When and How Do Seizures Kill Neurons, and Is Cell Death Relevant to Epileptogenesis?

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Abstract

The effect of seizures on neuronal death and the role of seizure-induced neuronal death in acquired epileptogenesis have been debated for decades. Isolated brief seizures probably do not kill neurons; however, severe and repetitive seizures (i.e., status epilepticus) certainly do. Because status epilepticus both kills neurons and also leads to chronic epilepsy, neuronal death has been proposed to be an integral part of acquired epileptogenesis. Several studies, particularly in the immature brain, have suggested that neuronal death is not necessary for acquired epileptogenesis; however, the lack of neuronal death is difficult if not impossible to prove, and more recent studies have challenged this concept. Novel mechanisms of cell death, beyond the traditional concepts of necrosis and apoptosis, include autophagy, phagoptosis, necroptosis, and pyroptosis. The traditional proposal for why neuronal death may be necessary for epileptogenesis is based on the *recapitulation of development hypothesis*, where a loss of synaptic input from the dying neurons is considered a critical signal to induce axonal sprouting and synaptic-circuit reorganization. We propose a second hypothesis – the *neuronal death pathway hypothesis*, which states that the biochemical pathways causing programmed neurodegeneration, rather than neuronal death *per se*, are responsible for or contribute to epileptogenesis. The reprogramming of neuronal death pathways – if true – is proposed to derive from necroptosis or pyroptosis. The proposed new hypothesis may inform on why neuronal death seems closely linked to epileptogenesis, but may not always be.

Keywords

Neurodegeneration • Epilepsy • Necrosis • Apoptosis • Autophagy • Phagoptosis • Necroptosis • Pyroptosis

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9.1 Seizures and Neuronal Death: When, Where, and What?

Debates and controversies concerning the interplay among seizures, neuronal death and epilepsy continue to occur. Over several decades, many epilepsy researchers have focused on various aspects of the issue of whether seizures cause neuronal death, and conversely, whether neuronal death is necessary and/or sufficient to cause epilepsy. For example, a classic – yet still ongoing – debate is the degree to which GABAergic interneurons are lost in tissue from patients and animal models of temporal lobe epilepsy, and the consequence of such loss. In spite of the longevity and intensity of the previous debates, the relationship between seizures, neuronal death and epilepsy remains one of the most disputed in translational neuroscience, particularly as it relates to possible mechanisms of acquired epileptogenesis and the clinical interactions and consequences of seizures and neuronal death. We will discuss, as the title implies, two important and longstanding hypotheses of contemporary epilepsy research – important because the degree to which seizures cause brain damage and the hypothetical role of neuronal death in the development of epilepsy are inter-related and could underlie the often quoted statement "Seizures beget seizures" [33]. These two issues are not "black and white"; rather, they probably form an interactive continuum and are quite complicated; and furthermore, technical limitations and interpretational difficulties plague any analysis of them. The key questions include ***when*** do seizures kill neurons, ***where*** in the brain are neurons most susceptible to seizure activity, and ***what*** is the identity of the neurons that are preferentially killed? Answers to a fourth issue – "***How*** do seizures kill neurons?" – may hold a key to understanding at least one component of epileptogenesis, as described below. We will begin with a brief summary of some of the key questions and controversial topics; then, we will review more recent views of the many possible mechanisms whereby seizures may kill neurons; and finally, we will conclude with a brief discussion of some of the ongoing issues and controversies in this area.

