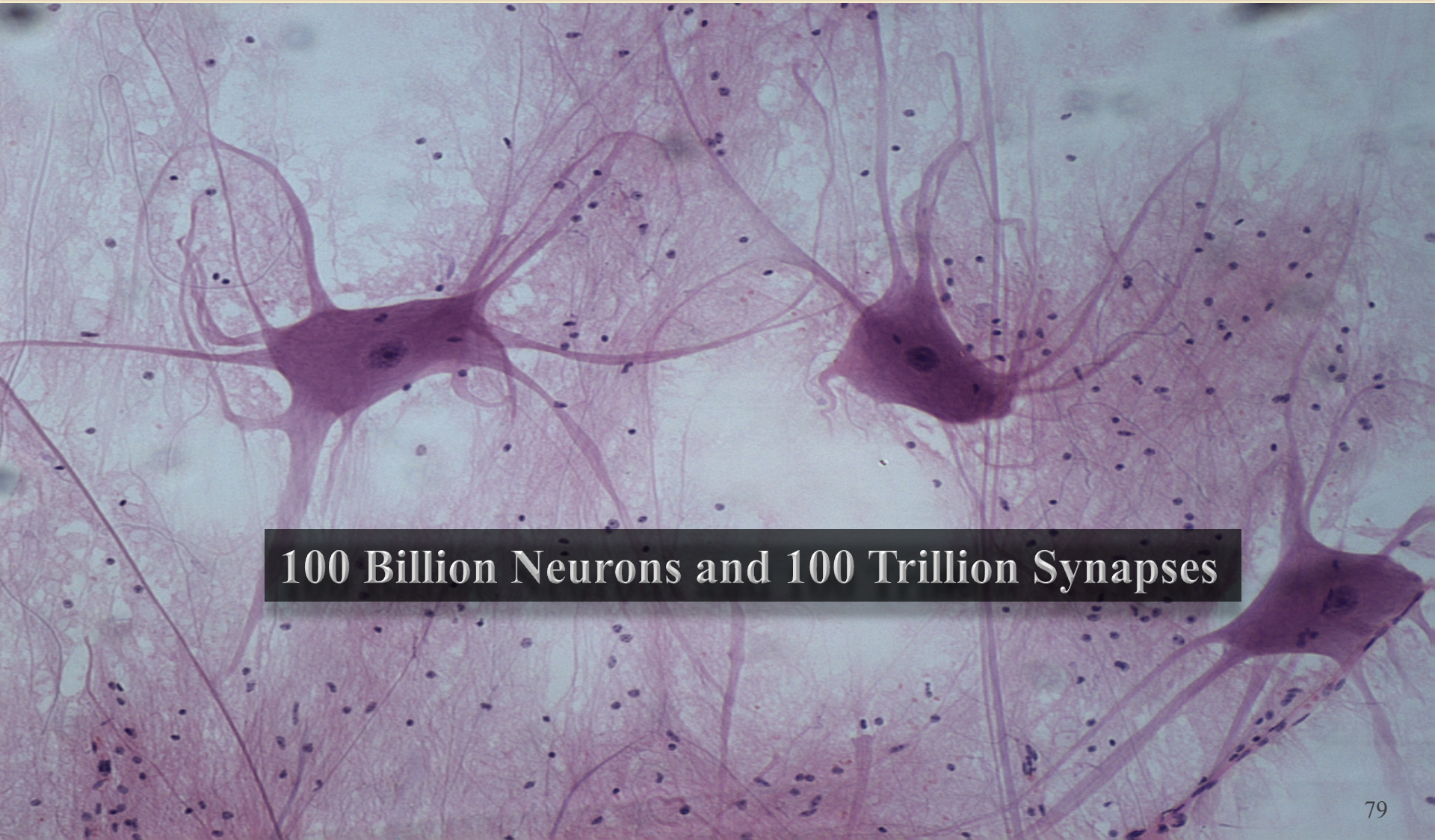
Traumatic Brain Injury:

