

# MECHANISMS UNDERLYING THE ONSET AND PROGRESSION OF TEMPORAL LOBE EPILEPSY

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## ABSTRACT

Epilepsy is a neurological condition marked by the spontaneous recurrence of seizures. Both genetic as well as environmental factors play a role in its etiology. Over the past two decades, the advent of sophisticated new technologies, better molecular and biochemical toolsets, and the establishment of a variety of excellent experimental models that recapitulate the human condition have done much to enhance our understanding of epilepsy. Herein, we present a succinct overview of some of the major themes that have emerged about the onset and progression of temporal lobe epilepsy.

Epilepsy is a neurological condition or syndrome that is marked by the unpredictable appearance of spontaneously recurring seizures. A seizure reflects a temporary change in a person's behavior and is promoted by the abnormal synchronized and repetitive firing of a cluster of neurons. Seizures which originate from a focal point in the brain are called partial seizures, while generalized seizures involve both cerebral hemispheres. Generalized seizures are accompanied by impaired consciousness, whereas simple seizures are not.

There are over 40 different types of epilepsies which reduce the quality of life for approximately 50 million people around the globe. About one-half of all epilepsies have no known cause and are called idiopathic; these types are mostly associated with genetic defects. Epilepsies where the cause is known are termed symptomatic epilepsy. The aim of this article is to discuss the etiology and progression of temporal lobe epilepsy (TLE), a prevalent form of epilepsy. However, before doing so it would be useful to have a clear understanding of the ionic basis of neural excitation and inhibition. This will be followed by a brief treatment of the role genes and environment play in epileptogenesis. Finally, mechanisms underlying the onset and progression of TLE will be discussed.

## IONIC BASIS OF NEURAL EXCITATION AND INHIBITION

At rest, neurons normally register a membrane potential of around -60 millivolts (mV) relative to the extracellular milieu. This is predominantly due to the fact that sodium and chloride ions are more concentrated outside the cell membrane, whereas potassium along with other organic ions and numerous proteins are found at higher concentrations intracellularly.

When excitatory neurons are exposed to glutamate, this results in the opening of glutamate specific ligand-gated ion channels that permit rapid influx of Na<sup>+</sup>. This process is further amplified through the opening of voltage-sensitive Na<sup>+</sup> channels and results in total collapse of membrane potential (which goes from negative to positive). This depolarization phase is transient and is rapidly followed by repolarization during which membrane potential reverts back to -60 mV. The repolarization step is facilitated by opening of voltage-sensitive K<sup>+</sup> channels that allow effluxed K<sup>+</sup> to reenter a cell. Opening of certain glutamate ligand-gated channels also permits Ca<sup>2+</sup> entry, which in turn plays an instrumental role in propagating the action potential by facilitating the release of neurotransmitter-containing vesicles from axons into the synaptic cleft.

Inhibition is a process exactly opposite to that of excitation. Here instead of sodium, neurons selectively allow Cl<sup>-</sup> to

enter through chloride-specific ion channels, hence increasing the membrane potential; hyperpolarized neurons require more intense excitatory input to be depolarized.

Glutamate and GABA (γ-amino butyric acid) are the two major neurotransmitters in the human nervous system that play opposite roles; glutamate is excitatory whereas GABA is inhibitory. Glutamate exerts its effect by binding to ionotropic (i.e., ligand-gated) and metabotropic receptors. The latter are G-protein coupled receptors (GPCR) whose stimulation activates signaling cascades via different second messenger systems, which in turn modulate gene expression and also influence the activity of different proteins.

NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4 isoxazole propionic acid) and kainate are the three types of ionotropic glutamate receptors which, upon stimulation, cause depolarization. In contrast, metabotropic receptor activation is a slow process, but its downstream effects are longer lasting as compared to ionotropic receptors, which respond quickly, but only have a transient effect on intracellular processes. GABA receptors are of three types namely, GABA<sub>A</sub>, GABA<sub>B</sub> and GABA<sub>C</sub>.<sup>1</sup> Among these GABA<sub>A</sub> is the ligand-gated ion channel which upon opening allows Cl<sup>-</sup> entry; GABA<sub>B</sub> and GABA<sub>C</sub> are metabotropic GPCRs.

