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BIOMOLECULAR MECHANISMS OF EPILEPTIC SEIZURES AND EPILEPSY: A REVIEW
Komang Trisna Sumadewi; Saktivi Harkitasari; David Christopher Tjandra
Acta Epileptologica

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AEPI-D-23-00028
BIOMOLECULAR MECHANISMS OF EPILEPTIC SEIZURES AND EPILEPSY: A REVIEW
Komang Trisna Sumadewi; Komang Trisna Sumadewi; Saktivi Harkitasari
Acta Epileptologica

Dear Ms Sumadewi,

Your manuscript 'BIOMOLECULAR MECHANISMS OF EPILEPTIC SEIZURES AND EPILEPSY: A REVIEW' (AEPI-D-23-00028) has been assessed by our reviewers. Although it is of interest, we are unable to consider it for publication in its current form. The reviewers have raised a number of points which we believe would improve the manuscript and may allow a revised version to be published in **Acta Epileptologica**.

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Reviewer reports:

Reviewer #1: The biggest difficulty in preventing and treating epilepsy is that people do not understand the mechanism of epilepsy formation and cannot develop new treatment methods based on the pathogenesis of epilepsy. Therefore, studying the causes of epilepsy formation is particularly important. The author of this article summarizes the research progress on epilepsy formation, which is very interesting and helpful to readers

Reviewer #2: In general, the author showed us molecular pathways involved in seizures and epilepsy, including inflammation, blood-brain barrier, mTOR pathway, Apoptotic Pathway, Ionic environment modifications, Neurogenesis, oxidative stress and miRNAs. However, the major fault is that the author only piles these data together, the whole paper is lacking logic and hierarchical, it will confusing to us. So I suggesting that the author should analyze the role of different molecules in different seizure periods. The following is the mirror detail suggestion :

1. The abstract descript The background of epilepsy is too much, and the content of the whole article is very simple. It is suggested that the background introduction in the front needs to be introduced, and the content of the following article needs to be introduced in detail.
2. In the whole paper the figure1/2/3 sign should marker in the text in the appropriate part.
3. Paragraph formatting needs to be adjusted. Some paragraphs do not have blank lines before them
4. Judging from the author's sub-title, the relationship between biological function and epilepsy described by the author in each paragraph lacks a sense of hierarchy, and the author should be structured in a digital hierarchical way, which will appear clearer. And some paragraph should adjust their position order.

Reviewer #3: After reading this paper, I think this paper is complete and contains all the necessary components. The structure is reasonable and logical. To further improve this manuscript, I have the following suggestions:

1. If it is possible, a figure, which illustrate the relationship between inflammation and epilepsy, should be made in the section "The role of

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2. In this article, the content is wordiness. It would be more convenient, if strengthen the use of abbreviations of professional terms. For example, Blood-Brain Barrier—BBB, Status Epilepticus—SE.
3. The section of "The Role of Thalamocortical Circuit Disruption in Epilepsy, Especially The Absence Type", which illustrate potential brain circuit changes of epilepsy. However, the thalamocortical circuit is just one of the pieces of evidence. It would be better, if add some new evidence. For example, hippocampus circuit.

Reviewer #4: The review is well summarize the biomolecular mechanisms of epileptic seizures and epilepsy. The structure and the logic of the paper is good, but there are still several points need to be improved.

1. The contents of the abstract need to be more refined, especially the description of the mechanism, which exhibits a lot of overlapping paragraph with the content of the introduction.
2. The introduction part of the article needs to present some general characteristics of the disease, like morbidity, clinical manifestations, pathogenic mechanism, prognosis..., and subsequently turn to introduce the primary contents of the review.
3. References that article cited are relatively too old.
4. There are some problems with sentence structure, verb tense and clause construction.

From editor:

Please make point-to-point response to reviewers and check the attachment for the revision of the article.
To make it clearer to readers, it is recommended to rearrange the structure of the manuscript. (a figure of schematic drawing or more logical and hierarchical heading sections will be helpful)
If you have any concerns or questions, please contact with the editor, E-mail: actaepilepsyeditor@163.com.

Acta Epileptologica

BIOMOLECULAR MECHANISMS OF EPILEPTIC SEIZURES AND EPILEPSY: A REVIEW

--Manuscript Draft--

Manuscript Number:	AEPI-D-23-00028R1
Full Title:	BIOMOLECULAR MECHANISMS OF EPILEPTIC SEIZURES AND EPILEPSY: A REVIEW
Article Type:	Review
Funding Information:	
Abstract:	<p>Epilepsy is a recurring neurological disease caused by the abnormal electrical activity in the brain. This disease has caused about 50 new cases in 100.000 populations every year with the clinical manifestations of awareness loss, bruising, and mobility abnormalities. Due to the lack understanding of the pathophysiology behind the illness, a wide variety of medications are available to treat epilepsy. Epileptogenesis is the process by which a normally functioning brain undergoes alterations leading to the development of epilepsy, involving various factors. This is related to the inflammation which is driven by cytokines like IL-1 and tumor necrosis factor-α TNF-α leads to neuronal hyperexcitability. Pro-inflammatory cytokines from activated microglia and astrocytes in epileptic tissue initiate an inflammatory cascade, heightening neuronal excitability and triggering epileptiform activity. The blood brain barrier (BBB) maintains central nervous system integrity through its tight endothelial connections, but inflammation impact BBB structure and function which leads to immune cell infiltration. The mTOR pathway's excessive activation influences epileptogenesis, impacting neuronal excitability, and synapse formation, with genetic mutations contributing to epilepsy syndromes and the modulation of autophagy playing a role in seizure onset. The apoptotic pathway contribute to cell death through glutamate receptor-mediated excitotoxicity, involving pro-apoptotic proteins like p53 and mitochondrial dysfunction, leading to the activation of caspases and the disruption of calcium homeostasis. Ionic imbalances within neural networks contribute to the complexity of epileptic seizures, involving alterations in voltage-gated sodium and potassium channels, and the formation of diverse ion channel subtypes. Epileptogenesis triggers molecular changes in hippocampus, including altered neurogenesis and enhanced expression of neurotrophic factors and proteins. Oxidative stress leads to cellular damage, disrupted antioxidant systems, and mitochondrial dysfunction, making it a key player in epileptogenesis and potential neuroprotective interventions. Thalamocortical circuitry disruption is central to absence epilepsy, the normal circuit becomes faulty and results in characteristic brain wave patterns.</p> <p>Keywords: Epileptic Seizures, Epilepsy, Epileptogenesis, Molecular mechanis</p>
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Response to Reviewers:	<p>Reviewer #1: The biggest difficulty in preventing and treating epilepsy is that people do not understand the mechanism of epilepsy formation and cannot develop new treatment methods based on the pathogenesis of epilepsy. Therefore, studying the causes of epilepsy formation is particularly important. The author of this article summarizes the research progress on epilepsy formation, which is very interesting and helpful to readers</p> <p>Author: Thank you for the feedback on this article</p> <p>Reviewer #2: In generally, the author showed us molecular pathways involved in seizures and epilepsy, including inflammation, blood-brain barrier, mTOR pathway, Apoptotic Pathway, Ionic environment modifications, Neurogenesis, oxidative stress and miRNAs. However, the major fault is that the author only piles these data together, the whole paper is lacking logic and hierarchical, it will confusing to us. So I suggesting that the author should analyze the role of different molecules in different seizure periods. The following is the mirror detail suggestion:</p> <ol style="list-style-type: none"> 1.The abstract describe The background of epilepsy is too much, and the content of the whole article is very simple. It is suggested that the background introduction in the front needs to be introduced, and the content of the following article needs to be introduced in detail. 2.In the whole paper the figure 1/2/3 sign should marker in the text in the appropriate part. 3.Paragraph formatting needs to be adjusted. Some paragraphs do not have blank lines before them 4.Judging from the author's sub-title, the relationship between biological function and epilepsy described by the author in each paragraph lacks a sense of hierarchy, and the author should be structured in a digital hierarchical way, which will appear clearer. And some paragraph should adjust their position order. <p>Author:</p> <ol style="list-style-type: none"> 1.We have made improvements to the abstract and introduction (can be seen on pages 1-2) 2.We have added captions to each of Figures 1, 2, and 3. Figure 1 caption can be seen on page 3, Figure 2 caption can be seen on page 9, and Figure 3 caption can be seen on page 10. Figure 4 has been marked on page 11 second paragraph. 3.We have adjusted the paragraph format according to the feedback provided. 4.We have tried to reorganize the content of the article and add images and captions. We have also added explanations to some paragraphs to make them easier to understand and more structured. <p>Thank you for the feedback on our article.</p> <p>Reviewer #3: After reading this paper, I think this paper is complete and contains all the necessary components. The structure is reasonable and logical. To further improve this manuscript, I have the following suggestions:</p> <ol style="list-style-type: none"> 1.If it is possible, a figure, which illustrate the relationship between inflammation and epilepsy, should be made in the section "The role of inflammation in epileptic seizure and epilepsy" for the readers' convenience. 2.In this article, the content is wordiness. It would be more convenient, if strengthen the use of abbreviations of professional terms. For example, Blood-Brain Barrier—BBB, Status Epilepticus—SE. 3.The section of "The Role of Thalamocortical Circuit Disruption in Epilepsy, Especially The Absence Type", which illustrate potential brain circuit changes of epilepsy. However, the thalamocortical circuit is just one of the pieces of evidence. It would be better, if add some new evidence. For example, hippocampus circuit. <p>Author:</p> <ol style="list-style-type: none"> 1.We have added Figure 1 which explains the role of inflammation in epilepsy. Figure 1 can be seen on page 3 2.We have corrected the words according to the abbreviations of professional terms. 3.We have added the involvement of hippocampal circuits in absence-type epilepsy. This explanation can be seen in the third paragraph of the subtitle "The Role of Thalamocortical Circuit Disruption in Epilepsy, Especially the Absence Type" on page 18. <p>Thank you for the feedback on our article.</p>