A Clinical Approach to Diagnosis and Treatment

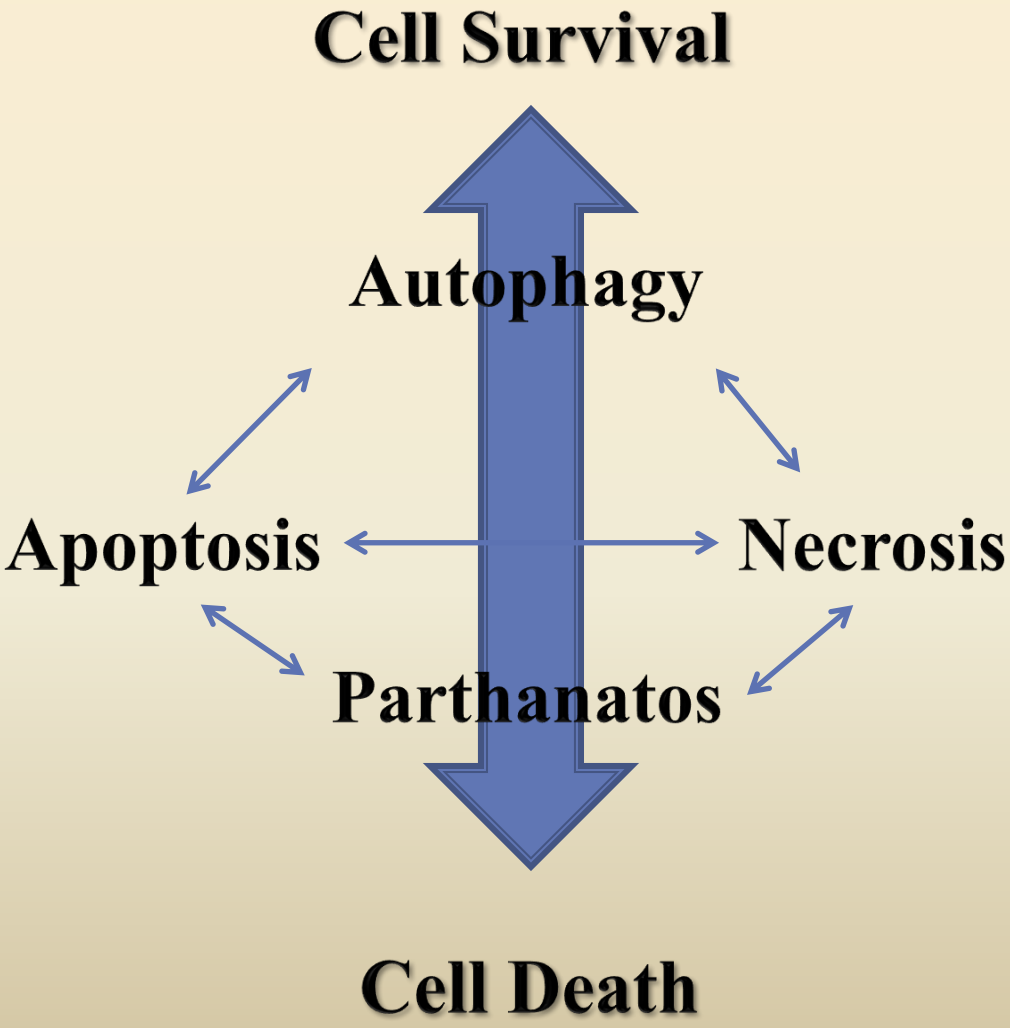
by Mark L. Gordon, M.D.

Chapter 3: Neuropathology

Neuropathology



A balance between life almost death and death



Cell Death Mechanisms Following Traumatic Brain Injury. Brain Pathology 2004;14:215-222. Ramesh Ragupathi, PhD. 2004

- Traumatic Brain Injury is a **complex neurodegenerative disease** resulting in preferential neuronal loss in the **Cortex**, **Hippocampus**, **Cerebellum**, and **Thalamus** **(Hypothalamus)**.
- The progressive increase in cortical lesion volume (cavitation), in the post-traumatic period suggests that delayed or chronic neuronal degeneration is a component of post-trauma pathology.
- **Cavitational lesions** expand over time due to the continued production of free radicals and heightened oxidative stress that induces further neuronal loss.

Cortical Functioning

- Loss of normal neocortical modulation may result in spasticity, tachycardia, gastrointestinal dysfunction, pyramidal patterns of muscle weakness, blood pressure changes, behavioral changes, changes in affect, and many other functional disorders.
- The associated muscle weakness patterns causing joint dyskinesia, may also result in primary musculoskeletal pain and dysfunction.

Hippocampal Functioning

- The hippocampus is critical for the formation of new autobiographical and fact memories. It may function as a memory "gateway" through which new memories must pass before entering permanent storage in the brain.
- Hippocampal damage can result in **anterograde amnesia**: loss of ability to form new memories, although older memories may be safe.

Cerebellar Functioning

- The cerebellum does not initiate movement, but it contributes to coordination, precision, and accurate timing of movement.
- It receives input from sensory systems of the spinal cord and from other parts of the brain, and integrates these inputs to fine tune motor activity.

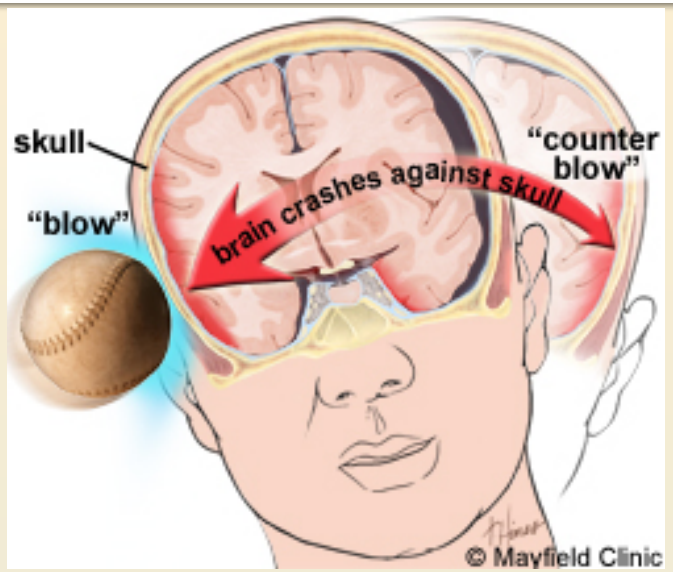
Thalamic Functioning

- Some of its functions are the relaying of sensory and motor signals to the cerebral cortex, and the regulation of consciousness, sleep, and alertness.

Hypothalamic Functioning

- The hypothalamus has a central neuroendocrine function, most notably by its control of the anterior pituitary trophic hormones (TSH, LH, FSH..ect).
- The hypothalamus controls body temperature, hunger, important aspects of parenting and attachment behaviors, thirst, fatigue, sleep, and circadian rhythms.

