

Development of the Cerebral Cortex: VIII. Apoptosis: Neuronal Hari-Kari

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It is a curious fact of CNS development that more than half of the neurons and glial cells that are born are destined to die. The elimination of these cells is accomplished by an orderly type of cellular suicide called programmed cell death or apoptosis (pronounced ap-a-tow'-sis). Although developmental biologists have long recognized the role of programmed cell death in metamorphosis and in sculpting vertebrate digits, the recent upsurge in interest in this field stems from discoveries showing that it is an active genetic process that is regulated via multiple signaling pathways. Apoptosis has been described for nearly every cell type in the body, and it occurs in diverse species from plants up the

evolutionary tree to humankind. It is a critical factor in the development of the cerebral cortex.

In the nervous system, final cell number is the sum of progressive developmental events such as neurogenesis and regressive events such as cell death. As described in earlier columns, CNS cells are generated during specific times of development within proliferating layers called the ventricular and subventricular zones. These layers surround the hollow core of the neural tube. After their final division, young neurons migrate away from these zones and maintain their postmitotic state for the life of the organism. Recent work has shown that during periods of cell genesis and the establish-

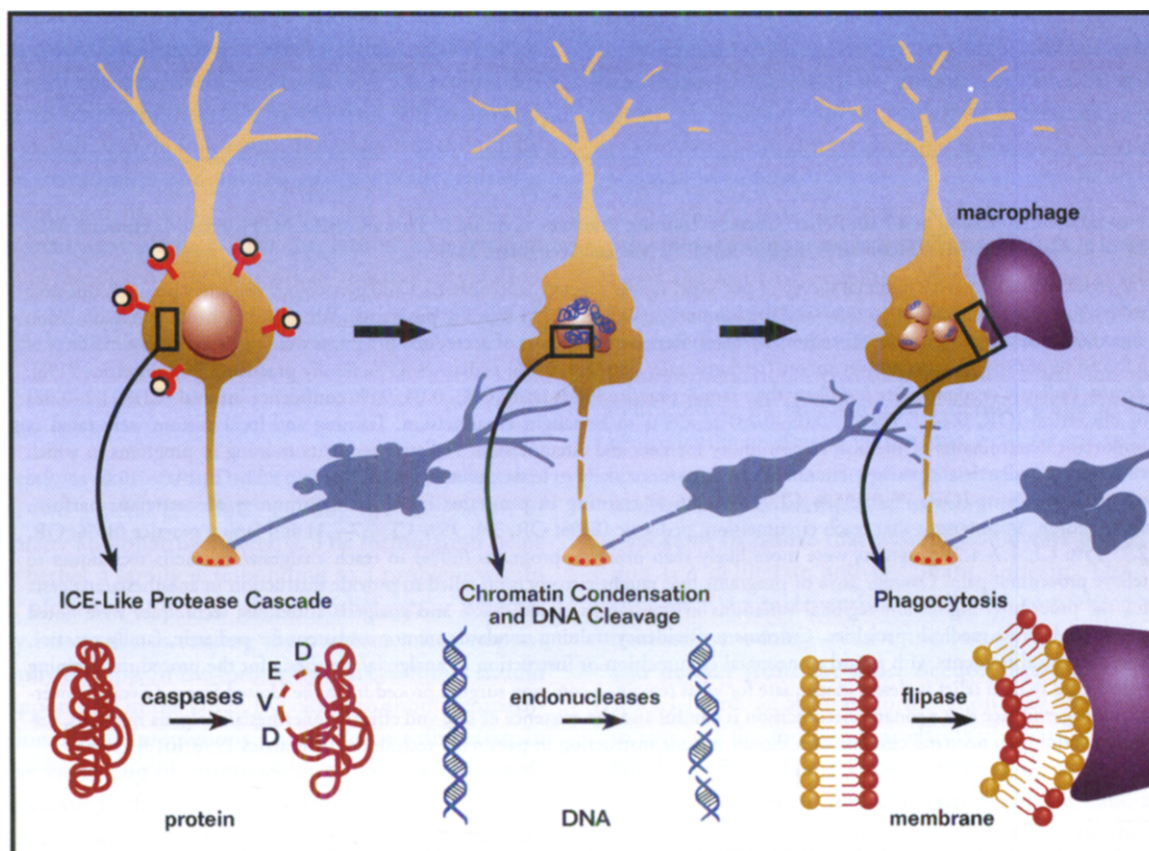


Fig. 1 A variety of conditions within a cell as well as extracellular signals can lead to apoptotic cell death. This highly regulated process involves activation of a cascade of cellular ICE-like proteases that cleave and activate additional proteases and other proteins. These enzymes, in turn, break the DNA into fragments, alter the cytoskeleton, and lead to changes at the cell surface that result in rapid phagocytosis of the dying cell. ICE = interleukin-1B converting enzyme.

ment of synaptic connections, widespread cell death occurs. Because apoptotic cell deaths are asynchronous and often occur during periods of cell genesis, it has been difficult to assess how much apoptosis occurs during CNS development. However, advances in methods for labeling dying cells *in situ* have led to current estimates that cell death removes more than half of the cells that are born during development.

Why might the cells of the CNS be overproduced in the first place? One explanation is that a surplus allows more exact matches between neuronal populations and their targets. During synaptogenesis, excessive numbers of neurons might create heightened competition for survival and for the growth factors that are produced in limiting amounts in the targets. Neurons are believed to take up these growth factors from their target only after they make the appropriate types of synaptic connections. Those neurons that acquire a sufficient level of growth factors will prosper, but those that fail will die. Growth factors responsible for cell survival in the developing nervous system include the neurotrophins (discussed in the past two columns). It appears that some growth factors repress intrinsic cell "suicide" programs. Failure to maintain sufficient levels of these factors allows the expression of cellular suicide genes. Once the initial gene cascade has been set in motion, cell death occurs within 24 to 48 hours. In this manner, only neurons that have established proper synaptic connections survive.