	<p>Reviewer #4: The review is well summarize the biomolecular mechanisms of epileptic seizures and epilepsy. The structure and the logic of the paper is good, but there are still several points need to be improved.</p> <ol style="list-style-type: none"> 1.The contents of the abstract need to be more refined, especially the description of the mechanism, which exhibits a lot of overlapping paragraph with the content of the introduction. 2.The introduction part of the article needs to present some general charateristics of the disease, like morbidity, clinical manifestations, pathogenic mechanism, prognosis..., and subsequently turn to introduce the primary contents of the review. 3.References that article cited are relatively too old. 4.There are some problems with sentence structure, verb tense and clause construction. <p>Author:</p> <ol style="list-style-type: none"> 1.We have improved the abstract to provide an overview of the mechanism of epilepsy (see abstract page 1). We have also improved the content of the article and reorganized it to reduce overlapping. 2.In the introduction section, we have added the general characteristics of epilepsy which can be seen in paragraph 1 of the introduction subtitle on page 1. 3.We have updated some of the references that are too old. This can be seen in the references section 4.We have tried to improve the sentence structure in this article. <p>Thank you for the feedback on our article</p>
Additional Information:	
Question	Response

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BIOMOLECULAR MECHANISMS OF EPILEPTIC SEIZURES AND EPILEPSY: A REVIEW

Komang Trisna Sumadewi¹, Saktivi Harkitasari², David Christopher Tjandra³

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²Neurology Department, Faculty of Medicine and Health Science, Warmadewa University, Denpasar, 80235, Indonesia

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Abstract

Epilepsy is a recurring neurological disease caused by the abnormal electrical activity in the brain. This disease has caused about 50 new cases in 100.000 populations every year with the clinical manifestations of awareness loss, bruising, and mobility abnormalities. Due to the lack understanding of the pathophysiology behind the illness, a wide variety of medications are available to treat epilepsy. Epileptogenesis is the process by which a normally functioning brain undergoes alterations leading to the development of epilepsy, involving various factors. This is related to the inflammation which is driven by cytokines like IL-1 and tumor necrosis factor- α TNF- α leads to neuronal hyperexcitability. Pro-inflammatory cytokines from activated microglia and astrocytes in epileptic tissue initiate an inflammatory cascade, heightening neuronal excitability and triggering epileptiform activity. The blood brain barrier (BBB) maintains central nervous system integrity through its tight endothelial connections, but inflammation impact BBB structure and function which leads to immune cell infiltration. The mTOR pathway's excessive activation influences epileptogenesis, impacting neuronal excitability, and synapse formation, with genetic mutations contributing to epilepsy syndromes and the modulation of autophagy playing a role in seizure onset. The apoptotic pathway contribute to cell death through glutamate receptor-mediated excitotoxicity, involving pro-apoptotic proteins like p53 and mitochondrial dysfunction, leading to the activation of caspases and the disruption of calcium homeostasis. Ionic imbalances within neural networks contribute to the complexity of epileptic seizures, involving alterations in voltage-gated sodium and potassium channels, and the formation of diverse ion channel subtypes. Epileptogenesis triggers molecular changes in hippocampus, including altered neurogenesis and enhanced expression of neurotrophic factors and proteins. Oxidative stress leads to cellular damage, disrupted antioxidant systems, and mitochondrial dysfunction, making it a key player in epileptogenesis and potential neuroprotective interventions. Thalamocortical circuitry disruption is central to absence epilepsy, the normal circuit becomes faulty and results in characteristic brain wave patterns.

Keywords: Epileptic Seizures, Epilepsy, Epileptogenesis, Molecular mechanism

Introduction

Epilepsy is one of the most prevalent neurological conditions that is caused by the abnormal electrical activity in the brain. It is a chronic, paroxysmal, and recurring disease marked by neurobiological, cognitive, psychological, and social factors that collectively contribute to recurrent seizures, alongside enduring risk factors for epileptic episodes (1,2). Epilepsy may significantly reduce quality of life, as it limits daily activities, social contacts, and even work chances. Moreover, this disease has caused about 50 new cases in 100.000 populations every year. About 75% of these cases start in childhood indicating the increased vulnerability of brain development to experience seizures (1,2). Clinical signs of epilepsy include loss of awareness, mobility abnormalities, sensation (vision, hearing, and taste), mood or cognitive capacities, bruising, and the danger of mortality. A study showed that medication will control the seizures of around 60% to 70% of persons with epilepsy. The general likelihood of epilepsy recurring after experiencing a solitary seizure ranges from 27% to 71%. Roughly 70% of children will enter a phase where they experience a span of at least 2 years without encountering a seizure. Due to the lack of a complete understanding of the pathophysiology behind the illness, a wide variety of medications are available to treat epilepsy. This circumstance results in a variety of distinct pharmaceutical agents to treat it. Epilepsy characterized by epileptic seizures starts in the cortex or subcortex's substantia grisea. Epileptic seizures results from an abrupt imbalance between excitation and inhibition in the network of cortical neurons. It starts when neurons exhibit high, coordinated, and sustained neuronal excitability activity.

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4 The term hypersynchrony and hyperexcitability are two different words found in the epilepsy pathophysiology
5 with a related significance among them. The abnormal reactivity of neurons to stimulation input is referred to as
6 hyperexcitability. This condition happens when neurons react too strongly when they receive signals from other cells
7 causing excessive firing of nerve cells in the brain. Neurons commonly start more than two or one electrical activity,
8 which is known as hypersynchrony. This involves several neighboring neurons in aberrant electrical bursts. In this
9 case, a small number of neurons first undergo abnormal stimulation. The normal inhibitory ion exchange and
10 membrane conductance of the synaptic gap are altered, which raises neuronal excitability and synchronization and
11 results in an epileptic seizure. Hyperexcitability is typically brought on by modifications to intrinsic neuronal
12 components and synapse function. According to developments in molecular genetics, several distinct types of human
13 epilepsy are considered to be brought on by mutations in ion channel genes (3–8). This article aims to comprehensively
14 delve into the intricate molecular underpinnings that give rise to epileptic seizures and the overarching epileptic
15 condition, thereby contributing to a more profound understanding of the etiological complexities inherent to
16 epileptogenesis.
17

18 **Epileptogenesis**

19 Epileptogenesis refers to the process through which a normal brain develops alterations that contribute to the
20 formation of epilepsy, alluding brain transformation that was previously functioning normally into a brain that
21 experiences seizures. Thus far, the definition of epileptogenesis remains a topic of ongoing discussion and lacks a
22 universally accepted characterization. This considered involving two processes, including the development of
23 epileptiform processes after brain injury and the process leading to the first spontaneous epileptic seizure. Research
24 shows that neurological alterations during the latent period persist long after epilepsy has been identified and aid in
25 the disease's development, it means that the mechanisms involved in both starting and developing epilepsy are counted
26 as part of epileptogenesis. The subsequent development or changes that occur following epileptogenesis is called
27 epileptic maturation. The processes of starting epilepsy and what happens afterward are different from each other, it
28 involves the establishment of enduring alterations in neural circuitry and excitability, also often characterized by an
29 increased propensity for recurrent seizures and the manifestation of clinical epileptic symptoms (4). Prior to the
30 occurrence of the epileptic seizure, the gradual changes of epileptogenesis occurs in neuronal excitability led to
31 changes in interconnections and structure. This include the neurodegeneration, neurogenesis, gliosis, axonal damage
32 or sprouting, dendritic plasticity, breakdown of the BBB, infiltration of inflammatory cells into the brain parenchyma,
33 re-organization of the extracellular matrix, and re-organization of the molecular architecture of individual neurons (9).
34

35 Excitability also occurs in the membrane or metabolic components changes of individual neurons. It includes
36 the ictogenesis from specific neurons, neuronal surroundings, and neuronal populations. This results from the
37 suboptimal environmental control, extracellular ion concentration, and neurotransmitters. A neuron can develop into
38 a population of hyperexcitable neurons through anatomical and physiological modifications (5,10). By blocking
39 synaptic and voltage-gated inhibitory conductance or activating synapse and voltage-gated excitatory conductance,
40 epileptic activity can be acutely produced. However, it differs from acute seizures in persistent epileptic seizures
41 which are unexpected and less often. In chronic epilepsy, a second mechanism is required to account for the timing of
42 the episodic change from regular activity to an epileptic seizure.

43 The biological processes, structural changes, and functional alterations play a crucial role in epileptogenesis.
44 The fundamental physiology of an epileptic seizure includes the instability of the cell membrane or the surrounding
45 or neighboring supporting cells. A contributing factor to cell membrane instability involves the deficiency of
46 adenosine triphosphate (ATP) acetate associated with ion transport or anomalies in potassium ion conductance. Some
47 examples of neurotransmitters that can boost excitability and expand neuronal activity are glutamate, aspartate,
48 acetylcholine, norepinephrine, histamine, corticotropin-releasing factor, purines, peptides, cytokines, and steroid
49 hormones. At the same time, dopamine and *γ-amino butyric acid* (GABA) restrict neuronal activity and growth.
50 During an epileptic seizure, there is an increased increased blood flow requirement to the brain, facilitating the
51 transportation of carbon dioxide and metabolic substrates essential for neuronal activity. Various forms of epilepsy
52 are associated with mutations in a multitude of genes. It encompasses several types that are correlated with genes
53 encoding protein subunits of voltage-sensitive and ligand-activated ion channels (4,8).