Primary Phase Injury



Macro-Damage: the Primary Phase of TBI

- Non-penetrating injures:
  - Contusions, micro-hemorrhages, Subdural Hematomas and Subarachnoid Hemorrhages.
  - Diffuse axonal injury.
  - Precipitation and Initiation of Secondary Cascades of injury.
- Penetrating injuries:
  - Fracture to the skull.
  - Loss of brain tissue.
  - Destruction or disruption of neuronal pathways.
  - Compromise of vasculature.
  - Precipitation and Initiation of Secondary Cascades of injury.

Micro-Damage: the Secondary Phase of TBI

- Cell death pathways; Necrosis, Apoptosis, Autophagy, and Parthanatos*.
- Free radicals and Oxidative stress*.
- Excitotoxicity*
- Hypoxia.
- Ischemia. Lactic Acidosis. Hypoglycemia*.
- Increased ICP with edema.
- Ionizing radiation (CT-Scan and plain x-rays)

Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases.

Acta Pharmacol Sin 2009 Apr; 30 (4): 379–387. Xiao-xia DONG, Yan WANG, Zheng-hong QIN* Department of Pharmacology and Laboratory of Aging and Nervous Diseases, Soochow University School of Medicine, Suzhou 215123, China.

- Excitotoxicity is defined as cell death resulting from the toxic actions of excitatory amino acids.
- Increased **extracellular Glutamate** levels leads to the activation of Ca^{2+} permeable NMDA receptors on myelin sheaths and oligodendrocytes, leaving oligodendrocytes susceptible to Ca^{2+} influxes and subsequent excitotoxicity by virtue of the elevated Ca^{2+}.
- Excessive activation of glutamate receptors by excitatory amino acids leads to impairment of calcium buffering, generation of free radicals, activation of the mitochondrial permeability transition and secondary excitotoxicity.

Neuronal Calcium Signaling Review

Neuron, Vol. 21, 13–26, July, 1998. Michael J. Berridge Siegesmund, 1968; Takahashi and Wood, 1970; Henkart The Babraham Institute et al.,1976). Babraham Laboratory of Molecular Signaling Cambridge CB2 4AT United Kingdom