9.1.1 When

A large and long-standing body of experimental and clinical data indicates that some types of seizures lead to neuronal death, while other types do not. In either experimental animals or humans, whenever seizures are long enough in duration and occur repetitively for prolonged periods, some neurons – particularly in adults – are killed. In terms of the temporal features of the seizures that are thought to cause neuronal death, relatively brief seizures – such as typical *absence* seizures in children (usually lasting 5–10 s) – do not appear to cause overt brain damage. However, the more prolonged seizures characteristic of temporal lobe epilepsy, such as the traditional complex partial seizures (i.e., dyscognitive focal seizures) that may progress to tonic-clonic convulsive seizures, are much more likely to lead to neuronal loss [84]. Finally, the prolonged and repetitive seizures that define status epilepticus typically cause brain damage, often with extensive neuronal death [10, 15, 29, 40, 57, 62, 67, 85]. Interestingly, however, status epilepticus in the immature brain causes far less neuronal death [16, 38, 59, 68, 74, 75, 80, 81], and appears less likely to cause epileptogenesis [51, 74]. The longstanding observation that experimental status epilepticus in laboratory animals, mostly rodents, leads to a chronic epileptic state raises the following question: Is the occurrence of neuronal death during status epilepticus a critical part of the epileptogenesis? In terms of epilepsy, one could view seizure clusters, where some of the interseizure intervals are much shorter than the typical interseizure intervals [36, 37], as essentially a reduced form of status epilepticus. The difference between status epilepticus and a seizure cluster in a patient with epilepsy is not always so clear. Thus, a fundamental question in clinical epilepsy is: Do the spontaneous recurrent seizures kill neurons – particularly when the seizures occur in clusters? If so, under what conditions does this contribute to a worsening of epilepsy? Are seizure clusters a particular concern in terms of neuronal death and brain injury? These are some of the unanswered questions that are both clinically important and can theoretically be addressed with animal models.