54 Epileptic seizures and epilepsy arise from a multitude of causes and sustained through a process of positive
55 reinforcement, where an initial imbalance between neural inhibition and excitation triggers further imbalances. The
56 initial triggering activity not only initiates the seizure but also fosters ongoing neural excitation while simultaneously
57 inhibiting the natural restraining mechanisms. This self-perpetuating cycle is facilitated by positive feedback,
58 effectively prolonging the epileptic seizure once it has been initiated (11). Epileptogenesis is influenced by factors
59 including oxidative stress, neurochemical alterations in the brain due to neurotransmitters and ion channels,
60 fluctuations in ion concentration, variations in cell surface receptors, and the presence of inflammation. Some targets
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in epileptogenesis include the Mammalian target of rapamycin (mTOR), P-glycoprotein (Pgp), and mutations in voltage-gated ion channels like hyperpolarization-activated cyclic nucleotide-gated channels (HCN) and Kv7 channel or (M-), Na-K-2Cl cotransporter (NKCC1) (8,12).

The Role of Inflammation In Epileptic Seizure and Epilepsy

Inflammation indeed plays a significant role in the progression of epileptogenesis. It reduces the threshold for the initiation of epileptic seizures, and it becomes further exacerbated under conditions of SE (8). Death of neuronal cells, malfunction of synapses, and the onset of hyperexcitability are all symptoms of brain injury brought on by concussions, hypoxia, or febrile convulsions. The release of cytokines, chemokines, lipid mediators, and protectins into the neuronal environment indicates that brain trauma has caused an organized cascade of biological processes. Depending on the part of the brain that is affected, dysregulation of these mediators and the expression of their receptors might result in clinical symptoms that include permanent neuronal injury (13).

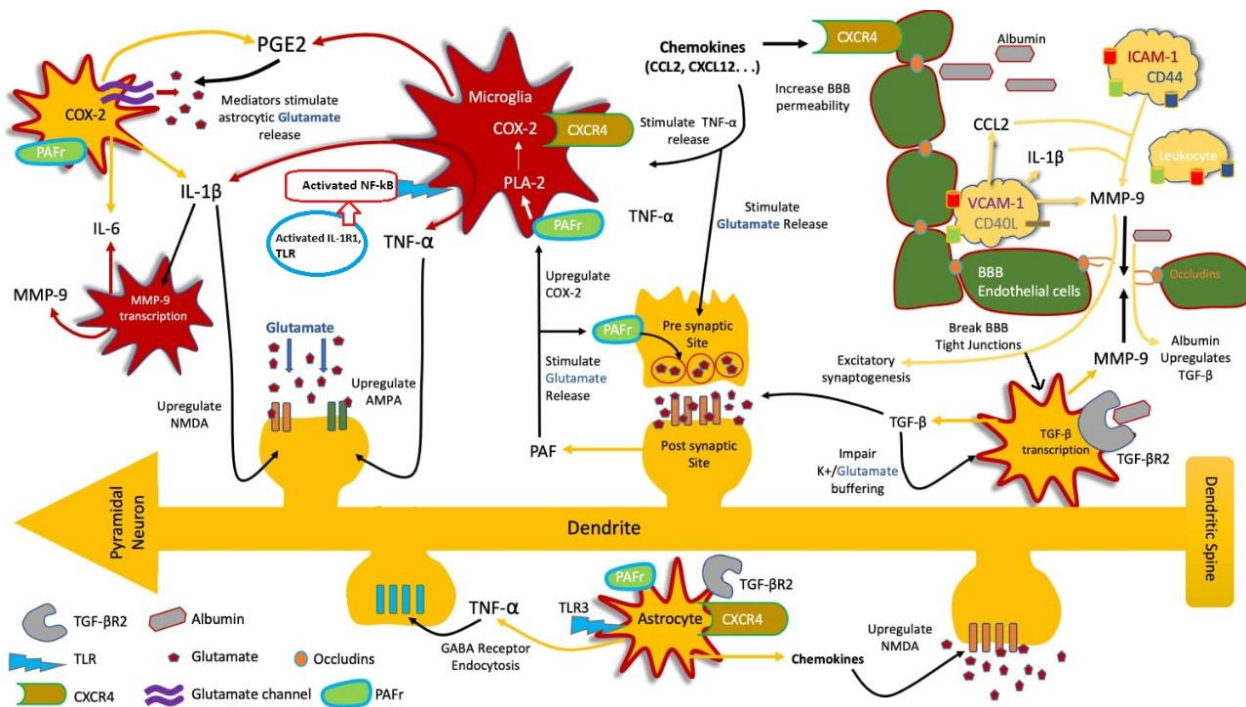


Figure 1. The Role of Inflammation in Epileptic Seizure and Epilepsy. A cascade of events unfolds after brain injury that triggers central inflammation and alters normal neuronal connections in the hippocampus. Meanwhile, systemic inflammatory diseases cause inflammation in the peripheral tissues, accelerating the accumulation of inflammatory agents. This dual inflammation, both in the peripheral and central systems, contributes to the weakening of the protective BBB by enhancing the levels of inflammatory agents. This compromised barrier permits the infiltration of immune cells, initiating heightened neuronal activity and further amplifying the production of inflammatory agents. The uncontrolled inflammation in both peripheral and central regions, coupled with the compromised blood-brain barrier, sets the stage for structural changes in synaptic connections within the hippocampus. These processes culminate in the progression of epilepsy. Permission was granted by Rana and Musto (©BMC [13]) to reuse this figure.

a. Brain Inflammation in People with Epilepsy

Epileptogenesis is closely linked to high degree of inflammation in the neural tissue's milieu both acutely and persistently. The central nervous system may be the source of the inflammatory response with possible damage to the BBB. This inflammation can spread from the central nervous system into the systemic circulation, and exacerbations of pre-existing chronic neuroinflammation may also occur as a result of acute neuroinflammation (13). Small amounts of the cytokine interleukin-1 (IL-1) are produced by activated astrocytes and microglia and expressed in the human central nervous system, these levels are increased during pathogenic processes. These cytokines cause astrocytes to consume less and release more glutamate, resulting in neuronal hyperexcitability.

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4 IL-1 is thought to cause epileptic seizures by increasing N-methyl-D-aspartate (NMDA) receptor expression in
5 post-synaptic cells through activation of the GluN2B subunit of the NMDA receptor (13–15).

6 Active astrocytes and microglia emit the pro-inflammatory cytokine Tumor Necrosis Factor- α (TNF- α).
7 Several research shows that TNF- α controls N-cadherin which is essential for the development and structure of
8 excitatory and inhibitory synapses. TNF- α also boosts glutaminase production at gap junctions, which in turn
9 causes microglia to release more glutamate. TNF- α also increase the the expression of α -amino-3-hydroxy-5-
10 methyl-4-isoxazolepropionic acid (AMPA) receptors, which amplifies glutamatergic transmission and results in
11 excessive calcium ion influx and neurotoxicity. It also triggers GABA receptor endocytosis which reduces the
12 inhibitory excitability, and results in significant alterations in excitability (13–15). Another multifunctional
13 cytokine that will increase along with the increment of TNF- α , IL-1 β , and interferon- γ (IFN- γ) is the interleukin-
14 6 (IL-6) which controls inflammation as immunological responses. The production of IL-6 in the central nervous
15 system remains in low level. However, it can be boosted by the activation of astrocytes and microglia. The
16 increasing levels of IL-6 expression reduces long-term potentiation (LTP), inhibits hippocampus neurogenesis,
17 and increases gliosis, which lead to epileptogenesis (13–15). The innate and acquired immune systems are
18 activated in patients with epilepsy. Analysis of brain samples from patients with refractory epilepsy demonstrated
19 heightened expression of IL-1 β and high-mobility group box 1 (HMGB1), along with their corresponding
20 receptors, namely interleukin-1 receptor type I (IL-1RI) and TLR4, in both glia cells and neurons. This clarifies
21 that patients with epilepsy experience activation of inflammatory mechanisms (8,13).
22

23 24 **b. Targets and Sources of Cytokines and Inflammatory Mediators in Epileptic Tissue**

25 Microglia and astrocytes as glial cells and non-neuronal parts of the central nervous system are the source
26 of pro-inflammatory cytokines in epileptic tissue. In epileptogenesis, glial cells control inflammatory and
27 immunological responses, it causes a rapid inflammatory response in epileptic seizures caused by chemo seizures
28 or electrical stimulation. Within the first 30 minutes of an epileptic seizure, activated astrocytes and microglia
29 trigger heightened levels of pro-inflammatory cytokines, such as IL-1 β , IL-6, and TNF- α . This process initiates an
30 inflammatory cascade that involves neurons and BBB endothelial cells, ultimately leading to the activation of the
31 acquired immune system. This cascade includes the activation of NF- κ B, COX-2, complement system,
32 chemokines, and acute phase proteins. These cytokines have the potential to influence susceptibility to stimuli that
33 trigger epileptic seizures, as well as contribute to the increased IL-1 β levels resulting from epileptic convulsions.
34 The initiation of this signaling cascade exacerbates epileptic seizures. The conversion of IL-1 β into its active form
35 is facilitated by the enzyme IL-1 β converting enzyme (ICE/caspase-1). Considering this, exploring ICE/caspase-
36 1 inhibitors as potential targets for anti-epileptic drugs holds promise (4,8,12).

