Preface

Neuronal apoptosis is a normal physiological process that is an essential and integral part of neurogenesis. The massive overproduction of neurons that occur during the development of the brain is followed by a programmed cell death of roughly one-half of the originally formed neurons. In this book, the authors present topical research in the study of neuronal cell apoptosis, including comparative microarray analysis of multiple neurodegenerative models; death of neuronal and glial cells induced by photodynamic treatment; cell death during cerebellar development and the fundamental mechanisms and pathophysiological implications of neuronal apoptosis.

Chapter I - Neurons have unique complex morphological and functional features. They have a characteristic cell body with plethora of surface ion channels, and greatly elongated processes (axons and dendrites), that consequentially transport signals for intracellular trafficking creating amazing web of connectivity. Could these features be considered as a double sword?. Although, neurons are considered among the most long-lived cell types, but they are encoded by various genetic programs involved in initiation, signaling, and execution of cell death during development and adult life. Thus, two main types of neuronal apoptosis can be distinguished, based on their timing and cellular targets; an early physiological apoptosis of dividing progenitors or immature neurons that is referred as naturally occurring neuronal death (NOND), and a late cell apoptois that strikes mainly postmitotic neurons and involved in the inappropriate elimination of cells under neuropathological conditions. The term apoptois of cell death was derived originally from the Greek words apo- "away" and ptosis- "fall". Apoptosis was defined as a distinct mode of programmed cell death (PCD) on the basis of a series of characteristic ultrastructural features according to a sequence of events including nuclear and cytoplasmic condensation, cell fragmentation and finally phagocytosis. In this type of death, the cells are responsible of their own demise, a reason for which apoptosis is commonly referred to as a "cell suicide". During vertebrate brain development, about 50% of the neurons do not survive into adulthood, most of which die by apoptosis, resulting in the loss of the cell and all its synapses and processes. Why so many neurons are generated in excess and eliminated shortly thereafter remains to be understood. This normal neurodegeneration is thought to be important for matching the size of neuronal groups to the size of their targets (other groups of neurons to which they are connected by axons and nerve terminal synapses). Although, the brain is only 2–3% of total body mass, but it consumes 20% of body oxygen. Neurons are particularly susceptible to oxidative damage due to high levels of polyunsaturated fatty acids in their membranes and relatively low activity of

endogenous antioxidant enzymes. Moreover, because neurons have limited glycolytic capacity, so they are highly dependent on aerobic oxidative phosphorylation by mitochondria for their energetic needs. The postmitotic differentiated neurons are completely devoid of replicative capability. Indeed they are believed to enter an "extended G0 phase" which, unlike other cell types, is irreversible. Contrary to this dogma, a growing body of evidence indicates that neurons have the capacity to re-enter the cell cycle. However, while neurons retain the ability to re-activate the cell cycle, engagement of the cell cycle machinery rarely leads to neuronal proliferation but typically induces apoptosis. Several different stimuli can initiate the apoptotic death of neurons. However, the finding that common morphological and biochemical alterations are observed independently upon the event that triggers apoptosis, suggests that most apoptotic pathways converge on a restricted number of common effector pathways. Basically two major pathways can be differentiated by the relative timing of caspase activation and mitochondrial release of cytochrome c. In the first, which is exemplified by activation of death receptors, an effector caspase is activated prior to mitochondrial alterations. In the other, cytochrome c is released from the mitochondrial intermembrane space prior to caspase activation. Regarding such molecular consequences, apoptosis is regulated by wide array of molecules within cells, some are antiapoptotic proteins (such as Bcl-2, Bcl-xL) and Proapoptotic proteins (such as Bax, Bcl-xS, Bad, Bid, Bik, caspases, p53). The molecular mechanisms for apoptosis involve the participation of at least three groups of proteins that are made from three different gene families. One set of proteins is the caspase family of cysteine-containing, aspartate-specific proteases (14 members have been identified to date). A second group includes the death promoting and death-suppressing proteins in the Bcl-2 family. A third group of proteins is designated as the inhibitor of apoptosis protein (IAP) family. Is the future is promising for exploring the causes of neurodegenerative disorders and identifying treatments? Unfortunately, until now there are no effective treatments are available to prevent or radically cure any of them. Thus more complete understanding of the characteristics and mechanisms of neuronal apoptosis within the CNS could provide such hope.