Both extrinsic signals and internal conditions within a cell can induce apoptosis. Changes in the local environment can produce signals that trigger apoptosis in cells that have served a particular function and are no longer needed. For example, in the developing nervous systems of insects and vertebrates, many early-generated neurons undergo apoptosis after they pioneer axonal pathways. In the mammalian cerebral cortex, neurons located in the subplate zone beneath the cortical plate provide incoming thalamic axons with a temporary synaptic target until cortical plate neurons have completed their own migrations to the overlying layers of the cortex. When thalamic axons grow beyond the subplate into the cortex, subplate neurons lose their temporary thalamic synaptic inputs and undergo apoptosis.

Extensive cell death in the proliferative zones of the developing cerebral cortex has recently been described. The locations of these dying cells suggest yet another reason for developmental neuronal death. Since the dying cells have not migrated, elaborated axons, or formed synapses, this type of neuronal apoptosis may occur following an error in cell division or differentiation. Once the apoptotic program is triggered, the intrinsic suicide program orchestrates distinctive histological changes that include rearrangements of the lipid bilayer at the cell surface, alteration of the cytoskeleton, and condensation of the chromosomes in the nucleus.

A number of genes have been identified that are activated during apoptosis. These include the caspases, a family of

cysteine proteases that cleave their substrates at specific amino acid sequences and thereby activate cellular proteins regulating the cell cycle and DNA repair. Members of this family have homology to interleukin-1 β converting enzyme, also called ICE. Members of the ICE family are synthesized as inactive precursor proteins that must themselves be cleaved to release active fragments (Fig. 1). It is interesting that some caspases activate other members of their family. A wave of active proteases is produced in response to a signal.

For apoptosis to occur, the cell's chromatin must come apart. DNA mechanisms that normally detect and rapidly repair DNA damage in healthy cells are repressed. When DNA from apoptotic cells is examined by electrophoresis in an agarose gel, it typically appears as a ladder with the chopped DNA fragments forming the rungs of the ladder. The enzyme responsible for fragmenting DNA has been identified as another caspase-activated protein, termed CAD. The gene that normally prevents CAD from destroying DNA in healthy cells was recently discovered (inhibitor of CAD, ICAD). As can be seen, there are many checks and balances to maintain a healthy cell.

