an inevitable consequence of a seizure. Recent human studies show signalling pathways associated with apoptosis may be altered in patient brain, offering possible therapeutic opportunities to target seizure-induced neuronal death in different ways.

Hippocampal sclerosis is the most common pathologic finding in temporal lobe epilepsy (TLE). However, there are patients with TLE with no apparent hippocampal damage and people with hippocampal sclerosis without TLE. If hippocampal sclerosis causes TLE, then efforts to prevent this lesion's development are critical. If epileptic seizures cause neuron loss, however, therapeutic efforts to prevent all seizures from occurring become more important. This chapter describes cell death and survival mechanisms after single and repeated brief seizures in animal models and humans. What is the etiology of hippocampal and extra-hippocampal cell loss in intractable TLE? Is there ongoing cell loss in refractory epilepsy? The question of whether single epileptic seizures damage the brain has been the subject of several previous reviews, to which the reader is referred.[1–3] The focus of this chapter is to present the evidence for and against cell death after brief seizures and the molecular mechanisms which may underlie such an outcome. We omit discussion of other forms of neuronal damage (including reversible injury) which may also have significant behavioural or cognitive implications, and the influence of repair mechanisms such as neurogenesis. Discussion of these issues can be found elsewhere.[4]

Evidence from animal models that single or repeated evoked seizures cause neuron loss

Evidence that single or repeated brief seizures could cause neuronal death emerged from work in animals using electrical stimulation of various brain regions. While "kindling" paradigms are not ordinarily associated with permanent neuron loss,[4] papers published in the early 1990s, particularly by Sutula's laboratory, showed that kindling-induced seizures caused reductions in neuron numbers.[5] Cavazos *et al.*, showed repeated stimulation of the perforant path, olfactory bulb or amygdala resulted in progressive decreases in neuronal density in multiple subfields of the hippocampus, including the hilus, CA1 and CA3, and parts of the entorhinal cortex.[6] The somatosensory cortex was unaffected and changes were not attributable to tissue volume changes.[6]

Other studies using electrically-evoked seizures have reported similar findings.[7–8] Not only is neuron loss progressive, but it may increase with secondarily generalized tonic-clonic seizures.[8] Reduced hippocampal neuron densities have also been reported after electroshock seizures,[9] and in addition to hippocampal neuron loss, a subpopulation of amygdala neurons may also be vulnerable.[10–11] Finally, recent studies by Sloviter and colleagues showed that sustained electrical stimulation of the perforant pathway leading to the hippocampus, which did not cause convulsive seizures or *status epilepticus*, produced extensive neuronal death and hippocampal sclerosis.[12] Thus, repeated brief seizures or sub-convulsive stimulation of the hippocampus in certain models can reproduce patterns of neuron loss similar to those found in human hippocampal sclerosis. (Table 1)

Table 1. Summary of findings on neuron loss after single or repeated brief seizures in experimental models.

Pathologic outcome	*Findings*
Neuron loss after repeated evoked brief seizures	Observed in many but not all models
Neuron death detected after repeated evoked brief seizures	Observed in some models
Neuron loss after seizures in spontaneously epileptic animals	Inconclusive
Neuron loss after seizures in animals with acquired epilepsy	Not currently supported by the evidence
Apoptosis-associated signaling	Modulation of Bcl-2 family genes, caspases

Evidence against single and repeated evoked seizures causing neuron loss

Studies in kindling models have shown that brief single seizures do not necessarily lead to cell loss. Thus, Bertram & Lothman reported reduced neuronal density after kindling, but attributed this to tissue volume expansion.[13] The possible role of tissue volume changes and changes in neuronal morphology in reports of seizure-induced neuronal loss has been emphasized by numerous authors.[2, 14–15] Brandt *et al.*, also argued that neuronal density reductions after extended kindling were due to volume changes and not neuronal death.[16] Other groups also failed to detect neuronal death after kindling in rats,[17–18] and mice.[19] Thus, studies in which neuron counts were used as the principal measure of whether cell loss occurred are not in agreement as to whether brief seizures cause neuronal death (Table 1).

Detection of acute cell death after evoked single and repeated brief seizures

Direct evidence that brief seizures cause acute neuronal death has been provided by biochemical analyses. Bengzon *et al.*, showed that a single seizure evoked by electrical stimulation of the hippocampus could cause hippocampal neurons to die, as detected by silver staining and staining of cells for irreversible DNA fragmentation using TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling).[20] Notably, more stimulations caused proportionately more cells to die.[20] Using similar methods, other groups have also reported that repeated brief seizures cause hippocampal and extra-hippocampal cell death.[11, 21–23] These studies confirm that brief evoked seizures can cause neuronal death in animal models (Table 1).

Do spontaneous seizures in epileptic animals cause neuron loss?