Ionotropic

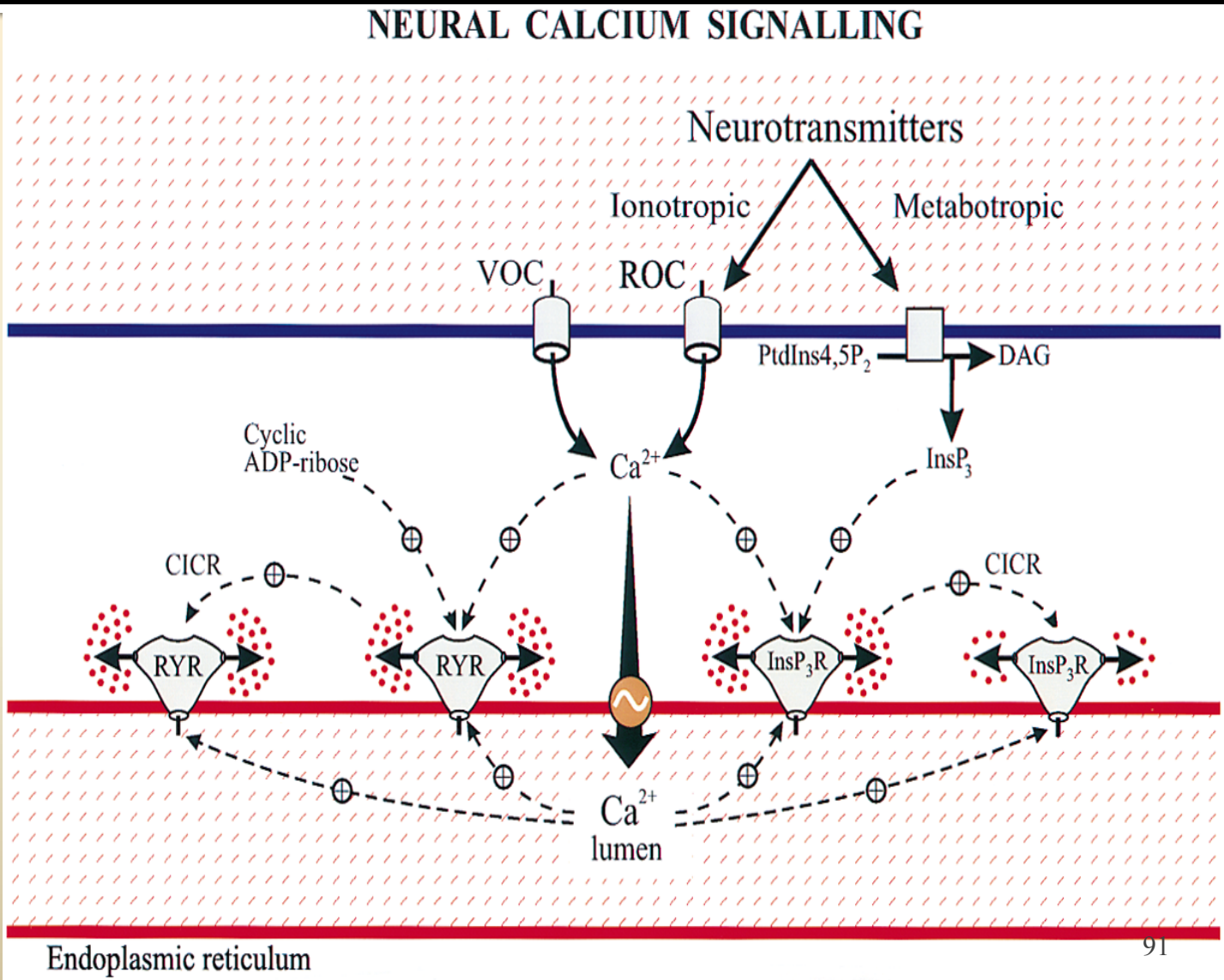
- Ion Channel

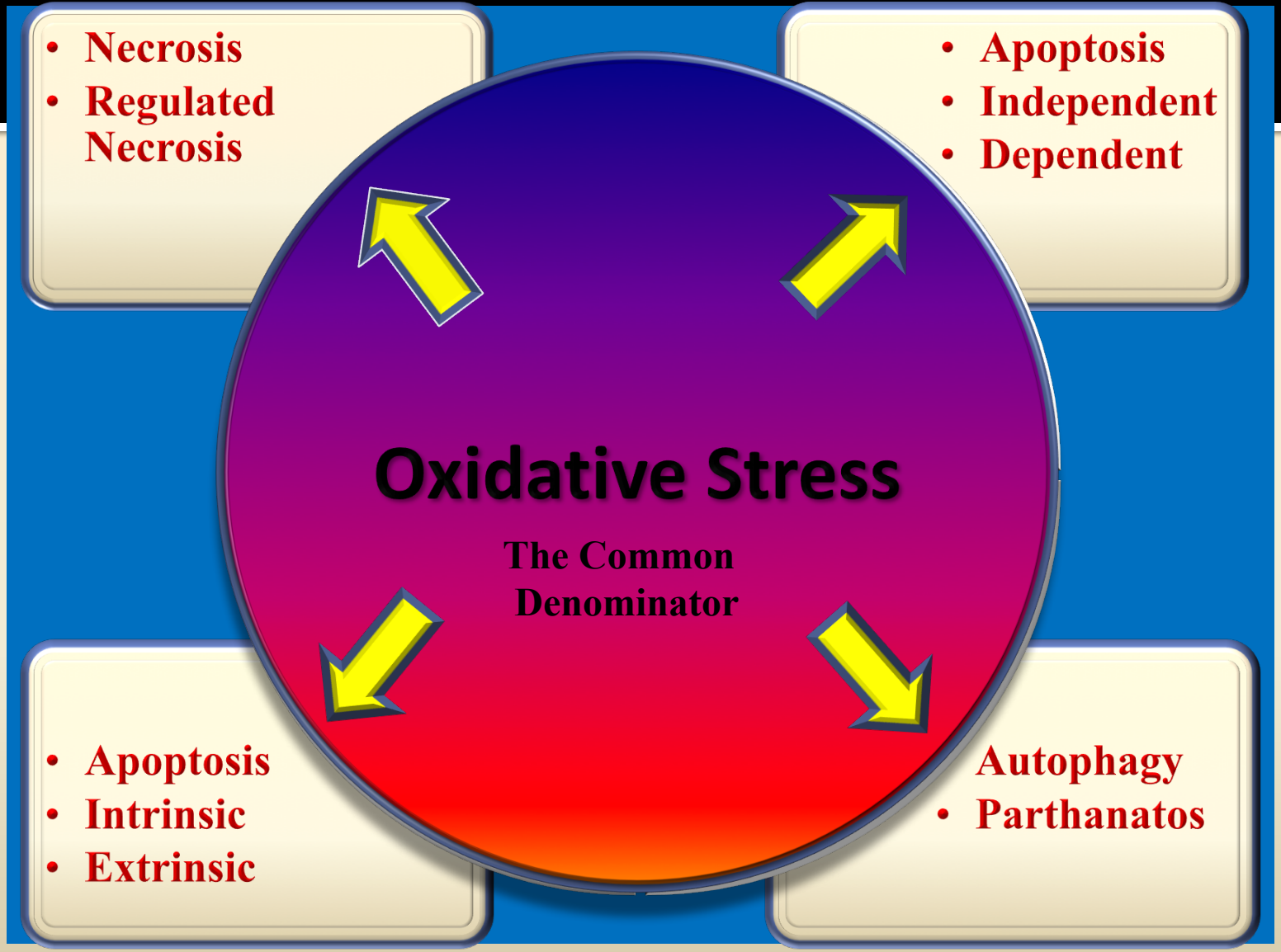
-Open allowing Ca+2 in.

Metabotropic

- G-Coupling

-Releases Ca+2 from ER.




• Necrosis
• Regulated Necrosis
• Apoptosis
• Independent
• Dependent
Oxidative Stress
The Common Denominator
• Apoptosis
• Intrinsic
• Extrinsic
Autophagy
• Parthanatos

Phase II: Oxidative Stress

Oxidative Stress
- Reactive Oxygen
- Reactive Nitrogen
- Lipid Peroxidase

Inflammation
- TH1
- Cytokines
- Chemokines

Disruption of BBB
- Hypoxia
- Ischemia
- Cerebral Edema

Neurosteroids
- Deficiencies
- Enzyme Inhibition
- Retarded production

Mitochondrial Dysfunction
BAX ► AIF, CytoC.

Excitotoxicity
- Glutamate
- Calcium

Disruption of Receptors
- NMDA, Sigma=1
- GABA-α and -β
- AMPA

Cell Death
- Necrosis
- Apoptosis
- Cavitation
- Loss of Brain

Traumatic Brain Injury: Oxidative Stress and Neuroprotection. Antioxidants & Redox Signaling Vol. 00, N0. 00, 2013.
Carolin Cornelius, Vittorio Calabrese, et al.

Ketones inhibit mitochondrial production of ROS following glutamate excitotoxicity by increasing NADH oxidation.