In a normally functioning brain a delicate balance exists between excitation and inhibition. Loss of inhibition, either through loss of GABA-ergic neurons, or due to poor expression of GABA receptor subunits, or one or more enzymes involved in synthesizing GABA, makes the brain hyper-excitable and susceptible to seizures. These conclusions stem from the observation that in the presence of picrotoxin, a GABA antagonist, pyramidal cells in hippocampal slices fire bursts of action potential more frequently than control slices. It has been estimated that without GABA blockade a neuron has about a 5% chance of firing and that this chance increases to 30% when inhibition through GABA is alleviated. Any significant compromise in GABA mediated inhibition is therefore likely to initiate a cascade of events that will frequently promote synchronized activation of excitatory neuronal ensembles. Thus, for a normally functioning brain it is essential that the excitation-inhibition balance remains as horizontal as possible. Genetic or environmental factors that tilt this balance towards excitation are likely to result in the development of various neuropathologies including epilepsy.

## GENETIC EPILEPSIES

Over 30% of epilepsy syndromes are due to defects predominantly in genes encoding ion channels.

Interestingly, many of these so called idiopathic epilepsies have specific ages of onset and offset, suggesting that there is a certain window of time during development in which the brain is more vulnerable to the effects of the mutated gene, or that the expression of the mutant gene during a certain phase of development makes the brain epileptic. Onset of idiopathic epilepsies is dependent on the type of affected genes and the locations of mutations within those genes; some of these epilepsies start in the neonatal period, some during late childhood and still others do not start until an individual reaches adolescence or even adulthood.

Epilepsy genes have been discovered either through cloning of the inherited disorders (in humans, *Drosophila melanogaster* and *Mus musculus*), or serendipitously after employing a targeted or random mutagenesis protocol.<sup>2,3</sup> To date, mutations in over 200 different types of genes have been shown to be responsible for causing epilepsy.<sup>4,5</sup> As expected, a vast majority of these epilepsy causing genes encode for voltage-sensitive Na<sup>+</sup> and K<sup>+</sup> ion channels, ligand-gated GABA<sub>A</sub> receptor subunits, and a nicotinic acetylcholine receptor subunit.<sup>6-8</sup> Since normal brain development follows a complex course that is driven by a heterogeneous lexicon of genes expressing proteins and un-translated RNAs, the list of epilepsy causing genes will continue to grow rapidly.

SCN1A encodes for a Na<sup>+</sup> channel subunit and represents one of the most intriguing examples of a gene in which mutations cause seizures. Carriers of this mutant gene exhibit a range of phenotypes which are dependent on the type and location of mutations. For example, various missense mutations in SCN1A have been found to cause generalized epilepsy with febrile seizures plus (GEFS+) in which the severity of seizures varies from benign febrile seizures to syndromes accompanied with various other seizure types.<sup>9</sup> Remarkably, however, the epileptic effects of this gene are lost completely by the time an individual reaches adolescence. More severe mutations within SCN1A that lead to the production of a truncated protein cause severe myoclonic epilepsy during childhood which is debilitating and refractory to therapy. KCNQ2 and KCNQ3 are examples of two additional genes where mutations cause benign familial neonatal seizures (BFNS) that start on the third day of life but which almost always disappear within a few months. Similarly, benign familial neonatal-infantile seizures (BFNIS) is a condition in which the period of seizure onset varies from the neonate to early infantile but no further seizures are experienced after the child reaches one year of age. This condition is caused by a mutation in the Na<sup>+</sup> channel encoding gene SCN2A which results in defective splicing of its mRNA.<sup>10</sup>

Not all epilepsy causing mutant genes encode for ion channels. In fact a battery of defective genes that cause

storage disorders (e.g., Tay-Sachs), chromosomal disorders (e.g., Fragile-X and Down's syndrome), mitochondrial disorders (e.g., MELAS and MERRF), amino acid disorders (e.g., phenylketonuria) and neural malformation disorders (e.g., tuberous sclerosis and double-cortex syndrome) also promote seizures. How these defective genes cause epilepsy remains unclear.