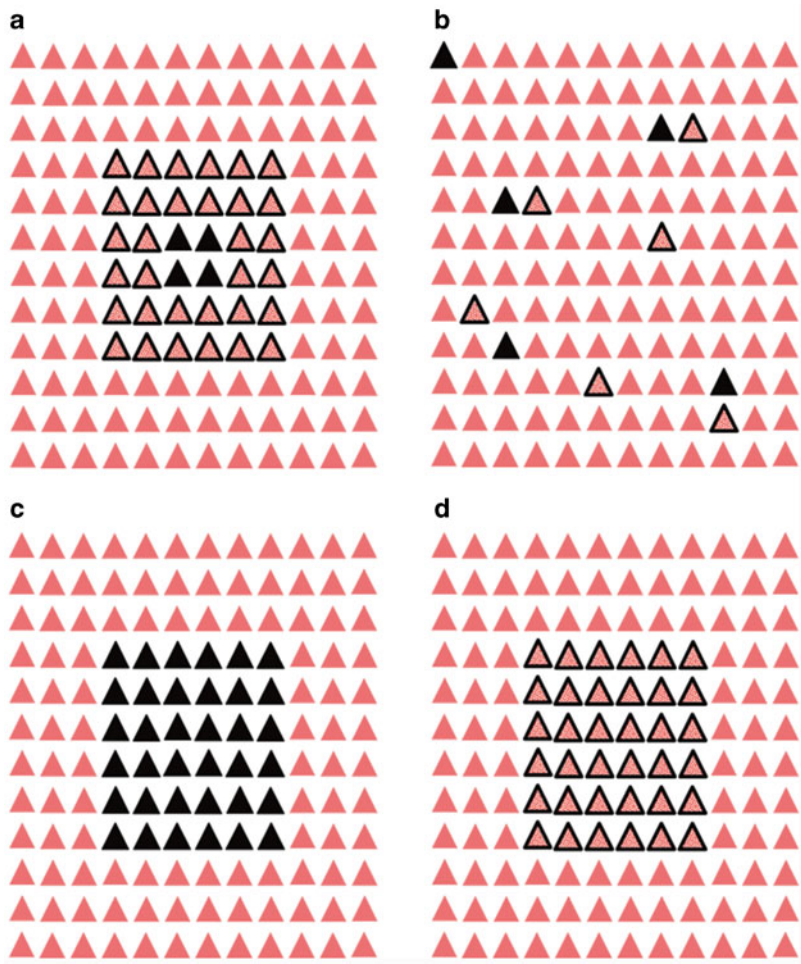

Fig. 9.1 Schematic diagrams showing hypothetical relationships of neuronal populations after a brain insult that activates cellular mechanisms of neuronal death. In the four panels of the figure, two or three populations of neurons are depicted in a schematic manner. Dead neurons (*filled black triangles*) are shown within a network of live and completely-normal neurons (*filled red triangles*). Among these two populations of cells is another group of neurons, which form the core of this hypothesis; these neurons have undergone only the initial steps of a neuronal-death and/or are under the molecular influence of the neuronal death process (*black triangular outline* with *red stiples* inside). (**a**) Focal neuronal loss. A small cluster of dead neurons is shown to be clumped together within a network of normal neurons, as would be expected to occur during an infarct. Between these two completely different neuronal populations is the group of neurons that are hypothetically epileptogenic, because they have undergone the first part of a neuronal-death process and/or are under the molecular influence of the neuronal death process. (**b**) Diffuse neuronal loss. Using the same code to define the members of the neuronal population, this diagram illustrates scattered neuronal loss, as would be expected to occur after status epilepticus (vs an infarct in (a)). (**c**) Occurrence of neuronal death without generation of neurons altered or influenced by death-process mechanisms, which theoretically represents the occurrence of frank brain damage without subsequent epilepsy. (**d**) Absence of neuronal death after a brain insult, but with the presence of death-pathway neurons. In this case, the death-pathway neurons are hypothesized to become epileptogenic, and they generate spontaneous recurrent seizures without the prior occurrence of overt neuronal death

9.1.2 Where?

If we focus on the seizures that characterize temporal lobe epilepsy and other forms of severe acquired epilepsy (e.g., after hypoxic-ischemic encephalopathy), many specific areas appear to be particularly prone to seizure-induced neuronal death. Depending on the etiology, neuronal death can be relatively circumscribed, as with an infarct (Fig. 9.1a), or it can be diffuse (Fig. 9.1b).

Seizures, particularly repetitive seizures, cause substantial brain damage in highly susceptible areas, such as parts of the hippocampus, entorhinal cortex, amygdala, thalamus and other limbic structures; however, neuronal death after seizures can be more widespread and is generally quite variable (e.g., [24, 77]).

9.1.3 What?

A focus in epilepsy research has been – and remains – the unequivocal identification of the type(s) of neurons that are killed: glutamatergic principal neurons, such as cortical pyramidal cells, and subpopulations of GABAergic interneurons, which comprise 5–10 % of the neurons in epilepsy-relevant brain regions and are highly heterogeneous in their anatomy and electrophysiology [4]. Regardless of the type of brain insult, the potential loss of interneurons is obviously a special case, because the loss of interneurons, if uncompensated by inhibitory axonal sprouting, can translate to a reduction in GABAergic tone.

9.2 What Are Some of the Important Technical and Experimental-Design Issues?

The challenges and controversies concerning how to evaluate whether neuronal death has occurred and how to quantify it are substantial. Even when one only considers a fraction of the methodological and protocol-related issues, the additional factors involving "what, where, and when" of neuronal death ("how" is discussed below) add further complexity to the potential analyses and interpretations. Additional disagreement surrounds the question "What is a seizure?" and the problem of what comprises an adequate animal model of acquired epilepsy.

An important issue in regard to considerations of neuronal death in epilepsy – as with most other research – involves the complimentary concerns of false positives (specificity) and false negatives (sensitivity). For example, two of the main approaches to analyzing neuronal death involve staining (1) those neurons that *remain* after seizures and (2) the neurons that are *destined to die*. Staining the remaining neurons involves a variety of traditional techniques such as cresyl violet staining of Nissl substance, and/or more specific methods including but certainly not limited to immunocytochemical staining of specific cell types, such as GABAergic interneurons. This most basic level of methodology has numerous caveats – some of which are obvious, and others not. For example, what does it mean when one finds no significant (i.e., statistical) difference between an experimental condition or animal model and the control group? On first principles, one has to ask: Does this mean that no neuronal loss (death) has occurred? Or, could it mean the amount of neuronal death was so small that it could not be detected? Issues such as how the tissue was sectioned (section thickness, where in the brain, but also orientation) are relevant, not to mention that extensive cell loss in epilepsy is associated with tissue shrinkage. Thus, cell number can be quite different than cell density. In regard to use of histological stains that mark "dying" neurons, such as FluoroJade B (FJB), one must also consider their advantages and disadvantages. For example, one has to question our confidence that they will actually die – can FJB-labeled neurons remain viable for a prolonged period before death? If we assume that all of the FJB neurons are going to die, or even most of them, then this approach has the important advantage that it can reveal situations in which only a small fraction of the neurons will die, which is simply not feasible with stains that mark the "remaining" neurons. Another issue, however, is that the FJB technique will only stain neurons that are dying at that particular time; so therefore, euthanasia, fixation, and staining must be performed at the appropriate time; neurons could have died at other times, and their death would not be detected with FJB [94]. Thus, although it is quite difficult to quantify neuronal loss, it is even more difficult – if not impossible – to show that neuronal loss has not occurred.