Chapter II - Apoptosis is the earliest identified mode of cell death characterized by its closely coordinated signaling mechanisms that put the cell to its demise without disruption to its microenvironment. It is the most reported cause of neuronal death seen in the pathogenesis of numerous neurodegenerative disorders. The current focus of this review revolves around the significance of the major biological processes (e.g. oxidative stress, endoplasmic reticulum/lysosomal disruption, mitochondrial respiratory dysfunction and ubiquitin-proteasome system inhibition) at work in the induction of neuronal apoptosis during neurodegeneration in various central nervous system diseases. To further complement and substantiate the existing findings, comparative microarray analysis was conducted on five neurodegenerative global gene profiles from the authors' laboratory to provide comprehensive temporal elucidation of the implication of these neuropathological mechanisms during neuronal apoptosis at the transcriptional level. These transcriptomic profiles were derived from the application of widely employed pharmacological agents on *in vitro* rodent models to mimic neuropathological hallmarks. They comprise of lactacystin (proteasomal inhibitor), Glu (excitotoxicity inducer), nitric oxide (source of reactive nitrergic and oxygen species), hypochlorous acid (source of reactive chlorinergic and oxygen species) and rotenone (mitochondrial complex I inhibitor) commonly used to model chronic neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis,

Parkinson's disease and Down syndrome as well as acute neurological disorders such as stroke and traumatic brain injury. Mediation of neuronal injury and apoptosis by any one agent is not mutually exclusive to any particular neurodegenerative condition, but at most times demonstrates simultaneous involvement across the neurodegenerative disease spectrum. This is an indirect indication of a significant commonality of signaling cascades in the pathogenesis of most neurodegenerative diseases, and that apoptosis plays a major role in the trigger of these downstream mechanisms. Subsequently, this provides the opportunity for identification of novel biological targets whose manipulation would provide beneficial broad-spectrum neurodegenerative disease therapeutic intervention.

Chapter III - Photodynamic therapy (PDT), an inducer of oxidative stress, is used for treatment of cancer including brain tumors. To study PDT effect on neurons and glial cells, authors used a simple model object - an isolated crayfish mechanoreceptor consisting of only two neurons enveloped by glial cells. PDT abolished neuronal activity and caused necrosis of neurons and necrosis and apoptosis of glial cells. Percent of necrotic neurons and glial cells progressively increased after PDT. At the ultrastructural level, the swelling of mitochondria, dictyosomes and endoplasmic reticulum was observed in neurons and glial cells after PDT. At 1 hour postirradiation these alterations were expanded throughout the cells. Neurons lost the "tigroid" morphology that was a result of segregation of the cytoplasm by Nissl bodies enriched with ER, mitochondria, ribosomes and polysomes, which are involved in protein synthesis and transport along neurites. The structures involved in glia-to-neuron (neuronal submembrane cisterns, glial protrusions, double-wall vesicles), intra-glial (tubular lattices) and intra-neuronal transport (Nissl bodies, Golgi apparatus, microtubular bundles) were also impaired at the earlier stages of the stretch receptor damage. Local inactivation of a neuron body by a focused laser beam enhanced glial apoptosis but not necrosis that was induced by following photosensitization. Therefore, the stretch receptor neuron prevented glial apoptosis. In order to find molecular signals involved in the neuroglial interactions, authors tested neurotrophic factors NGF, BDNF and GDNF. NGF and GDNF protected glial cells from PDT-induced necrosis and apoptosis. Inhibition of protein kinase JNK enhanced PDT-induced apoptosis of glial cells and prevented the anti-apoptotic effect of NGF. Hence, JNK was involved in the NGF-mediated protection of glial cells from PDT-induced apoptosis. Unlike, another MAP kinase, p38, was involved in PDT-induced glial necrosis. Calmodulin, calmodulin-dependent kinase II, phospholipase C, protein kinase C and adenylate cyclase also participated in PDT-induced necrosis of glial cells. Phospholipase C and glycogen synthase kinase also participated in PDT-induced apoptosis of glial cells, whereas adenylate cyclase, JNK, protein kinases A and C played the anti-apoptotic role in glial cells. The involvement of these signaling pathways in responses of neurons and glial cells to PDT suggests that their modulation can modify PDT efficacy. The scheme of participation of diverse inter- and intracellular signaling processes in responses of neurons and glial cells to PDT-induced oxidative stress has been created.