Neuroscience. 2007 March 2; 145(1): 256–264. Marwan Maalouf, Patrick G. Sullivan, Laurie Davis, Do Young Kim, and Jong M. Rho, Barrow Neurological Institute and St. Joseph's Hospital & Medical Center, Phoenix, Az. Spinal Cord and Brain Injury Research Center, Dept of Anatomy & Neurobiology, University of Kentucky, Lexington, Kentucky, USA

- Dietary protocols that increase serum levels of ketones, such as calorie restriction and the ketogenic diet, offer robust protection against a multitude of acute and chronic neurological diseases.
- Previous studies have suggested that the ketogenic diet **may reduce free radical levels in the brain**. Thus, one possibility is that ketones may mediate neuroprotection through antioxidant activity.
- Note: although caloric restriction will induce hypoglycemia which has been shown to increase apoptosis.

Mitochondrial Membrane Permeabilization in Cell Death. Physiol Rev 87: 99–163, 2007; Guido Kroemer, Lorenzo Galluzzi, and Catherine Brenner. Institut Gustave Roussy, Institut National de la Sante et de la Recherche Me dicale Unit "Apoptosis, Cancer and Immunity," Universite ´ de Paris-Sud XI, Villejuif; and Centre National de la Recherche Scientifique UMR 8159, Universite de Versailles/Saint-Quentin en Yvelines, Versailles, France.

- **Glucose**, favors the anti-apoptotic interaction between HKII and VDAC, exerts HK-mediated antioxidant effects, and promotes BAD inactivation* glucokinase.

- **Glucose**, at high concentrations, may have pro-apoptotic effects due to the local generation of ROS.

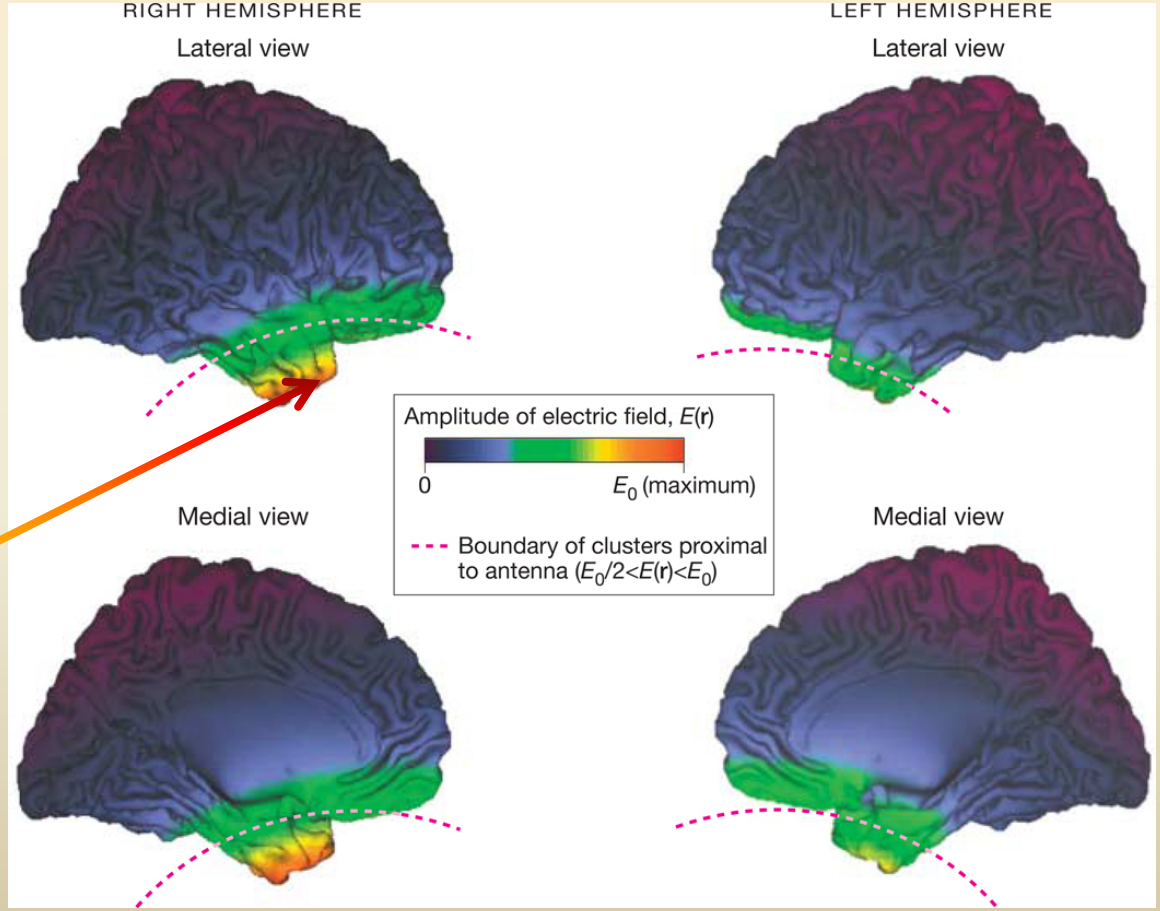
* BAD inactivation – stops opening of mitochondrial pores that allows for Cytochrome c and AIF to precipitate Apoptosis, Necrosis, and Autophagy.

Hypoglycemia

Effects of Cell Phone Radio-frequency Signal Exposure on Brain Glucose Metabolism

. JAMA. 2011 Feb. 23; 305(8): 808–813. Nora D. Volkow, MD, Dardo Tomasi, PhD, Gene-Jack Wang, MD, Paul Vaska, PhD, Joanna S. Fowler, PhD, Frank Telang, MD, Dave Alexoff, BSE, Jean Logan, PhD, and Christopher Wong, MS. National Institute on Drug Abuse, Bethesda, Maryland; National Institute on Alcohol Abuse and Alcoholism, Bethesda; and Medical Department, Brookhaven National Laboratory, Upton, New York.