Once DNA damage occurs, cells can no longer maintain their integrity. The cells do not completely fall apart, however. Leakage of cellular contents into the extracellular space is prevented by repackaging the cell contents into smaller membrane-bound apoptotic bodies that are rapidly recognized and consumed by macrophages in the vicinity. Macrophages thus prevent potentially toxic cell contents from damaging otherwise healthy neighboring cells and from triggering an inflammatory response. How apoptotic cells are recognized by macrophages is an area of intense investigation.

Knowledge about the mechanisms underlying apoptosis is likely to have enormous clinical relevance. For example, one of the genes linked with abnormal neuronal cell death is also involved in the suppression of tumors. Mutations in this gene are associated with a higher incidence of tumors and can cause a neurodegenerative disease of childhood called ataxia telangiectasia. The gene that is mutated, called ATM (ataxia telangiectasia mutated), is related to a family of genes whose normal function is to "guard the genome." Once they detect DNA damage, these proteins either halt the cell cycle until DNA repair occurs or initiate apoptosis. As these and other apoptosis-related genes are characterized, we increase our potential for learning how to regulate cell death for therapeutic purposes.

WEB SITES OF INTEREST

- Images of living cells undergoing apoptosis: <http://www.cellsalive.com/apop.htm>
Scientific American: Ask the Experts—Biology: <http://www.sciam.com/ask-expert/biology/biology14.html>
 NIH Apoptosis Interest Group: <http://www.nih.gov/sigs/aig/>
 Rockefeller University Cell Death Society: <http://www.celldeath-apoptosis.org/sites/isl.htm>

Information and contacts on ataxia telangiectasia: The AT Children's Project
Home Page: <http://www.med.jhu.edu/ataxia/>

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ADDITIONAL READINGS

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Cocaine-Induced Cerebral Vasoconstriction Detected in Humans With Magnetic Resonance Angiography. Marc J. Kaufman, PhD, Jonathan M. Levin, MD, MPH, Marjorie H. Ross, MD, Nicholas Lange, ScD, Stephanie L. Rose, Thelma J. Kukes, Jack H. Mendelson, MD, Scott E. Lukas, PhD, Bruce M. Cohen, MD, PhD, Perry F. Renshaw, MD, PhD

Context: Clinical observations and case reports suggest that there are important cerebrovascular complications of cocaine use, but no studies have documented a direct link. *Objective:* To determine whether low-dose cocaine administration induces cerebral vasoconstriction in healthy cocaine users. *Design:* Randomized controlled trial. *Subjects:* Twenty-four healthy and neurologically normal men (mean age, 29 years) reporting median cocaine use of 8 lifetime exposures (range, 3 to >40). *Intervention:* Double-blind intravenous administration of cocaine (0.4 or 0.2 mg/kg) or placebo, with cerebral magnetic resonance angiography performed at baseline and 20 minutes following infusion. *Main Outcome Measure:* Cocaine-induced angiographic change indicative of vasoconstriction, as independently and concordantly rated by 2 reviewers blind to treatment condition. *Results:* Cocaine-induced cerebral vasoconstriction in a dose-related fashion ($P = .03$), with angiograms indicative of vasoconstriction found in 5 of 8 and 3 of 9 subjects receiving 0.4- and 0.2-mg/kg cocaine, respectively, compared with 1 of 7 subjects administered placebo. Outcome stratification by frequency of self-reported lifetime cocaine use (3-10 times, 11-40 times, or >40 times) revealed a statistically stronger dose-related effect ($P < .001$), suggesting that greater lifetime cocaine use was associated with a greater likelihood of vasoconstriction. *Conclusions:* Cocaine administration induced dose-related cerebral vasoconstriction on magnetic resonance angiograms. These changes occurred at low cocaine doses and in the absence of other risk factors, including polydrug abuse, hypertension, or cerebrovascular disease. Outcome stratification by prior cocaine use statistically strengthened the relationship between cocaine administration and vasoconstriction, suggesting that cocaine may have a cumulative residual effect in promoting cerebrovascular dysfunction. *JAMA* 1998;279:376-380. Copyright 1998, American Medical Association.