## ACQUIRED EPILEPSIES

For a normal brain to develop properly, it is essential that different cell-types from different brain areas differentiate at the right time and make connections to their proximal as well as distant neighbors (some of which are non-neuronal) in order to produce a highly sophisticated circuit that is capable of readily responding to both endogenous as well as exogenous (i.e., environmental) cues. Putting together such a network requires a sophisticated plan which is executed and orchestrated by the temporal and spatial expression of a lexicon of different genes. An integral part of this development also involves programmed cell death (apoptosis) of almost half of the neurons. Given that the "pruned" human brain consists of approximately one billion neurons it is highly plausible that even miniscule changes in neural circuitry during ontogenesis will cause disease.

Whether in utero or after birth, any environmental insult that is intense enough to shift the excitation-inhibition equilibrium towards excitation is likely to cause epilepsy along with a set of other problems; a shift in the other direction (i.e., too much inhibition) may also be accompanied by neurological issues, such as anxiety and depression.

A remarkable feature of the developing brain is that despite being hyper-excitable, it does not produce seizures. There are two main reasons for this. One is that in the immature brain GABA, which is the major inhibitory neurotransmitter in adults, has excitatory (i.e., depolarizing) properties. Secondly, there is an overabundance of excitatory neurotransmitter receptors in the developing brain. Although these factors clearly perturb the excitation-inhibition balance, the immature brain does not experience spontaneous seizures; this is a dilemma.

Among the different forms of acquired epilepsies, most is known about the molecular and cellular biology of temporal lobe epilepsy (TLE), at least partly because of its prevalence (up to 40% of all epilepsies), and because its onset, pathophysiology and progression are relatively easy to mimic in experimental animal models. TLE refers to a chronic condition in which spontaneously recurring seizures originate primarily from one or both temporal lobes. The two main types of TLE are mesial (MTLE), which originates from hippocampus and amygdala, and lateral (LTLE) which arises from the neocortex and outer periphery of the

temporal lobe. MTLE is associated with generalized tonic-clonic seizures that are frequently caused by a fever in children less than 5 years of age. Early onset of MTLE normally causes hippocampal shrinkage that can be seen readily by magnetic resonance imaging (MRI) of the affected brain; interestingly, no hippocampal sclerosis is seen in brains of individuals in whom MTLE begins during adulthood. The etiology of LTLE is not as clear as that of MTLE but genetic factors as well as injury, tumors and infections (e.g., meningitis, encephalitis) are thought to be triggers. Since much is known about the onset and progression of MTLE, which is much more prevalent than LTLE, the subsequent discussion will focus entirely on MTLE.

## Mesial Temporal Lobe Epilepsy

The old tenet "seizures beget seizures" still holds true today. Although the prevalence of epilepsy worldwide ranges from 0.5-1.0%, it is estimated that over 5% of people experience a seizure at some point in their life. While these numbers refute the tenet somewhat because not all seizures in fact beget more seizures,<sup>11</sup> it does suggest that a single seizure significantly increases an individual's chance of experiencing additional seizures.

One way to defend the tenet would be to consider that it is the intensity of first seizure that really dictates the course of the syndrome. If the initial insult is weak (i.e., sub-threshold) it may not have any consequences (as in GEFS+). If the first seizure-catalyzing insult is intense (i.e., above a certain threshold) then it is likely to initiate a program that transforms a normal brain into one which is epileptic. Alternatively, sub-threshold insults may kill neurons cleanly via apoptosis while stronger insults might cause cell death through necrosis. Progression towards epilepsy might possibly be due to necrotic cell death because of a strong insult, while sub-threshold seizures may be inert because they only promote apoptosis.