37 Rapid HMGB1 release from neurons, microglia, and astrocytes after pro-convulsant-induced brain injury
38 was also shown to significantly influence the onset of brain inflammation and lower the threshold for epileptic
39 seizures. TLR signaling also plays a significant role in astrocytes and neurons. Injured or stressed cells emit danger
40 signals to warn the surrounding environment of impending or ongoing damage, including in the HMGB1. The
41 innate immune response and accompanying inflammatory pathways are activated in the tissue due to the interaction
42 between HMGB1 and its cognate TLR4 (4,8,12)
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44 **c. The Role of Cytokines on Neuronal Excitability**

45 Inflammatory cytokines play a role in changing neuronal excitability, producing toxic mediators, and
46 making the BBB more impermeable. The proconvulsant effects are produced by NMDA receptors and IL-1 β .
47 Specifically, IL-1 β can phosphorylate the NR2B subunit of the NMDA receptor via IL-1RI-dependent activation
48 of Src kinases and neuronal sphingomyelinases. This phosphorylation amplifies NMDA-mediated calcium ion
49 influx into neurons, thereby inducing neuronal hyperexcitability. Activated astrocytes release glutamate, which
50 serves as the primary source, initiating a gradual influx of ions into neurons. This process involves NMDA
51 receptors that incorporate the NR2B subunit. This influx of ions has a direct impact on excitatory events, exerting
52 a substantial influence on neural synchronization and potentially giving rise to action potentials within neurons.

53 An essential biological mechanism that intensifies epileptic seizures involves the protein IL-1 β .
54 Furthermore, IL-1 β has the capacity to reduce glutamate absorption by astrocytes, elevate glutamate release from
55 glial cells through TNF- α , and induce hyperexcitability. The presence of IL-1 β also leads to an inhibition of
56 glutamate uptake by nitric oxide. Similar to IL-1 β , TNF- α might boost the expression of AMPA receptors,
57 facilitating the entry of calcium ions into neurons, while concurrently diminishing the expression of GABA
58 receptors, thereby weakening the potency of inhibitory synapses. The signaling cascade mechanism governing
59 glutamate exocytosis from astrocytes involves the participation of tumor necrosis factor (TNF- α). This process
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4 engages tumor necrosis factor receptor 1 (TNFR1) activation within astrocytes, thereby amplifying the signaling
5 cascade responsible for glutamate release. This mechanism operates through the stimulation of CXCR4-dependent
6 signaling in both astrocytes and microglia. Furthermore, it works synergistically to facilitate the release of TNF- α
7 (4,8,12).

8 Inflammatory cytokines can lead to neuronal death through apoptosis, operating independently from
9 excitotoxicity. This effect is potentially facilitated by the release of neurotoxic mediators. Additionally,
10 glutamatergic excitotoxicity, which involves AMPA and NMDA receptors, can also contribute to neuronal demise.
11 Inflammatory responses can also change the BBB's permeability. When BBB is damaged, astrocytes take up serum
12 albumin. Within astrocytes, this albumin binds to TGF- β receptors, initiating a sequence of events that culminate
13 in neuronal hyperexcitability and epileptiform activity. The impairment of the BBB leads to increased abnormal
14 neuronal depolarization processes. The breach in the BBB allows leukocytes to enter the brain parenchyma and
15 proteins to leak from the serum. These exogenous inflammatory mediators collectively reduce the threshold for
16 epileptic seizures. This scenario underscores the significant impact of inflammatory processes on epileptogenesis
17 within the brain (4,8,12). The pathophysiology of epileptic seizures involves the adherence of inflammatory cells.
18 After a pilocarpine hydrochloride-induced epileptic seizure, there is an increase in the production of vascular cell
19 adhesion molecules (VCAM) and an increase in leukocyte adherence to endothelial cells in cerebral blood arteries,
20 which is mediated by leukocyte mucin P-selectin glycoprotein ligand-1 and leukocyte integrins. Consequently,
21 escalated leukocyte extravasation, brain inflammation, BBB leakage, increased neuronal excitation, and
22 ultimately, the onset of epileptogenesis ensue (4)

23 Peripheral inflammation has been correlated with the stimulation of gene transcription through NF- κ B, an
24 unconventional intracellular signaling pathway activated by pro-inflammatory mediators within epileptogenic
25 tissue. The recognition of this distinct mechanism provides insights into the genesis of epileptic seizures driven by
26 neuronal hyperexcitability. This pathway regulates a subset of interactions between neuronal and glial elements
27 that contribute to the reduction in the threshold for epileptic seizures (8,16). When pro-inflammatory molecules
28 activate other kinase families, such as mitogen-activated protein kinase (MAPK), protein kinase A (PKA), and
29 protein kinase C (PKC), voltage-dependent calcium, sodium, and potassium ion channels undergo rapid post-
30 translational changes that have a significant effect on neuronal excitability. Increased extracellular glutamate levels
31 is caused by IL-1's ability to promote glutamate release by glial cells and decreased glutamate absorption by
32 astrocytes through the generation of TNF- α . Furthermore, the amplified glutamatergic transmission, a
33 consequence of IL-1 β produced by both neurons and astrocytes, yields pro-convulsant effects. Recent findings
34 emphasize the critical role of astrocytic glutamate release in shaping the initiation and severity of epileptic
35 convulsions. Additionally, IL-1 impedes inhibitory transmission by blocking GABA-mediated chloride ion influx.
36 The presence of inflammatory compounds in the brain could also incite enduring transcriptional processes, thereby
37 activating genes integral to the plasticity mechanisms underlying epileptogenesis (8,16)

38 39 40 **d. The Role of P2X7 Receptors in Epilepsy**

41 The P2X7 receptor is a cation-permeable ATP-gated non-selective ionotropic receptor expressed only by
42 neurons and glia cells in the brain. The hippocampus's neuronal excitability was shown to be modulated by this
43 receptor's activation, which is also linked to microglia activation and neuro-inflammatory reactions. These
44 receptors play a role in the neurological system disorders, such as epilepsy. P2X receptors are ligand-gated ion
45 channels that ATP activates, and activating these receptors transfers signals between neurons and glia cells. The
46 extracellular space's potassium ion concentration rises during an epileptic episode. Hippocampal neurons and
47 astrocytes' Panx1 channels activate in response to this alteration, releasing ATP. Neuronal hyperactivity brought
48 on by the activation of ATP receptors leads to the onset of an epileptic seizure. Adenosine, as opposed to ATP,
49 exhibits anti-epileptic action via P1A1 receptors. These purinergic receptors are inhibited from releasing glutamate
50 when they are activated. Several P2X family receptors, including P2 x 2, P2 x 4, and P2 x 7, are damaged in the
51 hippocampus during status epilepticus (SE). Following SE, a reduction in P2X2 receptor expression was also
52 observed. In contrast, P2X7 receptor expression was higher in glial and neuronal injury regions. After SE, neuronal
53 death is reduced by a lack of P2X4 receptors (12).

54 55 **The Structural, Molecular, and Functional Alterations of BBB in Epileptogenesis and Epilepsy**

56 BBB, which is impermeable to a wide range of chemicals, toxins, and cells due to the presence of non-
57 fenestrated endothelial cells forming inter-endothelial tight connections, serves as a protective shield for the central
58 nervous system, preventing any immune system reactions. The BBB is composed of both chemical and structural
59 barriers. It comprises neovascular units such as the basement membrane, endothelial cells that line the blood arteries,
60 neurons, astrocyte processes, tight junctions, and pericytes. These components collectively form the structural barrier
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4 of the BBB. Nectrin 1, functioning as a laminin-like protein responsible for regulating glial cell motility, is secreted
5 by endothelial cells in response to signals from astrocytes, which concurrently produce the sonic hedgehog protein. In
6 a concerted effort to fortify the BBB and diminish the infiltration of leukocytes, netrin 1 plays a crucial role in
7 enhancing endothelial junctional proteins. These include occludins, claudins, junctional adhesion molecules (JAMs),
8 and endothelial selective adhesion molecules (ESAM). This ensemble of proteins collectively widens the intercellular
9 gaps, constituting the foundational elements of tight junctions and intercellular barriers. Members of the
10 immunoglobulin superfamily, such as ESAM and JAMs, play a pivotal role in both the formation and upkeep of tight
11 junctions. The basement membrane, constituting a foundational structure, embeds components like type 4 collagen,
12 fibrillin, laminin, fibronectin, extracellular matrix proteins, and parasite matrix proteins, which collectively give rise
13 to the basal capillaries or basement membrane. Within the brain capillaries, tight junctions are safeguarded by efflux
14 transporters, forming a chemical barrier. These transporters, operating at the junctions between endothelial cells,
15 necessitate the presence of ATP. Prominent among these are Pgp and breast cancer resistance protein (BCRP),
16 functioning as flux transporters. Their active mechanism involves pumping potentially harmful lipophilic compounds
17 back into the bloodstream, thereby restricting the assimilation of certain medicinal substances into the brain
18 parenchyma. Furthermore, the chemical barrier of the BBB derives additional strength from the drug-metabolizing
19 enzyme cytochrome P450 (CYP450), which is localized within the endothelial cells of brain capillaries. Studies by
20 Rana & Musto (2018) and Löscher & Friedman (2020) have underscored these aspects. During epileptic seizures, the
21 release of glutamate, facilitated by NMDA and COX-2 receptors, is accountable for the escalation in Pgp activity (12).