Chapter IV - Development of the nervous system not only involves the generation of new neurons and glia, but also the death of cells that are no longer required and/or are produced in excess. Because the process of cell death follows a complex framework of regulated events during normal development, it is usually referred to as programmed cell death. Programmed cell death is a gene-regulated process that plays important roles in several normal and pathological conditions. It takes several different forms, but, most commonly, eukaryotic cells die following a conserved series of molecular and cellular modifications usually referred to as

apoptosis, a form of "cell suicide" most often found during embryonic development, but also in normal cell and tissue turnover during adulthood and senescence. Another form of cell death is autophagy, a catabolic process involving the degradation of cellular components through the lysosomal machinery. There is no conclusive evidence as regarding the involvement of autophagy in apoptosis, and a causative relationship between autophagy and cell death has not yet been established in full. Nevertheless, observations of cells with autophagic features undergoing programmed cell death have led to define an autophagic type of death (also known as cytoplasmic cell death or type II cell death), with morphological features somehow different from those of apoptosis. Proliferative and/or apoptotic events have been extensively characterized not only during embryonic development but also in several areas of the post-natal and adult brain such as the cerebellar cortex. The post-natal cerebellar cortex undergoes significant structural remodeling mainly because of the generation, differentiation, migration, and apoptosis of the granule cells. Therefore, it has been widely investigated as a general model for studying neuronal cell apoptosis. The existence of two waves of apoptotic cell death has been demonstrated in cerebellum and other areas of the central nervous system, affecting different types of neurons in normal development and in certain pathological conditions. The first wave regards neuronal progenitors and pre-migratory neuroblasts, the second post-migratory neuroblasts and mature neurons. In parallel, autophagic phenomena have also been observed to mainly affect the granule cells and the Purkinje neurons. These may be of particular relevance in the granule cells, for the regulation of the cellular levels of B cell lymphoma-2, one of the most important anti-apoptotic proteins in mammals.

Chapter V - Brain cells fundamentally differ, structurally, biochemically and functionally from other cell types of the body. In the brain, neuronal apoptosis is a normal physiological process that is an essential and integral part of neurogenesis. The massive overproduction of neurons that occur during the development of the brain is followed by a programmed cell death of roughly one-half of the originally formed neurons. This precisely controlled neuronal demise is highly conserved in diverse organisms. However, apoptosis when becomes aberrant has been implicated in various neurological disorders as diverse as Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis, spinal muscular atrophy, diabetic encephalopathy, and ischemic and traumatic brain injuries. At the fundamental level, the main focus of the recent research has been in the area of understanding the functional mechanisms of the death receptor mediated 'extrinsic' and mitochondria mediated 'intrinsic' apoptotic pathways, and the roles of caspases and protein members of the Bcl-2 family. Neuronal apoptosis is classically viewed as an active and highly regulated form of neuronal death, whereas neuronal necrosis is considered as an accidental form of cell death. Also, the recent concept of a programmed necrosis has considerable scope in neuronal cell death while autophagy, a caspase independent apoptosis related mechanism in which the cytoplasm is destroyed by lysosomes has been implicated in cell death as well as in cell protection. The main purpose of this chapter is to provide the basic details and update the current understanding of neuronal apoptosis and its implications in neurodegenerative, ischemic and traumatic conditions. Thus, this chapter focuses on two major areas (a) the fundamental mechanisms of neuronal apoptosis and (b) the pathophysiological implications of neuronal apoptosis.