9.3 Mechanisms of Seizure-Induced Neuron Injury

In order to explore how seizures could kill neurons, it is first necessary to review cell-death pathways; our understanding of them has expanded well beyond the traditional mechanisms of *apoptosis* and *necrosis*. This seemingly simple endeavor is complicated by the observation that some of the newly identified cell-death pathways share criteria used for identification. For clarity, we classify cell-death processes as non-inflammatory (apoptosis, autophagy, phagoptosis) and inflammatory (necrosis, necroptosis, pyroptosis) (Table 9.1).

9.3.1 Apoptosis

A controlled, programmed process of packaging internal components of the cell for clearing by phagocytes characterizes the apoptotic process. As such, intracellular molecules with the potential to activate immune responses are disposed of rapidly, without initiating an immune response [2]. Apoptosis is also characterized by chromatin and cytoplasmic condensation, plasma membrane blebbing, formation of apoptotic bodies as well as fragmentation of cellular compartments and DNA. Apoptosis occurs naturally during development and serves as a means to facilitate cellular turnover in healthy tissue, and also in response to hormone withdrawal [47]. This programed series of events is reliant upon the effector functions of activated caspases −3, −6, and −7, which enzymatically cleave intracellular organelles, proteins and DNA. The degraded cellular corpse is then packaged in preparation for phagocytosis by macrophages or microglia [26]. Processing of intracellular compartments and subsequent removal of cellular debris during apoptosis does not result in a secondary inflammatory response in surrounding tissue as inflammatory mediators are largely sequestered and degraded [2]. Changes in mitochondrial membrane permeability [50] and release of mitochondrial proteins are also observed [87]. Another characteristic of apoptotic cells is the exposure of phosphatidylserine on the extracellular leaflet of their plasma membrane. While phosphatidylserine is normally found exclusively on the cytoplasmic side of the plasma membrane, apoptotic cells present phosphatidylserine on the extracellular surface to serve as an "eat-me" signal for neighboring phagocytes [32, 70]. Cellular shrinkage, likely due to caspase-mediated proteolysis of cytoskeletal proteins, also typifies apoptotic cells [49].

9.3.2 Autophagy

Although autophagy usually serves a protective role, in extreme stress conditions it can contribute to cell death. In similar fashion as apoptosis, autophagic pathways also progress in a series of cellular steps that involve programmed degradation of cellular components. However, intracellular autophagic, largely non-caspase, enzymes are responsible for degradation of organelles or other cytoplasmic proteins within double-membrane vesicles known as autophagosomes [54]. The autophagosome then fuses with intracellular lysosomes to facilitate degradation of the contents within the autophagosome by acid hydrolases. In contrast to apoptosis, caspase activation is not required and chromatin condensation is minor [11].

Table 9.1 Six mechanisms of cell death

Death process	Programmed?	Inflammatory lysis?	Effector	Shape Δ	TUNEL?
Necrosis	No	Yes	Non-caspase	Swell	No
Necroptosis	Yes	Yes	TNF-α RIPK1	Swell	No
Pyroptosis	Yes	Yes	Caspase-1	Swell	Yes
Autophagy	Yes	No	Lysosomes	?	No
Phagoptosis	Yes	No	Microglia	No	No
Apoptosis	Yes	No	Caspase-3/6/7	Shrink	Yes

In addition to contributing to the death of a cell, autophagic mechanisms also contribute to cellular function and homeostatic maintenance. For example, in the immune system, antigen-presenting cells utilize autophagy to digest intact proteins, creating smaller antigens for subsequent presentation to T lymphocytes [21, 54]. Moreover, mice deficient in proteins involved in autophagy develop spontaneous neurodegeneration [35, 48]. Taken together, these findings indicate that, in addition to cell death, autophagy mediates an important role in the organism's response to pathogens as well as maintenance of cellular homeostasis.