22
23 Seizures or the processes underlying them have the potential to impact the BBB, brain capillaries, and the
24 neovascular unit in diverse ways. This can encompass thickening of the basement membrane, alterations in the
25 constituent endothelial cells of the BBB, modifications in the neurons comprising the neovascular unit, and changes
26 in the astrocyte processes forming the glia limitans—a secondary barrier. Epileptogenic brain injury can lead to the
27 leakage of blood albumin and immune cells into the brain parenchyma. Notably, the escalated expression of efflux
28 transporters such as Pgp and breast cancer resistance protein (BCRP) on the apical surface of endothelial cells, along
29 with heightened activity of the enzyme cytochrome P450 (CYP450) on these cells, has been associated with BBB
30 damage. Concurrently, the presence of persistently activated microglia clusters may contribute to the disruption of the
31 BBB. Furthermore, post brain injury, astrocytes undergo alterations in both morphology and function. These changes
32 can collectively lead to a complex interplay of factors influencing the integrity and functionality of the BBB and
33 related systems (17).

34 The mechanism by which epileptic convulsions impact the BBB remains uncertain. Observations in epilepsy
35 patients and experimental animal models suggest that BBB failure can occur before, during, or after an epileptic
36 seizure. Epileptic convulsions induce temporary changes in both the structural and functional aspects of the BBB.
37 Within 30 minutes of an epileptic episode, there is a rapid increase in permeability that persists for hours. Elevated
38 levels of glutamate during epileptic episodes disrupt the BBB. The expression and activity of MMP-2 and MMP-9 at
39 the BBB also rise during seizures, contributing to BBB dysfunction. These matrix metalloproteinases influence the
40 integrity of the BBB by altering the extracellular matrix surrounding brain capillaries and degrading the tight junction
41 proteins that line the endothelium. The extracellular matrix, in turn, affects processes like AMPA receptor
42 mobilization, paired-pulse depression, L-type voltage-dependent calcium channel activity, and the long-term
43 potentiation (LTP) process. Changes in the expression of hyaluronic acid, a crucial component of the neuronal
44 extracellular matrix, can lead to dysregulation of calcium ions and neuronal hyperexcitability. Inducing epileptic
45 seizures can also permit ions to enter the parasite, thereby modifying its function and the constituents of the BBB both
46 in vitro and in vivo. These defense system breaches, causing epileptic seizures, can establish a positive feedback loop.
47 In this loop, seizures resulting from these breaches lead to a continuation of epileptic episodes, ultimately culminating
48 in epilepsy. The breach of the cerebral blood barrier defense can trigger epileptic episodes and contribute to the
49 development of epilepsy (6,14,17).

50 The BBB serves as a crucial barrier to maintain the peripheral immune cells in a healthy state, preventing their
51 entry into the central nervous system. However, when breached, the BBB can allow both innate and acquired
52 peripheral immune cells, including monocytes, neutrophils, B cells, and T cells, to infiltrate the central nervous system.
53 These immune cells then carry out specific functions that could be either neuroprotective, neurotoxic, or even a
54 combination of both. A significant facet of neuro-inflammation involves the entrapment of these immune cells within
55 the central nervous system through the BBB. This orchestrated process hinges on adhesion interactions between
56 lymphocytes and capillary endothelium. When immune cells come into contact with the activated endothelium,
57 selectins facilitate loose binding, causing the leukocytes to roll along the endothelial surface. Additionally,
58 chemokines present on the luminal surface of the endothelium trigger the activation of leukocytes, leading to the
59 engagement of leukocyte integrins and subsequent halting of leukocyte movement on the endothelial surface. Once
60 the appropriate adhesion occurs, immune cells can traverse into the perivascular space through either of two
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4 transmigration processes: the trans-cellular route, where they navigate through the endothelial cell body, or the
5 paracellular route, utilizing the endothelial cleft. Upon infiltrating the perivascular space, these immune cells interact
6 with resident perivascular immune cells before being guided by chemoattractants like chemokines and cytokines
7 toward the brain parenchyma. Employing an enzyme-mediated mechanism, these infiltrating lymphocytes breach the
8 glia limitans and access the brain parenchyma. Once within the brain parenchyma, they become stimulated to produce
9 cytokines, contributing to the cascade of immune responses and inflammation. The BBB serves as a sentinel that
10 regulates the entry of peripheral immune cells into the central nervous system. Breaching the BBB leads to a series of
11 orchestrated events involving adhesion, activation, and migration of immune cells, ultimately culminating in their
12 interaction with brain parenchymal cells and the release of cytokines (17).

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14 Albumin extravasation plays a significant role in both epileptogenesis and the migration of immune cells across
15 a compromised BBB. The initiation of acute epileptic seizures can be attributed to BBB impairment, and the
16 progression from epileptic episodes to epilepsy is facilitated by the osmotic disruption of the BBB. Once extravasated,
17 albumin enters the brain parenchyma and binds to various cellular components, including neuronal cells, astrocytes,
18 and microglia. This binding triggers a sequence of events, including albumin's entry into astrocytes through TGF- β
19 receptors. Consequently, the expression of critical components such as the Kir 4.1 channel, the water channel
20 aquaporin 4 (AQP4), and glutamate transporters is reduced. This alteration results in a decreased ratio of extracellular
21 potassium to glutamate, creating an environment that facilitates NMDA receptors in triggering epileptiform activity
22 and amplifying neuronal hyperexcitability. The impact of TGF- β signaling extends further, encompassing changes in
23 the extracellular matrix, excitatory synaptogenesis, pathological plasticity, and inflammation-related transcriptional
24 alterations. These cumulative effects collectively contribute to the lowering of the threshold for epileptic seizures
25 during the process of epileptogenesis. In essence, albumin extravasation sets in motion a complex cascade of events
26 that involve multiple cellular components and signaling pathways. This interplay ultimately heightens neuronal
27 excitability and paves the way for epileptogenesis and the progression towards epilepsy (17). Albumin binding to
28 TGF-R2 receptors on astrocytes triggers the activation of the TGF- β signaling pathway, leading to the production of
29 TGF- β . This, in turn, activates astrocytes, causing a disruption in the balance between cellular potassium and glutamate
30 levels. Additionally, the ALK5/TGF- β pathway is responsible for inducing excitatory synaptogenesis (as indicated by
31 reference 13). In models of epileptogenesis, augmenting TGF- β signaling has been shown to have a preventive effect
32 on the development of epilepsy. Furthermore, another study established a connection between compromised BBBs
33 and irregular neuronal activity (17).

34
35 Damage to the BBB triggers gliosis and activation of astrocytes. After inducing SE, astrocytes positive for glial
36 fibrillary acidic protein (GFAP) become activated within a span of 24 to 48 hours. Reactive gliosis becomes evident
37 around three to four months following the initial insult. The inability of these reactive astrocytes to effectively manage
38 extracellular glutamate leads to hyperexcitability and neuronal damage. Furthermore, these activated astrocytes release
39 C-C motif chemokine ligands 2, 3, and 5, fostering interactions with other inflammatory cells and giving rise to the
40 production of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α . This cascade contributes to heightened
41 neuronal excitability, the onset of seizures, cell death, and the propagation of neuro-inflammation (13).

42 **The Role of mTOR Pathway in Epileptic Seizure and Epilepsy**

43
44 The excessive activation of the mTOR signaling pathway directly influences the progression of epileptogenesis
45 and neuronal excitability. This process involves the phosphorylation of molecules within this pathway. Additionally,
46 the mTOR pathway plays a role in governing the expression of glutamate receptors. Recent research further
47 underscores the potential for disrupted neural signaling, as mTOR contributes to both synapse formation and the
48 mechanisms underlying plasticity (12). Moreover, mTOR exerts control over the expression of diverse ion channels
49 and neurotransmitter receptors, as well as influencing synaptic plasticity, neuronal morphology, and synaptic
50 transmission (18).

51
52 In prevalent causes of hereditary epilepsy such as focal cortical dysplasia (FCD) and tuberous sclerosis complex
53 (TSC), the involvement of the mTOR pathway in epileptogenesis is notably evident. Beyond these conditions, the
54 mTOR pathway plays a role in the development of both inherited and acquired epilepsies, encompassing conditions
55 like Lafora disease (LD), temporal lobe epilepsy, brain injuries, and experimental epileptic states induced by
56 chemically triggered convulsions. Additionally, the mTOR pathway is implicated in systemic lupus erythematosus,
57 an inflammatory disorder that stands as a prominent contributor to epileptic seizures (19). Genetic mutations impacting
58 various components of the mTOR pathway, such as TSC1 and TSC2, STE20-related kinase adaptor- α (STRADA),
59 phosphatase and tensin homologue (PTEN), AKT isoform predominant in the brain (AKT3), phosphatidylinositol-
60 4,5-bisphosphate 3-kinase- α (PIK3CA), and MTOR itself, result in the hyperactivity of the mTOR pathway. This
61 hyperactivity, in turn, triggers the initiation of epilepsy syndromes (20–24). Conversely, the inhibition of mTOR can
62 forestall the onset of epilepsy. The modifications in autophagy driven by mTOR are interconnected with the process
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4 of epileptogenesis. Following mTOR hyperactivation, autophagy experiences suppression, and the commencement of
5 spontaneous epileptic seizures is associated with the breakdown of autophagy. Autophagy governs diverse processes
6 including axon guidance, synapse development, dendritic spine architecture and pruning, vesicular release, and
7 synaptic plasticity. It also exercises control over the proliferation and migration of both interneuronal and neuronal
8 cortical progenitors.