Cell against Right Ear.



Necrosis-Apoptosis-Autophagy-Parthnatos

	Necrosis	Apoptosis	Autophagy	Parthanatos
Nucleus Fragmentation	NO	YES	NO	Yes
Chromatin Condensation	NO	YES	NO	Yes
Apoptotic Body Formation	NO	YES	NO	No
Cytoplasmic Vacuolation	YES	NO	YES	No
Organelle Degradation	YES	NO	YES	No
Mitochondrial Swelling	YES	Late	Variable	?
Cytoplasmic Swelling	YES	NO	NO	No
Caspase Activity	NO	**YES**	NO	No
PARP-1 (poly ADP ribose polymerase)	**YES**	Cleaved	NO	**YES**
Cathepsin B (Lysosomal)	NO	NO	YES	?
Bcl-2 Proteins/Cytochrome c	NO	YES	NO	No
Death Associated Protein	YES	YES	YES	?
PARP-1/AIF	?	YES	NO	YES

Caspases the “Messengers of Death”

- **Caspases**, are a family of cysteine proteases that play essential roles in necrosis, apoptosis, and inflammation.
- Caspases are essential in cells for apoptosis and have been termed "executioner" proteins for their roles in causing cell death.
- Some caspases are also required in the immune system for the maturation of lymphocytes.

Molecular mechanisms of "regulated" necrosis. Seminars in Cell & Developmental Biology. 2014 Nov;35:24-32. Galluzzi L, Kepp O, Krautwald S, Kroemer G, Linkermann A

- Up until 2005, cellular Necrosis was looked upon as an uncontrollable process leading to cell death and the precipitation of collateral damage by the release of inflammatory chemistry.
- Dr. Junying Yuan, discovered a protein called **Necrostatin** which could regulate the process of Necrosis.
- If Necrosis could be regulated then it was no longer a default mechanism lacking control, but one that is triggered by a chemical cascade that can now be influenced by pharmacology.

Cell Death by Necrosis: 1

- Necrosis is characterized morphologically by vacuolation of the cytoplasm, breakdown of the plasma membrane and an induction of inflammation around the dying cell attributable to the release of cellular contents and pro-inflammatory molecules.
- Cells that die by necrosis frequently exhibit changes in nuclear morphology but not the organized chromatin condensation and fragmentation of DNA that is characteristic of apoptotic cell death.
- It has become increasingly clear that Necrosis and Apoptosis share the same biochemical networks.

Cell Death by Necrosis: 2

- The final form of a cell's death is highly dependent on its physiologic context at the time when the death signal is received.
- Linking the term "programmed" or “regulated” to the word "necrosis" implies that cellular signaling pathways initiate necrosis in response to specific cues rather than "by accident".
- Programmed necrosis may not simply be a backup when apoptosis fails, but has a biological function under conditions where an immune reaction to the dying cell is desirable, such as in microbial infection.

Autophagy, Apoptosis, Mitoptosis and Necrosis: Interdependence Between Those Pathways and Effects on Cancer.

Arch. Immunol. Ther. Exp. (2013) 61:43–58. Wiem Chaabane, Sırma D. User, Mohamed El-Gazzah, Roman Jaksik, Elaheh Sajjadi, Joanna Rzeszowska-Wolny, Marek J.Łos

- 10 million cells per day undergo apoptosis in a healthy adult human.
- **During apoptosis**; chromatin condenses, cells lose their attachment to the surrounding tissue and shrink, becoming a part of a cell membrane bleb or Apoptotic Body.
- **Phosphatidylserine**, embedded in the plasma membrane, is exposed on the outer side of apoptotic bodies (ABs) and acts as an "eat me" signal, attracting macrophages to get efficiently phagocytized.

Apoptosis and autophagy: regulatory connections between two supposedly different processes

. Apoptosis (2008) 13:1–9. Department of Pharmacology, University of Colorado at Denver and Health Sciences Center, Aurora, CO 80045, USA. Andrew Thorburn. **APOPTOSIS**