9.3.3 Phagoptosis

Many of the identified physiological cell death pathways involve phagocytosis of either whole cells doomed to die or of fractured cellular components. As such, the process of phagocytosis has been viewed as a secondary event, occurring after the death of the cell [70]. However, the process of phagocytosis can also kill living cells. Recent studies have identified a pathway, termed "phagoptosis", wherein phagocytes such as activated microglia actively contribute to the death of viable neurons and other cells [8]. Similar to apoptosis, the otherwise viable cell presents "eat-me" signals, such as phosphatidylserine, on the outer leaflet of its cellular membrane. The "eat-me" signals are then recognized by nearby phagocytes, and cellular uptake ensues followed by digestion of the viable cells. Importantly, cell death can be prevented during phagoptosis by inhibiting phagocytosis [28, 60]. This is because "eat-me" signal exposure is transient and reverses when phagocytosis is prevented. Therefore, neuronal insults not severe enough to initiate apoptotic pathways might be a trigger for phagoptosis due to the temporary exposure of eat-me signals on stressed but viable neurons [8, 28].

9.3.4 Necrosis

In contrast to these non-inflammatory modes of neuron death, during necrosis cells lyse, effectively spilling their internal contents into the interstitial fluid and releasing molecules that can initiate inflammatory cascades. This uncontrolled release of intracellular molecules can potentially damage surrounding tissue and cells [76, 79]. Necrotic cell death is typically initiated by extreme physiological stress or trauma that kills cells quickly. Biochemically, caspase is not involved. Morphologically, condensation or digestion of internal cellular compartments is not observed. Instead, organelles and the entire cell undergo extensive swelling. The cell eventually bursts, spilling its internal contents into the surrounding environment, triggering robust inflammation in the neighboring tissue [45].

9.3.5 Necroptosis

While necrosis leads to an uncontrolled cellular death, a variant of necrosis, which has some controllable features, has recently been described. This programmed pathway, termed necroptosis, exhibits characteristics of both programmed cell death and necrosis. The main characteristic distinguishing necrosis from necroptosis is that the latter is initiated by TNF-α and other death receptor activators, which promote the assembly of receptor-interacting protein kinase 1 (RIP1) with RIP3 [86]. Thus, kinase activity controls necroptosis [45]. Interestingly, RIP1 and RIP3 assemble into a functional kinase-containing cell-death complex only in the absence of functional caspase 8 [25, 46]. While the physiological impact of necroptosis is currently under investigation, it is conceivable this pathway may be relevant in the event caspase activity is impeded and thus canonical apoptosis is not possible.

9.3.6 Pyroptosis

Perhaps the most extreme example of inflammation-related cell death is pyroptosis (i.e., caspase 1-dependent programmed cell death). While this form of cell death was first described

after infectious stimuli, such as *Salmonella* and *Shigella* infection [6, 18], caspase-1-dependent cell death also occurs in myocytes after myocardial infarction [27] and in the central nervous system [53, 103]. The primary distinguishing feature of pyroptosis is the formation of the inflammasome, an intracellular multimolecular complex that is required for the activation of inflammatory caspases, particularly caspase 1. The activated inflammasome culminates with production of enzymatically active caspase 1, which in turn mediates the maturation and secretion of active IL-1β and IL-18 [2]. Secreted pro-inflammatory cytokines can subsequently influence nearby cells with potentially adverse consequences, such as blood-brain barrier breakdown and possible leukocyte entry into the brain. Although TUNEL-positive breaks in cellular DNA typify both apoptosis and pyroptosis, the latter is entirely reliant upon caspase-1 [7, 17]. This is important for classification purposes because caspase-1 is not involved in apoptosis. Mitochondrial release of cytochrome c, a hallmark of apoptosis, also does not occur during pyroptosis. In contrast to the coordinated packaging of intracellular components observed in apoptosis, cellular lysis and release of inflammatory effector molecules occur during pyroptosis [26].