9 Evidence points towards mTOR-mediated autophagy failure giving rise to aberrations in the clustering of
10 GABAA receptors at synapses, thereby leading to an imbalance between excitation and inhibition—an essential factor
11 in epileptogenesis. Perturbations in the dopamine system's irregular activity additionally influence the functioning of
12 mTOR-mediated autophagy, contributing to epileptogenesis. This intricate interplay arises from bidirectional
13 communication and shared biochemical pathways, involving cross-reactions between the immune and neurological
14 systems. The connection between mTOR and cell-clearing mechanisms responsible for regulating the metabolic
15 processes of lymphocytes and other immune cells, as well as the antigen-processing capabilities of antigen-presenting
16 cells (APCs) in both the central and peripheral nervous systems, further bolsters this notion. Changes in mTOR-
17 associated cell-clearing processes can disrupt and misalign the communication between the immune and neurological
18 systems (19). In parallel with its impact on autophagy, epilepsy also exerts effects on the ubiquitin-proteasome system
19 (UPS), a regulatory network governing neuronal excitability, synapse plasticity, and neuro-inflammation/immunity.
20 Disturbances in the UPS within the context of epilepsy are associated with mTOR hyperactivation, akin to the
21 phenomenon observed with autophagy disruption. Recent investigations have elucidated the role of mTOR in
22 orchestrating the convergence of autophagy and UPS morphological pathways, as well as their collective cell-clearing
23 mechanisms. These findings underscore that mTOR-mediated autophagy and UPS activities collectively contribute to
24 epilepsy by influencing brain plasticity and promoting neuroprotection against excitotoxicity. This interplay sheds
25 light on the multifaceted mechanisms through which epilepsy shapes neurological processes and underscores the
26 significance of mTOR regulation in these contexts (19).

27 Autophagy operates at the interface of the GABA, dopamine, and glutamate systems, contributing to the
28 molecular mechanisms underlying epileptogenesis and the neuronal changes prompted by epileptic convulsions. In
29 instances of autophagy failure, heightened p62 levels conceal the surface presentation of GABAA receptors, leading
30 to a reduction in GABA signaling. Furthermore, the degradation of NMDAR and AMPAR glutamate receptors no
31 longer takes place, resulting in irregular glutamate signaling and an influx of calcium ions. This disarray disrupts the
32 delicate equilibrium between excitatory and inhibitory neurotransmission pivotal to epileptogenesis. On the
33 dopaminergic front, autophagy's role extends to curtailing dopamine release through the disintegration of synaptic
34 vesicles at the dopamine terminal. In cases where autophagy falters, anomalous dopamine release ensues,
35 accompanied by the stimulation of dopamine D1 receptors. This, in turn, exacerbates autophagy suppression through
36 mTOR hyperactivation. This detrimental cycle leads to the accumulation of damaged cellular components, which
37 combine with glutamate-associated excitotoxicity, ultimately culminating in neuronal demise (19).
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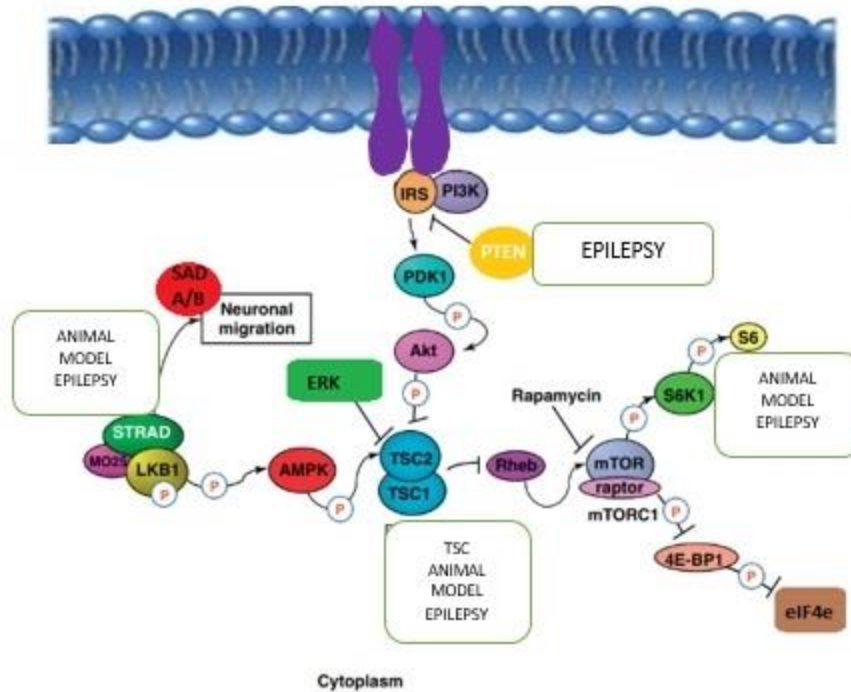


Figure 2. The Role of mTOR Pathway in Epileptogenesis (Dysmorphic neurons and balloon cells, which lack dendrites or axons, undergo alterations in neurotransmitter receptor subunit expression and uptake site distribution in conditions like TSC, FCD, and Hemimegalencephaly (HME). Notably, changes are prominent in GluR, NMDAR, and mGluR subtypes. The direct causation of these alterations—whether they arise from heightened mTOR signaling, tissue cytoarchitecture changes, or adaptations stemming from recurrent seizures—remains uncertain. The accompanying diagram illustrates various locations where dysregulation of mTORC1 (influenced by both phosphoactivation and phosphoinhibition effects) is implicated in human neurological disorders collectively referred to as "mTORopathies," which encompass epilepsy. Components such as PTEN, STRADA, TSC1/TSC2, and S6K1 might play roles in this context. Experimental animal models involving these proteins also exhibit compromised seizure susceptibility and/or deficits in social and behavioral functions. Rapamycin and other mTOR inhibitors emerge as primary potential therapeutic agents for modulating mTORC1 signaling. These interventions hold promise for regulating the mTOR pathway, offering prospects for addressing the underlying mechanisms of mTOR-related neurological disorders, including epilepsy (21,25). Permission was granted by Crino (©Cell Press [21]) to reuse this figure.

The Role of Apoptotic Pathway in Epileptic Seizure and Epilepsy

Beyond its pivotal role in neuronal development, differentiation, and synaptic plasticity (26,27), glutamate receptor-mediated excitotoxicity is also recognized as a major mechanism contributing to cell death in various disorders of the central nervous system (CNS). These include conditions like brain injury, neurodegenerative diseases, and epilepsy (28). Elevated levels of glutamate receptor activity have the potential to induce cell damage and subsequent death through mechanisms involving apoptosis or autophagy. As a consequence of heightened glutamate receptor activity, the expression of pro-apoptotic proteins such as p53 is triggered (29–31). This activation of pro-apoptotic proteins is often prompted by immediate excitotoxic injury (32,33).

Excessive activation of glutamatergic receptors or their analogs leads to a phenomenon known as excitotoxicity, which ultimately results in cell death. When these receptors experience an overstimulation, an excessive influx of calcium ions (Ca²⁺) occurs, with a significant portion becoming sequestered within mitochondria. Consequently, cellular metabolism becomes impaired, giving rise to the generation of free radicals. This excessive calcium influx also triggers the activation of enzymes like proteases, phospholipases, endonucleases, and nitric oxide synthase. Furthermore, the process inhibits protein synthesis, exacerbating the cascade of detrimental events within the affected cells (34). Disruption of calcium homeostasis leads to an accumulation of calcium within the

mitochondrial matrix. This accumulation has the effect of depolarizing the mitochondrial membrane through the activation of mitochondrial transition pores, which normally function to redistribute calcium back to the cytosol. This process unfolds in two primary steps. Initially, the elevation in positive ion concentration within the mitochondrial matrix diminishes the chemo-osmotic potential across the membrane. This reduction in potential subsequently slows down the rate at which ATP synthesis occurs. In the second step, irreversible damage takes place at the mitochondrial membrane. This disruption in calcium regulation and the ensuing impact on mitochondrial function represent critical components of the excitotoxic cascade (35,36).

The complexity of neuronal cell death associated with neurodegeneration is not mitigated by neuronal cell death triggered by epileptic seizures. However, the precise nature of neuronal cell demise, whether through necrosis or apoptosis, remains a subject of debate. While necrosis is frequently cited as the principal cause of neuronal cell death following epileptic seizures and is widely considered the predominant mechanism, the discussion persists. Biochemical investigations have unveiled the involvement of the B-cell lymphoma 2 (Bcl-2) family and caspases in the cell death process during epileptic seizures. Consequently, the potential contribution of apoptosis cannot be dismissed. Indicative of apoptosis, DNA fragmentation and activation of endonucleases have been identified in the cell death program. Additionally, there is evidence of p53 accumulation in the nucleus in tandem with increasing concentrations of cell death receptors and ligands. These indications point to the involvement of apoptotic pathways in contributing to cell death (37,38).

