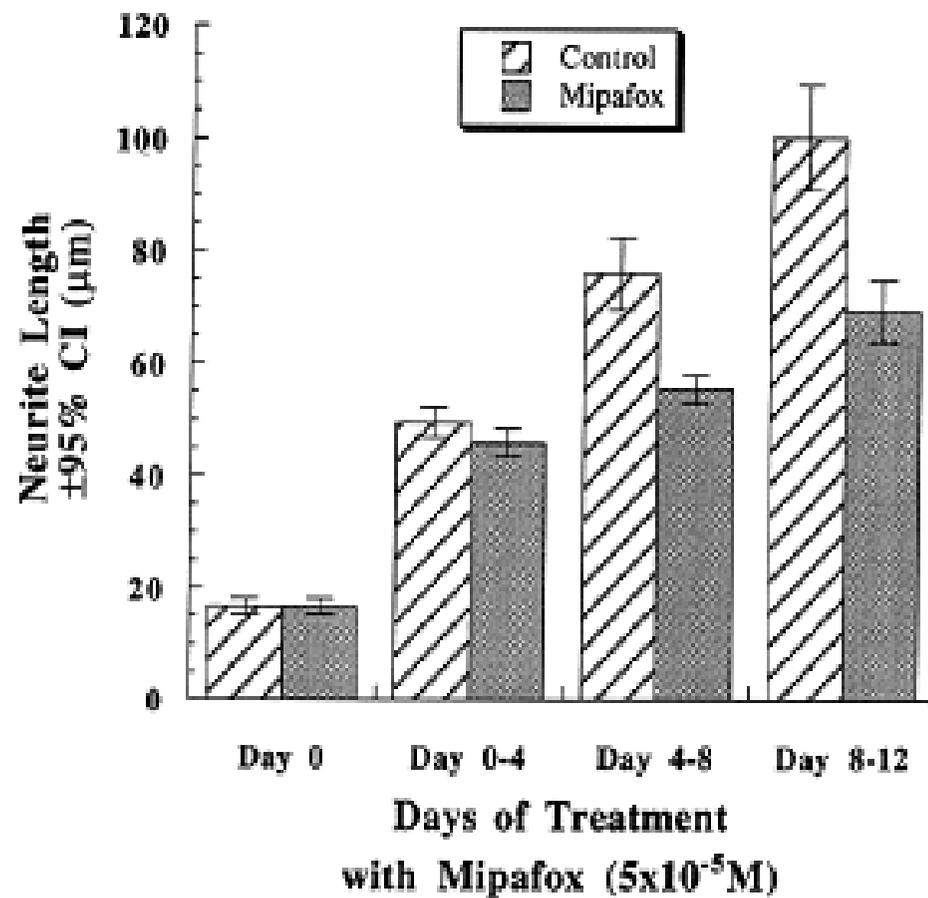
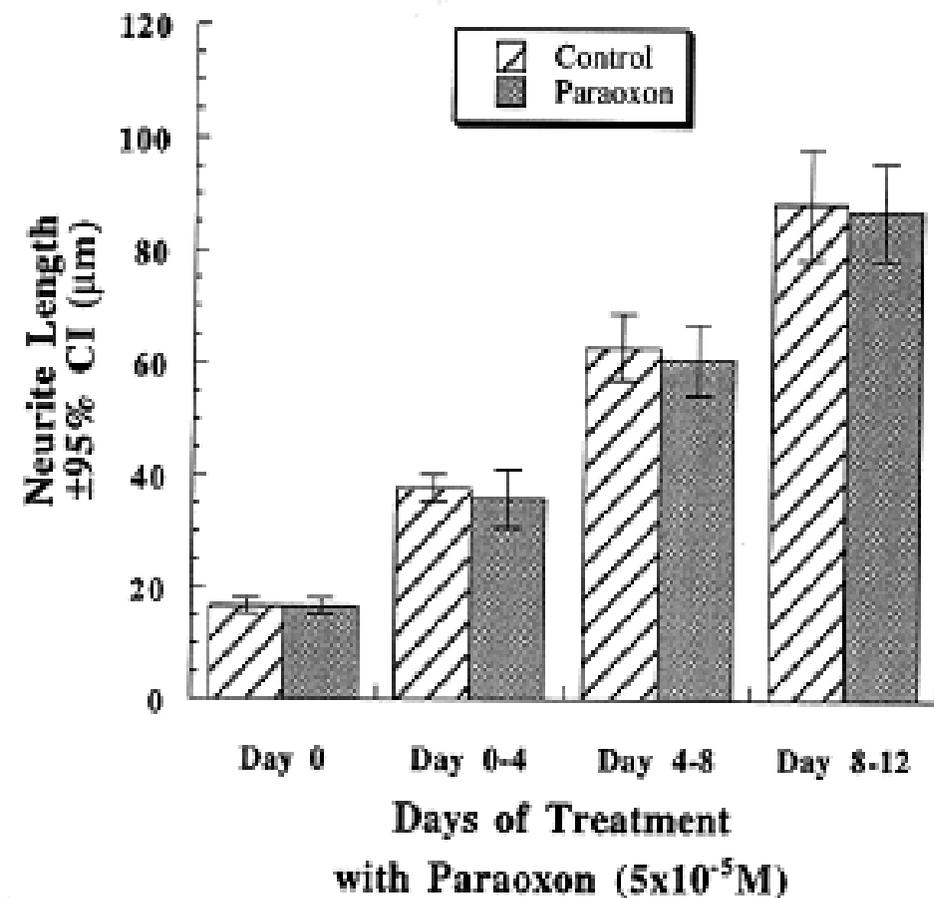
7 ng NGF/ml

1 ng NGF/ml

0 ng NGF/ml

(Hong and Tiffany-Castiglioni, unpublished)

Effect of Paraoxon and Mipafox on Neurite Length in SY5Y Cells



(Hong and Tiffany-Castiglioni, unpublished)

Research Needs

- Mechanistic integration of any known behavioral effects of space radiation with their molecular and cellular substrates
- Morphologic, physiologic, and molecular effects of space radiation on synaptogenesis, neuronal plasticity, and regeneration
- Complete chronological effects of space radiation on tumorigenesis in brain
- Differences in radiation sensitivity between immature and mature astroglia
- Effects of space radiation on oligodendroglial cells in the adult nervous system
- Interactions of space radiation with other toxic insults or endogenous metals

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