9.4 How Might Seizure-Induced Neuronal Injury Promote Epileptogenesis?

9.4.1 Overview of Two Competing Hypotheses

We envision two conceptually distinct answers to this question. *First*, maladaptive new circuits among neurons could form to replace synapses *lost* during neuronal death. This mechanism, potentially involving axonal sprouting within excitatory pathways and amplified by loss of inhibitory interneurons, has been described in numerous previous studies and can be termed the "*recapitulation of development*" hypothesis. If replacement of lost synapses is the critical factor underlying this mechanism, then neuronal death would seem to be an essential component of the process. *Second*, rather than neuron death *per se* being responsible, molecular signals from upstream pathways that mediate some of the more newly recognized forms of cell death might underlie or contribute to epileptogenesis. We call this the "*neuronal death pathway*" hypothesis. We will focus on potential roles for IL-1β and TNF-α. We will also consider whether the inflammasome pathways (caspase-1 activation leading to synthesis of IL-1β and IL18), normally considered a feature of myeloid cells and innate immunity, might be involved in epilepsy-related neurodegeneration.

In some cases focal inflammation produced by lytic cell death, perhaps involving only a small number of neurons undetectable by normal Nissl stains (e.g., Fig. 9.1b), could promote increased neuronal excitability and perhaps synchronous activity. However, in the absence of any neuronal death (Fig. 9.1d), how might inflammatory cascades be initiated? Understanding how microglia, the innate immune cells of the CNS, respond to injurious or danger signals may provide insights into this undoubtedly complex process. Microglia in the intact, healthy brain continuously palpate the surrounding tissue for subtle disturbances [61], and can rapidly respond to tissue injury or danger signals by altering morphology, proliferating and expressing a wide variety of inflammatory cytokines and chemokines [19, 69]. Microglial activation can be initiated by injured neurons through the release of molecules collectively known as alarmins [4].

One well-characterized alarmin, prostaglandin E2, is released by highly active neurons in a COX-2-dependent process. Cyclooxygenase 2 (COX-2) is rapidly upregulated in hippocampal pyramidal cells and dentate granule cells after seizures [55, 73, 98], but the impact of *neuronal* COX-2 has remained elusive because astrocytes, endothelial cells and probably other cell types in the CNS also express COX-2. To determine the role of neuronal COX-2 after status epilepticus, a neuron specific conditional knockout mouse was utilized wherein principal neurons of the hippocampus, dentate granule cells, amygdala, thalamus and layer-specific neurons in the piriform

and neocortex (layer 5) are devoid of COX-2, while the remaining cell types of the CNS still express functional protein [43, 72]. Interestingly, conditional ablation of COX-2 from neurons resulted in less severe damage to hippocampal neurons after status epilepticus produced by pilocarpine. The intensity of status epilepticus was not diminished in the COX-2 conditional knockouts, as judged by the temporal evolution of behavioral seizures and by cortical EEG [78], making it unlikely that neuroprotection was caused by a less severe seizure episode. Neuroprotection was accompanied by reduction in multiple markers of neuroinflammation as well as preserved integrity of the blood-brain barrier, suggesting that neuronal COX-2 mediates a broad deleterious role after status epilepticus. These findings provide strong evidence that the neuron itself can contribute to the neuroinflammatory milieu [78]. The beneficial effects of the conditional ablation of COX-2 from principal forebrain neurons were completely recapitulated by systemic administration of a novel antagonist of EP2, a receptor for PGE2 [44].