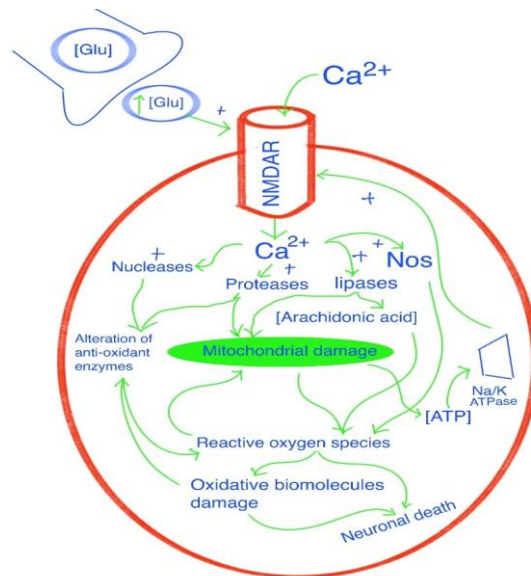


Figure 3. The Mechanism of Excitotoxicity. The sustained activation of N-methyl-D aspartate receptors (NMDAR) due to excessively high concentrations of glutamate (Glu) results in a substantial influx of calcium into the cell. This influx, in turn, triggers the activation of lytic enzymes and nitric oxide synthase (NOS). Concurrently, heightened levels of arachidonic acid and mitochondrial impairment foster the generation of reactive oxygen species, initiating a cascade of biomolecular damage. Ultimately, these processes lead to the activation of apoptotic death programs within cells. The impact of energy shortages compounds the degenerative process. Energy deficits prolong membrane depolarization by impairing the energy supply to the sodium/potassium pump (Na/K ATPase). This, in turn, maintains NMDAR in an active state, rendering the cell more susceptible to the typical glutamatergic inputs from the cerebral cortex (38). Permission was granted by Lorigados et al. (©Editorial Elfos Scientiae [38]) to reuse this figure.

The p53 gene emerges as the initial apoptotic regulator disrupted by an epileptic episode. Subsequent to an epileptic seizure, the process involves the binding of p53 to DNA. This is accompanied by heightened expression of Bcl-2-associated X (Bax) protein, which in turn leads to increased levels of both mRNA and protein. Protection against excitotoxicity induced by kainic acid was observed when inhibitors of p53 production were administered. Studies involving p53-deficient mice's neurons indicated heightened resilience to apoptosis induced by excitotoxicity and epileptic seizures. Given the multifaceted roles of p53, it remains unclear precisely how p53 is altered in epileptic seizures that trigger cell death. However, the prevailing evidence predominantly points toward caspases, Bcl-2, and p53 as integral components of the pathways underlying neuronal cell death induced by epileptic seizures (38).

In mammals, the regulation of the apoptotic process revolves around two principal gene families: caspases and the Bcl-2 family of proteins. The caspase family, composed of caspases 2, 8, 9, and 10 as initiators, and caspases 3, 6, and 7 as executors, serves pivotal roles within the apoptotic pathway. On the other hand, the Bcl-2 family of proteins exerts crucial control over whether a cell will survive or undergo apoptosis. This family can be categorized into three distinct types of proteins, each contributing uniquely to the apoptotic process.

The first group encompasses anti-apoptotic proteins featuring four Bcl-2 homology (BH) domains: BH-1, BH-2, BH-3, and BH-4. The second category includes three BH domains (Bax/Bak, 1, 2, and 3). The third set is marked by the presence of the BH3 domain (BH3 alone) (Bid, Bad, Bim, Bik). Among these, the BH3 alone family and the initial three members of the Bcl-2 BH family act as pro-apoptotic factors. The anti-apoptotic Bcl-2 family proteins such as Bcl-2 and Bcl-XL bind to the pro-apoptotic protein Bax/Bak, inhibiting their capacity to form mitochondrial membrane pores. Conversely, pro-apoptotic proteins like Bid, Bad, Bim, and Bik detach Bax/Bak from the Bcl-2-Bax/Bak or Bcl-XL-Bax/Bak complex, thereby initiating Bax translocation to the mitochondrial membrane and facilitating pore formation. Upon release from the anti-apoptotic protein connection, Bax/Bak dimerizes and remains in the active mitochondrial membrane. Subsequently, the creation of mitochondrial pores leads to the release of crucial proteins like cytochrome C, pivotal for triggering apoptosis through the mitochondrial pathway. Alongside caspases and Bcl-2 family proteins, other prominent players, including p53, TNF, Fas ligand, and NF- κ B, play significant roles in orchestrating the cell death pathway (4).

The process of excitotoxicity involves the hyperactivation of glutamatergic receptors and their analogs, leading to cell death (Figure 4). The excessive influx of calcium ions into the cell, as a result of this hyperactivation, accumulates within mitochondria. This accumulation triggers a chain of events including metabolic dysfunction, the generation of free radicals, activation of enzymes like proteases, phospholipases, endonucleases, and nitric oxide synthase, as well as the inhibition of protein synthesis. Additionally, disruptions in calcium ion homeostasis stem from malfunctions in the calcium ion pump, sodium and calcium ion exchange mechanism, and calcium-balancing proteins. When this delicate balance is perturbed, surplus calcium ions amass in the mitochondrial matrix. There are two primary mechanisms through which this buildup occurs. Firstly, an elevated concentration of positive ions within the mitochondrial matrix reduces the chemo-osmotic potential across the membrane, slowing down the rate of ATP synthesis. Secondly, the activation of the mitochondrial transition pore aims to transport calcium ions back to the cytosol. However, if the returned calcium ions are not effectively cleared, it results in irreversible membrane depolarization. The heightened concentration of calcium ions in the mitochondrial matrix further fosters the generation of free radicals. This, in turn, leads to lipid membrane peroxidation, nitric oxide production, and the activation of enzymes involved in the breakdown of proteins, phospholipids, and nucleic acids. Notably, nitric oxide can also act as a retrograde messenger, amplifying glutamate release from the terminal presynapse, thereby exacerbating its excitotoxic effects (38).

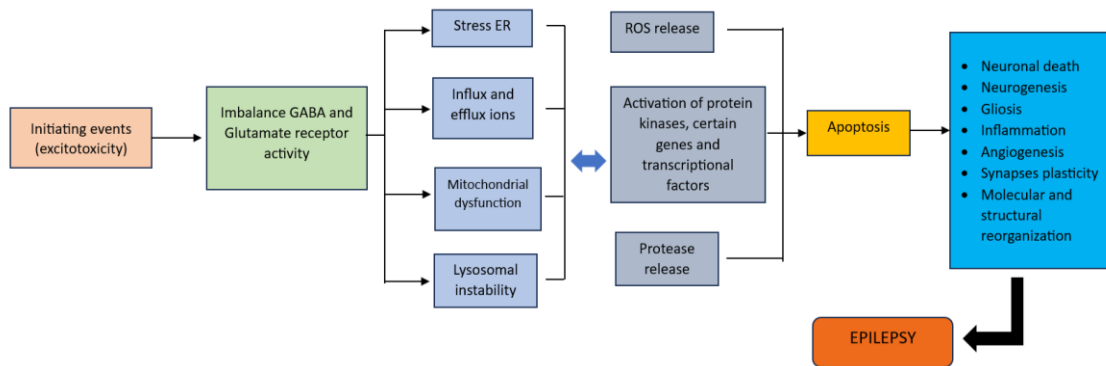


Figure 4. The Mechanism Scheme of Excitotoxicity Induces Epilepsy. ER: Endoplasmic Reticulum, ROS: Reactive oxidantive stress. Permission was granted by Lorigados et al. (©Editorial Elfos Scientiae [38]) to reuse this figure.

Ionic Environment Modifications Causing Epileptic Seizures and Epilepsy

While certain forms of epileptic seizures might originate in subcortical regions, the majority of such seizures stem from the cortical and hippocampal structures. Various factors, including the site of disruption, the timing and duration of the electrical bursts, and the associated clinical manifestations, collectively contribute to the complexity of epileptic seizures. Beyond instigating abnormal activity within individual neurons, alterations in excitability, intrinsic to epileptogenesis, encompass networks of hyperexcitable cells. These cells function cohesively, demonstrating a high degree of coordination via both functional and potentially dysfunctional pathways (39).

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4 Upon membrane depolarization, voltage-gated sodium channels are activated, leading to a pivotal shift in the
5 action potential phase. Concurrently, various voltage-gated potassium channels, integral to the repolarization phase,
6 initiate their activity. Following their activation, sodium ion channels transition into an inactive state due to the ball-
7 and-chain mechanism, which closes the channel pore, effectively halting further sodium ion passage. Notably, as the
8 action potential reaches its zenith, the majority of sodium ion channels are already inactive, with an exception being
9 the persistent sodium channels (I_{NaP}) that remain open. Even this subtle continuation of sodium ion conduction by I_{NaP}
10 channels suffices to propel the membrane past the action potential threshold. This mechanism forms the basis of the
11 intrinsic electrical impulse observed in layer V pyramidal neurons. Consequently, this inherent electrical discharge
12 extends throughout axon branches, fostering synaptic connections with proximate neurons and preserving their
13 synchronized activity (39)

14 Ion channels are integral proteins that form pores within a cell's lipid membrane. These pores facilitate the
15 selective passage of specific ions, thereby contributing to the maintenance of the cell's negatively charged resting
16 membrane potential. These membrane proteins, termed hetero-oligomeric ion channels, consist of two to six subunits.
17 Each subunit features transmembrane segments and differing domain counts, culminating in the creation of diverse
18 channel subtypes. The assembly of these subunits generates a range of channels, each equipped with distinct
19 functionalities. Ion channels are classified as either voltage-gated or ligand-gated, contingent upon how they respond
20 to alterations in the membrane potential. Ligands such as GABA and the neurotransmitter acetylcholine can activate
21 ion channels, serving as prime examples. Cation channels play a pivotal role in initiating the action potential, thus
22 contributing to neuronal excitability. Conversely, anion channels are instrumental in executing inhibitory processes
23 that counteract neuronal stimulation. Disturbances in ion charge equilibrium resulting from anomalies in anion or
24 cation channel function, known as channelopathies, have the potential to trigger epileptogenesis (4,39,40).