Chapter VI - The cells that make up the nervous system die at two distinct times during the lifetime of an organism. These periods of neuron loss occur during embryonic

development and with advancing age, occurring at an accelerated rate in neurodegenerative disorders. The major risk factor for many neurodegenerative diseases is ageing. During development, neuronal apoptosis is essential for the correct shaping and hard-wiring of the nervous system. However, in a number of neurological conditions, apoptosis causes a devastating, and currently irreversible, loss of neurons. This review considers the extracellular and intracellular signals that determine which neurons live, and which neurons die, during development. It then asks the question of whether the authors' understanding of apoptosis during neurodevelopment can lead to novel treatments to protect the nervous system from neuron loss due to pathology.

Chapter VII - The term apoptosis, from the Greek word for "falling off" of leaves from a tree, is used to describe a process in which a cell actively participate in its own destructive processes. Apoptosis, or programmed cell death, is a physiological process observed in many organs and cells, which may also occur in pathological conditions such as diabetic neuropathy. The apoptotic program is characterized by certain morphological features, that include changes in the plasma membrane such as loss of membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. In the final stages, the dying cells becomes fragmented into "apoptotic bodies" which are rapidly eliminated by phagocytic cells without eliciting significant inflammatory damage to surrounding cells.

Chapter VIII - Neuronal apoptosis is an underlying cause of many neurodegenerative diseases such as amyotrophic lateral sclerosis and Parkinson's disease, as well as, in acute neuronal injury such as stroke. The two main pathways that induce apoptosis in neurons are the intrinsic (mitochondrial) and extrinsic death receptor cascades. These pathways are not mutually exclusive and the intrinsic apoptotic cascade can function as an important amplification loop to the extrinsic apoptotic pathway. Evidence also suggests that endoplasmic reticulum (ER) stress is a significant contributor to neuronal apoptosis. Central to each of these pathways is the B-cell lymphoma-2 (Bcl-2) family of proteins which function as crucial regulators of programmed cell death. The Bcl-2 family is comprised of three distinct subgroups: the pro-survival subgroup (e.g., Bcl-2 and Bcl-xL); the pro-apoptotic, multi-domain subgroup (Bax and Bak); and the Bcl-2 homology-3 domain (BH3)-only pro-apoptotic subgroup (e.g., Bim, Bad, and Puma). Regulation of apoptosis is largely mediated by finely tuned interactions between pro-survival and pro-apoptotic Bcl-2 family proteins. An essential role of Bcl-2 family proteins in neuronal survival has been underscored by evidence demonstrating that either loss of pro-survival Bcl-2 function or gain of Bax/Bak function are causally linked to neuronal apoptosis and neurodegeneration. More recently, a prominent role has been established for BH3-only proteins in neuronal apoptosis associated with chronic neurodegenerative diseases or acute neuronal injury. At least thirteen BH3-only proteins have been described in mammals and evidence suggests that these proteins function as sensors of intracellular and extracellular cell death signals that tip the delicate balance between pro-survival and pro-apoptotic Bcl-2 proteins towards apoptosis. In this review, authors discuss the roles of BH3-only proteins in the intrinsic, extrinsic, and ER stress apoptotic cascades, as well as hierarchical BH3-only pathways and regulation of BH3-only proteins. Authors also examine specific evidence of a role for BH3-only proteins in *in vitro* models of neuronal apoptosis, animal models of neurodegeneration or acute neuronal injury, and in human neuropathology. Given their crucial roles in neuronal apoptosis, BH3-only proteins represent

a novel class of therapeutic targets for the treatment of neurodegenerative diseases and acute neuronal traumas such as stroke.