Injured neurons might indirectly contribute to inflammation after status epilepticus through cell-to-cell signaling with microglia. Multiple lines of evidence indicate that the local microenvironment plays an important role in regulating the microglial phenotype wherein microglia activation is constitutively inhibited by repressive forces [34, 65, 69]. For example, surface proteins on microglia, such as CD200R and CX3CR1 (the fracktalkine receptor), normally interact with the neuronal surface protein ligands, CD200 and CX3CL1 (fracktalkine), respectively [14, 42]. If interactions between CD200R and CD200 [42, 102] or CX3CR1 and CX3CL1 [3, 13] are disrupted by signals released during neuronal damage or distress, then microglia are unleashed from this constitutive state of inhibition and a more florid microglial response ensues. Enhanced microglial activation is likely attributed to the presence of ITIM motifs (immunoreceptor tyrosine-based inhibitory motif) on both CD200R and CX3CR1 as these motifs function as activators for SHP-1 and SHP-2 phosphatases that can repress further inflammatory signaling [5]. Indeed, CX3CR1-deficient mice exhibit microglia-mediated neurotoxicity, through enhanced IL-1β secretion, after immune challenge [14]. Interestingly, altered expression of CX3CL1 has been reported in both epileptic patients and animals models after status epilepticus [97].

In addition to the above-mentioned studies, viable neurons might also induce inflammatory cascades. Studies in *Drosophila melanogaster* originated this concept, wherein damaged cells, prevented from dying, release mitotic signals that prompt neighboring cells to divide. Cells in the wing of flies were triggered to die by X-rays, but they were blocked from completing the death process by expression of anti-apoptotic proteins. The authors describe the resulting cells as "undead". The neighboring cells divide in an apparent attempt to fill the void in the tissue expected to be left by the dying cells [64]. Do similar situations occur in human disease? Interestingly, neuronal populations expected to degenerate in the brains of Alzheimer's Disease (AD) patients re-express proteins typically encountered in a mitotic cell cycle [12, 58, 92, 93]. Importantly, DNA replication accompanies cell cycle entry [99]. Transgenic mouse models of AD also recapitulate neuronal cell cycle entry [88, 101], suggesting that the same "stressors" that provoke neuronal cell cycle entry in the human AD brain are phenocopied in the mice. However, cycling neurons exhibit little atrophy [100] and robust neuronal loss is absent in AD mice [41, 56], indicating that re-expression of mitotic proteins and DNA synthesis in a post-mitotic neuron is not sufficient to induce death, at least in the lifetime of the mouse. It has been proposed that cycling neurons also might send out mitotic signals, pressuring otherwise healthy neurons to enter the "undead" state [39].

9.4.2 Inflammatory Pathways and Epileptogenesis

How might inflammatory signaling upstream of neurodegeneration increase excitability and subsequent synchronicity? Immune responses in the brain are initiated, maintained and terminated by soluble effector proteins known as cytokines. Although a strong correlation between seizures

and elevated inflammatory cytokines or their mRNA transcripts has been reported [90], emerging experimental evidence indicates that inflammatory cytokines can in turn alter neuronal excitability and synchronicity by modulating receptor function and expression [31, 89]. For example, the pro-inflammatory cytokine TNF-α has also been shown to promote the recruitment of AMPA receptors to postsynaptic membranes. Interestingly, the recruited receptors preferentially lack the GluR2 subunit [52, 63, 82] and consequently the calcium conductance underlying EPSPs is increased. Additionally, TNF-α causes endocytosis of $GABA_A$ receptors from the cellular surface, decreasing inhibitory synaptic strength [82]. Taken together these findings demonstrate that TNFα can have a profound impact on circuit homeostasis in a manner that can provoke the pathogenesis of seizures.

In addition to TNF-α, multiple lines of evidence directly implicate IL-1β in lowering the seizure threshold, and perhaps in epileptogenesis. First, hippocampal application of IL-1β can increase seizure intensity threefold. This proconvulsant effect is attributed to IL-1β-mediated engagement of Src-family kinases in hippocampal neurons. The activated kinases subsequently phosphorylate the NR2B subunit of the NMDA receptor, leading to seizure exacerbation [1]. Second, IL-1β can inhibit calcium currents through protein kinase C, at least at low concentrations [66]. Finally, IL-1β can also inhibit $GABA_A$ receptor current, which could underlie neuronal hyperexcitability [95]. These studies, coupled with the findings that pharmacological treatments targeting IL-1β or its activation result in robust anticonvulsant effects [20, 71, 90, 91], indicate that inflammation might play an important role in epileptogenesis and is a viable therapeutic target class.