Temporal lobe epilepsy begins early in life after either of two hippocampi is damaged because of a prolonged seizure precipitated by infection or fever. Since babies have a poorly developed thermoregulatory system, a fever increases the core body temperature, thus evoking a seizure. Commonly, these seizures last for only 1-2 minutes but in some cases the convulsions can go on for as long as one hour; such a prolonged seizure enhances an infant's chances of developing TLE.

How can one intense seizure accomplish this? Studies based on a variety of different experimental animal models have demonstrated clearly that even a single intense seizure is capable of causing excitotoxic death of numerous hippocampal neurons.<sup>12,13</sup> That the NMDA receptor is directly involved in neuronal cell death is demonstrated by

Table 1: Important dates and events associated with epilepsy

Year	Event
Pre400BC	Epileptics thought to be possessed by demons
400BC	Hippocrates was the first to recognize epilepsy as a brain disorder
1494	Seizures seen as a characteristic of witches
1859-1906	Recognized as the beginning of modern era of epilepsy due to the work of Jackson Reylonds and Gowers; seizures were defined scientifically and their effect on behaviour studied and documented
1904	Term 'epileptologist' coined by William Spartling
1912	First anticonvulsant drug phenobarbital is introduced under Luminal
1920	Ketogenic diet comprised of high fat and low carbohydrate is used for treating children with epilepsy
1929	Electroencephalogram, used to monitor brain's electrical activity and useful for diagnosing epilepsy is discovered by Hans Berger
1939-1963	Anticonvulsants phenytoin, carbamazepine, ethosuximide and sodium valporate were introduced in treatments
1968	Epilepsy foundation of America (EFA) is founded
1970	Veterans Administration establishes epilepsy centers in the US
1993-2000	Anticonvulsants felbamate, gabapentin, lamotrigine, topiramate, tiagabine, levetiracetam, zonisamide and oxcarbazepine are introduced for clinical use. Vagus nerve stimulation also approved
2000	EFA organizes conference on 'Curing Epilepsy' and sets aggressive goals for the prevention, treatment and cure of epilepsy.

the fact that co-injection of MK-801 (a noncompetitive antagonist of the NMDA receptor) with kainate does not kill hippocampal neurons as extensively as kainate alone; furthermore, pharmacological blockade of the NMDA receptor has been found to reduce epilepsy progression in vivo in several of the rodent animal models of epilepsy including those based on kindling, kainate and pilocarpine.<sup>14-16</sup> The excitotoxic cell death of hippocampal neurons is caused by  $Ca^{2+}$  which at physiological levels plays a variety of important roles in cellular homeostasis but when present in abnormally high amounts can be toxic.<sup>17</sup>  $Ca^{2+}$  enters cells through the NMDA and AMPA receptors, as well as through the voltage-sensitive  $Ca^{2+}$  channels (e.g., L, T, N, P and Q); the incoming  $Ca^{2+}$  further promotes release of more  $Ca^{2+}$  from endogenous mitochondrial and endoplasmic reticulum reservoirs. These abnormally high  $Ca^{2+}$  levels promote activation of different signaling pathways and enzymes (e.g., proteases, nucleases, etc.); calcium-induced activation of nitric oxide synthetase (NOS) and generation of excess free radicals that damage membranes and nucleic acids are also thought to play important roles in promoting cell death.<sup>18</sup> Additionally, glutamate released from neurons that have experienced sudden death through necrosis will hyper-excite other neurons in its vicinity and this process might also cause more cell death. Intense seizures, along with the debris released by the dead cells, are therefore capable of initiating signaling cascades in the surviving cells which in turn influence the expression of genes encoding proteins and enzymes involved in neural plasticity.