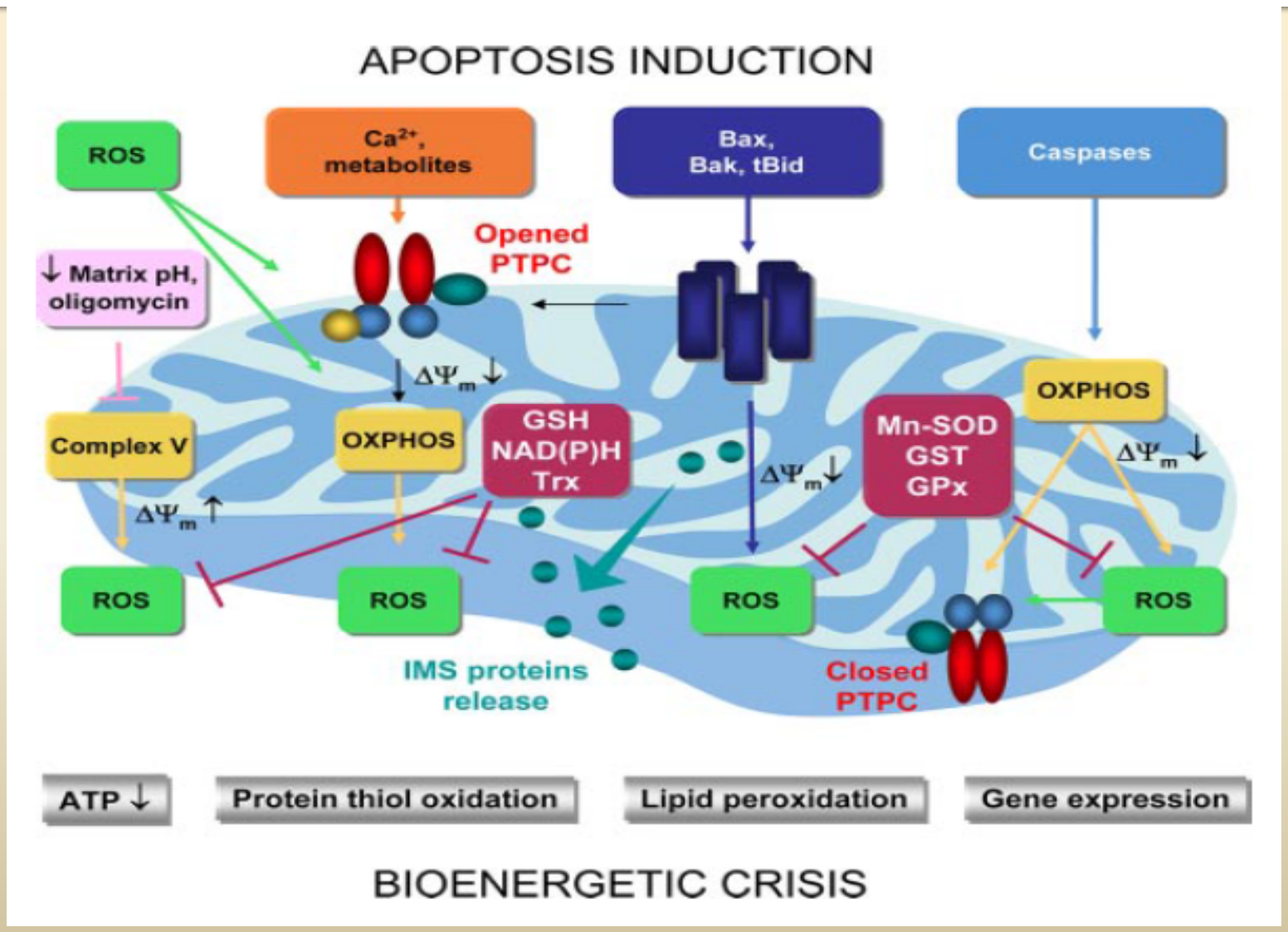
- The loss of mitochondrial ATP production culminates in the cells inability to regulate intra-cellular metabolism and mechanisms for cell survival.
- The lack of energy allows Ca+ stores to rise, leakage of cytochromes from the inner membrane space, and release of AIF (apoptotic inducing factor).
- In a death rattle, the mitochondria try to make ATP only to generate more toxic forms of ROS. There is no alternative left other than to die by Apoptosis.

Mitochondrial Dysfunction and Reactive Oxygen Species in Excitotoxicity and Apoptosis : Implications for the Pathogenesis of Neurodegenerative Diseases.

Neurochemical Research, Vol. 28, No. 10, October 2003, pp. 1563–1574. A. Cristina Rego, and Catarina R. Oliveira.

- The role of mitochondria, not only as ATP producers through oxidative phosphorylation but also as regulators of intracellular Ca^{2+} homeostasis and endogenous producers of ROS.
- Mitochondrial Ca^{2+} overload resulting from excitotoxicity is associated with generation of superoxide radicles that induce the release of pro-apoptotic mitochondrial proteins, proceeding through DNA fragmentation, condensation and culminating in cell demise by apoptosis and/or necrosis.

Loss of ATP production



Androgens selectively protect against apoptosis in hippocampal neurons.

J Neuroendocrinol . 2010 Sept ; 22(9): 1013–1022. Thuy-Vi V. Nguyen, Anusha Jayaraman, Allison Quaglino, and **Christian J. Pike** Neuroscience Graduate Programme and Davis School of Gerontology, University of Southern California, Los Angeles, CA 90089

- Androgens directly activate a neuroprotective mechanism specific to inhibition of cell death involving apoptosis.

Apoptosis and **Autophagy**: regulatory connections between two supposedly different processes.

Apoptosis (2008) 13:1–9. Department of Pharmacology, University of Colorado at Denver and Health Sciences Center, Aurora, CO 80045, USA. Andrew Thorburn. **AUTOPHAGY**

- One suggested mechanism is the sequestration of damaged Mitochondria to avoid release of Apoptotic activating chemicals (AFIP, AIF, Cytochrome c).
- As long as energy can be generated (increased glycolysis caused by elevated GAPDH), cells can use autophagy to survive MOMP^ and the release of cytochrome c and other apoptogenic proteins and recover to continue to grow.

^ Mitochondrial outer membrane permeabilization (MOMP) is considered the 'point of no return' as this event is responsible for engaging the apoptotic cascade in numerous cell death pathways.