9.5 Implications of the New Concepts on Neuronal Death for Epileptogenesis

The long-standing *recapitulation-of-development* hypothesis essentially states that neuronal death in acquired epilepsy is linked to a re-activation of developmental processes, which replace the synapses lost through neuronal death [30]. Initially, most experimental and clinical epileptologists viewed this hypothesis as "mossy fiber sprouting", which causes the formation of new recurrent excitatory circuits among dentate granule cells. This hypothesis is discussed by Buckmaster [9] and a more general view would be that neuronal death in many areas of the brain, particularly in seizure-sensitive regions, causes multiple networks to form new local excitatory circuits [22, 23, 83]. The data reviewed above suggest a new hypothesis, the *neuronal-death-pathway* hypothesis, whereby the biochemical pathways causing programmed neurodegeneration, rather than neuronal death *per se*, are responsible for or contribute to epileptogenesis. This hypothesis is consistent with the view that frank brain damage (i.e., cases where obvious neuronal death has occurred) leads to epilepsy, and further, that the likelihood of developing intractable epilepsy is linked somehow to the severity of the brain injury. In addition, however, this hypothesis may begin to explain why brain injuries that clearly induce neuronal death do not always appear to lead to epilepsy, since the critical hypothetical mechanism for acquired epileptogenesis would be the linkage between the to-be-defined mechanisms *within the pathways responsible for neuronal death*, as opposed to neuronal death itself (Fig. 9.1c). The identification of these hypothetical processes is an area ripe for future investigation. Finally, this hypothesis could also explain how epilepsy may occur when neuronal death is absent or appears minimal (Fig. 9.1d). It is conceivable that these molecular mechanisms may be aborted or reversed before neuronal death actually occurs, for example, so that specific signaling molecules direct some of the surrounding neurons toward an epileptogenic phenotype, even though the processes of neuronal death may not reach completion. The key point here is the proposal or hypothesis that molecular/genetic signals from neurons that are on a "death pathway" could initiate epileptogenesis independent of the final outcome (i.e., neuronal death).

9.6 Concepts and Conclusions

Although much has been learned about when seizures do kill neurons and the conditions when they appear to cause less damage, it is extraordinarily difficult to *rule out* that neuronal death has occurred after seizures. One problem is both a conceptual and technical one, namely, showing that something has not occurred is particularly challenging, if not impossible. We simply do not know if a threshold exists whereby a few, brief seizures – possibly in the seizure-resistant immature brain – cause absolutely no neuronal death. In terms of the question, "When?", there is no way to show that neuronal death has not occurred during and/or after seizures, except to count the remaining neurons in control and experimental groups; however, the potential error – even in well-powered studies, can be 10 % or more ([10] [see Table 1 and Fig. 2A-B]; [96] [see Fig. 6]) – and yet a loss of just a few percent of the neurons within a brain structure could have a substantive epileptogenic effect. If one considers the problem of "Where?", it becomes obvious that the answer is "Almost anywhere!". For the animal models of repetitive seizures and status epilepticus – whether induced by hypoxia, pilocarpine, or some other precipitating insult – numerous seizure-sensitive areas of the brain show neuronal loss, and the structure could be different for individuals within a similarly-treated cohort of animals, further supporting the idea that it is extremely difficult to exclude a role of neuronal death. In terms of, "What types of neurons may be lost?", excluding loss of part of the critical interneuron pool generally requires specific staining techniques, such as immunohistochemistry with stereology (e.g., [10]). As important, however, is the discovery of new neuronal death pathways that could lead to neuron loss in ways that have previously not been appreciated. This latter set of observations opens up the possibility that a gateway to seizure-induced neuronal loss involves signaling pathways that represent or are influenced by early neuron-death pathways. Thus, we propose that – in addition to the previously proposed *recapitulation-of-development* mechanisms – another hypothesis could be the *neuronal-death-pathway* hypothesis, whereby the early steps of neuronal death generate signals that promote epileptogenesis even if the neurons ultimately do not die. An attractive feature of this hypothesis is that it could lend itself to classification by molecular markers that reflect these neuronal pathway molecules. This hypothesis might also explain why neuronal death seems so important to acquired epileptogenesis, yet might in some cases be unnecessary.

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