The hippocampus is the structure that is central to learning and memory but which is the brain region affected most by seizures. Studies have shown that repeated seizures cause hippocampal sclerosis presumably through excitotoxic death of a large number of neurons via excessive activation of glutamate receptors. Seizure-induced cell death has been reported to occur during the early stages of epileptogenesis but not in the chronic stages.<sup>19,20</sup> The granule cells which make up most of the dentate gyrus have axons called mossy fibers. Seizure-induced death of some of the sensitive granule cells in the hilar region alters gene expression patterns, beginning with master regulatory proteins like c-Fos, Jun, zif-268, etc., which promote the sprouting of mossy fibers. Ultrastructural analyses have shown that this sprouting is associated with the formation of new synapses that innervate dendrites of granule cells; these aberrant connections therefore produce a neural circuit that is easily synchronizable and hypersensitive to excitatory stimuli. That this newly established hyper-excitabile circuit is the underlying cause of epilepsy is demonstrated by the fact that surgical removal of the sclerotic hippocampus leads to dramatic improvement and, in some cases, a complete cure. Mossy cells are the other class of cells that are located in the hilus region and are also extremely sensitive to seizure-induced cell death. These neurons are thought to be excitatory and relatively easier to activate than granule cells by low threshold perforant path stimulation. Several studies have suggested that it is the death of the GABA-ergic neurons that tips the balance towards excitation. Interestingly, detailed immunohistochemical analysis of sclerotic hippocampii from humans and animal models has revealed that these

GABA-ergic interneurons are much more resilient to seizure-induced excitotoxic cell death as compared to mossy cells. This observation has become the basis of the so-called dormant basket cell hypothesis which states that the death of mossy cells eliminates functional excitatory projections into the GABA-ergic basket cells, causing loss of inhibition because these "unconnected" basket cells lie dormant. In light of the findings that excitatory mossy cells are not consistently damaged by seizures and that the inhibitory basket cells are also sometimes prone to seizure-induced cell death, this hypothesis has been revised.<sup>21</sup>

Interestingly, there is also strong experimental basis for believing that the death of neurons is accompanied by neurogenesis. It is well known that in normal adults there is a certain amount of neurogenesis that constantly goes on. However, recent findings have indicated that the number of granule cells increases significantly in the dentate gyrus after seizure-induced cell death.<sup>22-24</sup> Whether dentate neurogenesis takes place once status epilepticus has been reached remains an open question.<sup>25</sup> Although some of these newborn cells are capable of finding their correct positions, there are numerous others which do not reconnect to their neighbors normally and therefore end up forming synaptic networks composed of easily excitable ensembles.

## CONCLUSIONS

The onset of idiopathic epilepsies and their progression is dependent entirely on the type of gene carrying the mutation. Some of the genetic epilepsies are volatile which appear during childhood but then disappear in a few months without leaving an apparent effect on the child. Other types of idiopathic epilepsies have a different age of onset and severity, and most are treatable. It is important to note that some of the idiopathic epilepsies are also accompanied by hippocampal sclerosis. With regard to temporal lobe epilepsy, a theme that has emerged over the past decade is that it progresses in three stages. The first step of epileptogenesis begins when a prolonged seizure is induced by fever, trauma, hypoxia, severe perinatal injury, stroke, developmental or post-infection lesions, and tumors. The initial insult is sufficient to slightly tilt the balance towards excitation through a combination of excitotoxic cell death, cell birth, and changes in gene expression patterns within surviving seizure-resistant neurons. This is then followed by a quiescent (latent) period during which alterations in the hippocampal circuitry take place in a way which reduces seizure threshold within a neuronal ensemble; in this phase an individual experiences seizures periodically which cause more significant morphological changes in hippocampal circuitry in a way which further lowers seizure threshold. This cascade eventually culminates in the establishment of a

chronic state commonly known as status epilepticus during which an individual experiences intense seizures frequently. The currently available portfolio of anticonvulsant drugs is not ideal for treating TLE patients because these drugs have severe side-effects; in extreme cases sufferers must resort to surgery to gain relief. In the absence of a good small molecule as therapy for combating TLE, there is an urgent need to identify molecules involved in TLE onset and progression that can serve as suitable targets for drug development.

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