While brief evoked seizures in nonepileptic animals are useful models they do not capture all aspects of the pathophysiology of spontaneous (i.e. epileptic) seizures. Is there evidence that spontaneous seizures in epileptic animals can cause neuron loss? This would be directly relevant to the etiology of progressive damage in human mesial temporal sclerosis. Two types of model have been studied in this context; animals that are spontaneously epileptic and animals which acquired epilepsy as the result of an initial precipitating injury. With the exception of certain genetically-altered mice with active neurodegeneration, neuron loss does not appear to occur in spontaneously epileptic animals. For example, the hippocampus of spontaneously epileptic EL mice, which experience multiple complex partial seizures with secondary generalization on a weekly basis, shows no obvious neuron loss.[24] Evidence of subfield-specific seizure-induced hippocampal neuron loss has been reported in spontaneously epileptic rats,[25] although no acute cell death after a seizure or a biochemical marker thereof was detected.[25]

Studies in animal models of acquired epilepsy also suggest spontaneous seizures do not cause further neuron loss. Pitkanen *et al.*, reported that a longer duration of epilepsy was not associated with lower numbers of neurons in epileptic rats.[26] Moreover, no acutely degenerating neurons were found in any of the chronically epileptic animals, despite some experiencing more than 10 seizures per day.[26] Other studies appear to corroborate these data; hippocampal damage may continue for some time following *status epilepticus*, but neuron loss does not progress once animals are epileptic.[27–29] (Table 1).

MOLECULAR MECHANISMS OF CELL DEATH FOLLOWING SINGLE AND REPEATED BRIEF SEIZURES

The molecular mechanisms underlying cell death following single and repeated brief seizures are not as well researched as they have been in models of *status epilepticus* (reviewed in refs. [30–32]). Glutamate-mediated excitotoxicity is the principal mechanism driving neuronal death after *status epilepticus,* whereby excessive

glutamate release leads to intracellular calcium overload, oxidative stress, organelle swelling and rupture of intracellular membranes, activation of proteases and necrosis.[33–34] Is glutamate-mediated toxicity the cause of neuron death after single or repeated brief seizures? We assume that it is, and necrosis has been detected after brief seizures,[11] but there have been no studies using appropriate pharmacological tools demonstrating that cell death can be prevented by glutamate receptor antagonists. Instead, there is biochemical and morphological evidence supporting cellular apoptosis occurring after brief seizures.[7, 20–21] Notably, administration of the *N*-methyl-D-aspartate glutamate receptor antagonist MK801 (which is neuroprotective in models of *status epilepticus*) did not prevent cell death after brief seizures.[20] The pathophysiologic changes caused by brief seizures are no doubt glutamate-driven and may feature perturbed intracellular calcium homeostasis,[35] but through other pathways. These might include non-NMDA receptor-gated calcium entry and disruption of endoplasmic reticulum or mitochondrial function. Thus, apoptosis, which may have overlapping mechanisms of activation with necrosis, contributes to cell death after single or repeated brief seizures.

Molecular control of apoptosis

Apoptosis is a form of programmed cell death used to dispose of unwanted or damaged cells in a controlled manner. Excess neurons are removed during brain development by apoptosis and apoptosis also occurs after the developing or mature brain is exposed to, or deprived of, certain substances. For example, ethanol exposure triggers widespread apoptosis in the developing rat brain,[36] and adrenalectomy triggers apoptosis of dentate granule neurons.[37]

Two main molecular pathways control apoptosis - extrinsic and intrinsic.[38–39] The extrinsic pathway is triggered by surface-expressed death receptors of the tumor necrosis factor (TNF) superfamily on binding their ligands (secreted cytokines such as TNFα). The intrinsic pathway is mitochondria-mediated, and activated by an array of intracellular stressors including DNA damage and perturbation of intracellular calcium homeostasis or organelle function.[40–41] This pathway is regulated by members of the Bcl-2 gene family at the point of initiation. Both pathways result in the downstream activation of a group of enzymes called caspases.

Caspases

The caspases are a family of cysteinyl aspartate-specific proteases expressed in healthy cells in an inactive zymogen form. Caspases share a common structure comprising an *N*-terminal pro-domain followed by a large ~20 kD subunit and smaller ~10 kD subunit. Caspases regulating apoptosis are typically organized into two functional groups: The upstream initiators, have long pro-domains. Activation of these requires protein-protein binding interactions between the pro-domain and scaffolding molecules activated in response to pro-apoptotic stimuli. For example, the pro-domain of caspase-8 binds to regions on signalling molecules recruited to the intracellular side of activated death receptors, whereas the pro-domain of caspase-9 associates with the apoptotic protease activating factor 1 (Apaf-1) forming the so-called apoptosome in association with released cytochrome *c* from mitochondria.[42] Activated initiator caspases then cleave and remove the short pro-domain of apoptosis effector (or executioner) caspases, thereby activating them.[42] Caspase-3 and other effector caspases such as caspases 6 and 7 then cleave numerous proteins within the cell, including structural proteins (a full listing can be found at http://bioinf.gen.tcd.ie/casbah/). Collectively, the caspase system results in hallmark morphological changes, DNA fragmentation (which can be detected by TUNEL), and eventual dispersal of the cell within membrane-enclosed apoptotic "bodies" to be phagocytosed by surrounding cells.

Bcl-2 family proteins

Bcl-2 family proteins function as critical regulators of apoptosis by controlling the release of intra-mitochondrial apoptogenic molecules via effects on outer mitochondrial membrane permeability. The Bcl-2 family comprises both pro- and anti-apoptotic members which share one or more Bcl-2 homology (BH) domains. Anti-apoptotic members include Bcl-2 and Bcl-xL, which possess four BH domains in common and a transmembrane