Poly(ADP-ribose) Signals to Mitochondrial AIF: A Key Event in Parthanatos

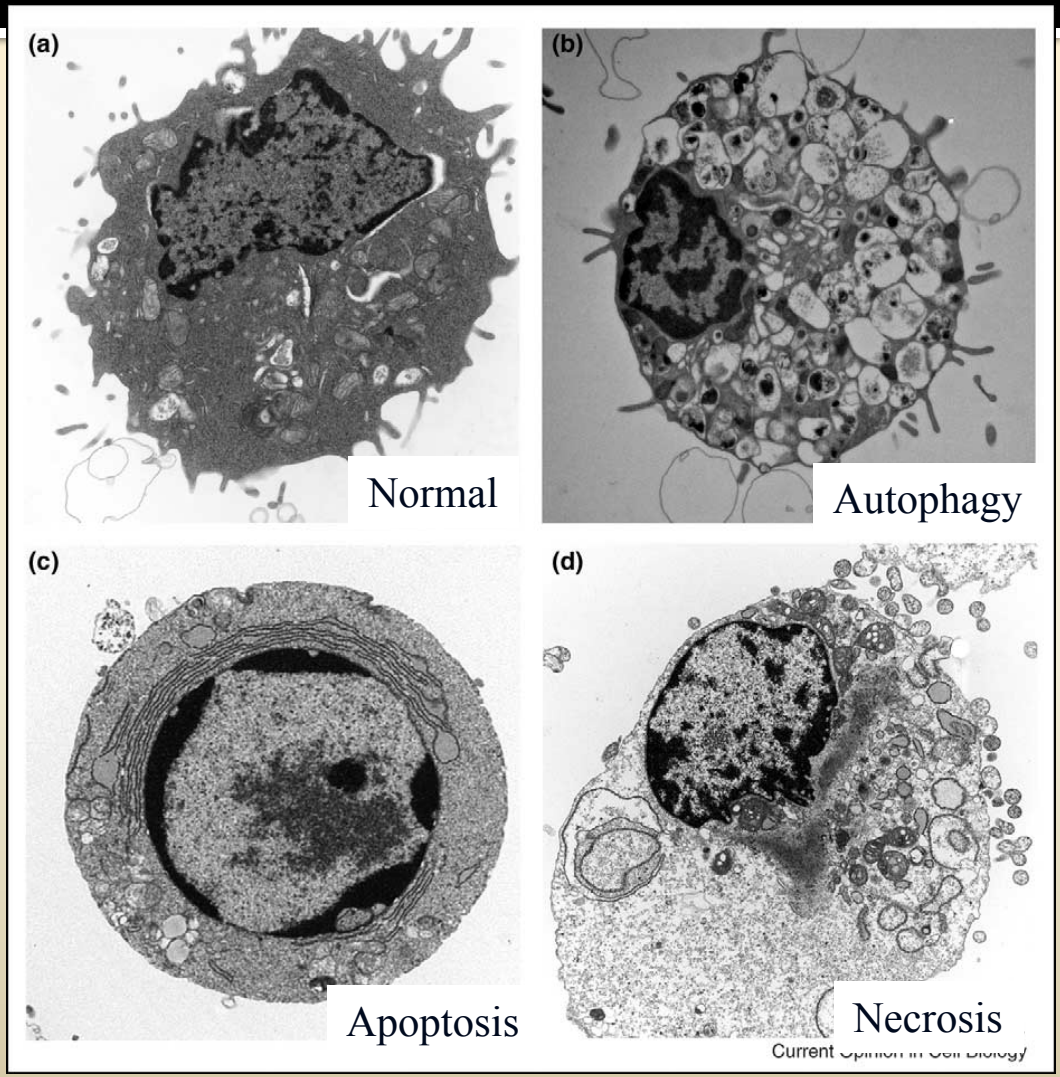
. Experimental Neurology. 2009 August ; 218(2): 193–202. Yingfei Wang, Valina L. Dawson, and Ted M. Dawson, Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, Johns Hopkins. University School of Medicine, Baltimore, MD, USA Departments of Neurology, Neuroscience, and Physiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

- Poly(ADP-ribose) polymerase-1 (PARP-1) plays a role in a number of neurologic diseases by mediating caspase-independent cell death. (Apoptosis)
- Mitochondrial apoptosis-inducing factor (AIF) release and translocation into the nucleus is the commitment point for Parthanatos.
- Intra-nuclear activation of PARP-1 then translocates to the mitochondria to mediate the release of AIF.
- In Parthanatos, the PARP signaling to mitochondrial AIF is the key event initiating the deadly crosstalk between the nucleus and the mitochondria.

Death by design: apoptosis, necrosis and autophagy. Current Opinion in Cell Biology 2004, 16:663–669Aimee L Edinger and Craig B Thompson. University of Pennsylvania, Abramson Family Cancer Research Institute, 450 BRB II/III, 421 Curie Blvd, Philadelphia, Pennsylvania 19104, USA

Morphological features of autophagic, apoptotic and necrotic cells. (a) Normal, (b) autophagic, (c) apoptotic (d) and necrotic cells. Whereas the morphologic features of apoptosis are well defined, the distinction between necrotic and autophagic death is less clear.

The bioenergetic catastrophe that culminates in cellular necrosis also stimulates autophagy as the cell tries to correct the decline in **ATP levels by catabolizing its constituent molecules**. Thus, vacuolation of the cytoplasm is observed in both autophagic cells (b) and in cells stimulated to undergo **programmed necrosis** (d). By contrast, ATP levels are maintained in normal (a) and apoptotic cells (c) consistent with the limited number of autophagic vacuoles in their cytoplasm.



Summary

- The primary trauma initiates a secondary processes which leads to disruption of regulatory communication between cells and regions of the CNS.
- It is the accumulation of the secondary traumas that allow for continuation and expansion of the primary trauma and prevents repair and regeneration.
- Cell death by any programmed or unprogrammed means is used to remove non-viable or potentially damaging cells and their contents from the brain.
- **Therefore, the key for optimal treatment is to diminish the secondary phase of Traumatic Brain Injury via limitation of inflammation and the production of free radicals.**