25 A. Sodium Ion Channels

26 The movement of sodium ions through the fast channel's inactivation propels the action potential phase
27 upward. This phenomenon is intertwined with the inactivation of the slower sodium ion channel component, a
28 process pivotal in governing membrane excitability. The presence of toxins that hinder the rapid inactivation of
29 sodium ion channels can additionally amplify the inactivation of the slow component. This dynamic shift leads
30 pyramidal neurons in the neocortex to transition from regular spiking to burst firing. This alteration can be
31 epileptogenic in two distinct ways. Firstly, it can trigger paroxysmal depolarizing shifts (PDS) inducing electrical
32 bursts within the affected neurons. Secondly, it can enhance synchronization stemming from intrinsic electrical
33 bursts in the pyramidal neurons of the neocortex's layer V. As a consequence, a substantial cohort of neurons
34 becomes engaged, thereby laying the foundation for an epileptogenic process to take root (39,40).

35 B. Potassium Ion Channels

36 Calcium ion influx triggers the activation of potassium channels, playing a pivotal role in the subsequent
37 repolarization of the cell membrane following excitation. In fact, the calcium ion-dependent flow of potassium
38 ions maintains its distinct contribution to post-action potential hyperpolarization in cells intrinsically stimulated,
39 such as pyramidal neurons within the CA area. This potassium ion movement encompasses the M current, named
40 due to its inhibition by muscarinic acetylcholine receptor activation—an integral mechanism for regulating
41 neuronal excitability. The M current holds significance in governing membrane excitability below the threshold
42 and the ability to respond to synaptic input. Its activation relies on a slow conductance that occurs between -60
43 mV and -20 mV. Blocking the M current by muscarinic receptor binding induces a shift in the neuronal membrane
44 towards greater depolarization. Consequently, this alteration can lead to the occurrence of repetitive action
45 potentials interspersed with periods of burst activity. In the context of benign neonatal familial convulsions, genetic
46 factors linked to M current deficiency play a pathogenetic role. This M current is governed by two potassium ion
47 channel subunits, namely KCNQ2 and KCNQ3, and mutations within the genes encoding these subunits—located
48 at chromosome positions 20q13 and 8q24, respectively—contribute to M current disorders that are associated with
49 the phenotype of benign neonatal familial convulsions (39,40).

50 C. Sodium Ion Channels

51 The movement of sodium ions through ion channels, facilitated by the fast channel's inactivation, leads to
52 a rise in the action potential phase. This phenomenon is intricately linked to the inactivation of the slow sodium
53 ion channel component, which also exerts a significant influence on membrane excitability. Toxins that impede
54 the fast inactivation of sodium ion channels can additionally intensify the inactivation of the slow component,
55 inducing a shift in pyramidal neurons within the neocortex from regular spiking to burst firing. This alteration can
56 exhibit a proepileptogenic impact through two distinct pathways. Firstly, it can give rise to PDS-inducing electrical
57 bursts within the affected neurons. Secondly, it has the potential to amplify synchronization resulting from intrinsic
58 electrical bursts in pyramidal neurons within the neocortex's layer V. Consequently, this action can set the stage
59 for an epileptogenic process to ensue, involving a substantial ensemble of neurons (39,40).

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4 D. Potassium Ion Channels

5 Calcium ion influx initiates the activation of potassium channels, which are vital for the subsequent
6 repolarization of the cell membrane following excitation. Remarkably, the flow of potassium ions governed by
7 calcium ions maintains its unique contribution, distinct from the post-action potential hyperpolarization observed
8 in intrinsically stimulated cells, such as pyramidal neurons within the CA area. Within the realm of potassium ion
9 movement, the M current plays a pivotal role. This name originates from its inhibition by muscarinic acetylcholine
10 receptor activation—a pivotal mechanism for modulating neuronal excitability. The M current is especially adept
11 at controlling membrane excitability beneath the threshold and facilitating responsiveness to synaptic input. This
12 is accomplished through its reliance on activating a slow conductance spanning the range of -60 mV to -20 mV.
13 In the event that the M current is obstructed due to muscarinic receptor binding, the neuronal membrane
14 experiences an increase in depolarization. This phenomenon can lead to the generation of repetitive action
15 potentials characterized by interposing gaps and burst activity in proximity. The genetic factors associated with M
16 current deficiency are evident in cases of benign neonatal familial convulsions, where such factors have a
17 pathogenetic influence. Regulation of the M current hinges on two subunits of potassium ion channels—KCNQ2
18 and KCNQ3. Mutations within the genes coding for these subunits (located at chromosomal positions 20q13 and
19 8q24, respectively) result in M current disorders that are intimately linked to the phenotype of benign neonatal
20 familial convulsions (39,40).

21 E. Calcium Ion Channels

22 Calcium ion channels play a crucial role in transient and persistent cell membrane depolarization, driven
23 by their threshold and kinetic activation properties. However, understanding the involvement of calcium ion flux
24 in epileptogenesis poses several challenges. Firstly, due to its essential role in protein synthesis and metabolism,
25 the majority of calcium ions are stored within the endoplasmic reticulum. Manipulating calcium ion concentrations
26 artificially, as often done in experimental setups, can trigger the release of stored calcium ions from their
27 compartments, thereby introducing complex effects on cell excitability that are further compounded by metabolic
28 influences. Secondly, when assessing the impact of calcium ion concentration on membrane excitability, it's
29 important to consider its role in mediating vesicle fusion in nerve terminals. This fusion process is vital for
30 neurotransmitter release and synaptic function, adding another layer of complexity to the relationship between
31 calcium ions and excitability. Thirdly, intentional reduction of calcium ion concentration can disrupt various types
32 of calcium-dependent potassium currents that contribute to cell excitability. This disruption can lead to indirect
33 and unpredictable outcomes that directly influence calcium ion movement through the cell membrane. This
34 interconnectedness makes it challenging to isolate the precise effects of altered calcium ion concentrations on
35 cellular excitability. Comprehending the role of calcium ion flux in epileptogenesis is intricate due to its
36 multifaceted functions. Manipulating calcium ion concentrations can trigger cascading effects on cell excitability,
37 neurotransmission, and potassium currents, making it challenging to draw definitive conclusions about its
38 contribution to the epileptogenic process (39,40).

39 F. Excitatory Amino Acid Receptors

40 In the cerebral cortex, the two primary excitatory amino acid neurotransmitters are glutamate and aspartate.
41 These neurotransmitters exert their effects through distinct receptor subtypes. The specific level of ion permeability
42 and the corresponding rates of ion flow are governed by the composition of their respective subunits (41,42). Two
43 distinct types of receptors exist: NMDA and AMPA (with kainate and quisqualate subtypes). The ionophores
44 associated with these receptor types allow the permeation of sodium ions, while the NMDA receptor type also
45 permits calcium ion entry. NMDA receptors are blocked by magnesium ions, which remains dependent on the
46 membrane's polarization state. To activate this channel, sufficient depolarization of the membrane must first occur
47 to remove the magnesium blockage. Glutamate binds to metabotropic receptors as well, which serve as second
48 messengers in addition to ionotropic receptors. The processes involving sodium and calcium ions in the AMPA
49 and NMDA components of excitatory postsynaptic potentials (EPSPs) are potent enough to sustain membrane
50 depolarization, significantly extending and amplifying EPSPs. Anomalously formed synaptic connections,
51 resulting from sprouting mossy fibers, can lead to the creation of additional excitatory feedback circuits. This
52 feedback loop augments conductance facilitated by NMDA receptors (39,43).

53 F. GABA Receptors

54 Chloride and potassium ionophores are attributed to the GABAA and GABAB subtypes, respectively,
55 among the primary classes of ionotropic GABA receptors. While it has been proposed that the third receptor
56 subtype, GABAC, may serve physiological functions, this remains uncertain. GABA binding to its receptors
57 induces membrane hyperpolarization and generates inhibitory postsynaptic potentials (IPSPs), accomplished
58 through the entry of chloride ions and the exit of potassium ions. GABA-mediated IPSPs have a notable ability to
59 quell neuronal electrical bursts, primarily due to their connection with reduced input membrane resistance. Local
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inhibitory circuits comprised of Golgi type 2 GABAergic neurons regulate the main population of pyramidal neurons in the cerebral cortex. In individuals with refractory temporal lobe epilepsy, reduced GABAergic neuron density and impaired GABAergic circuit function have been observed in brain tissue. This underscores the role of GABA-inhibitory abnormalities in the progression of epilepsy (39).

G. Acetylcholine Receptors

Nicotinic acetylcholine receptors hold particular significance due to their presence in the central nervous system. These receptors form pentameric structures composed of three subunits: one subunit from the α group ($\alpha 1 - \alpha 8$) and another subunit from the β , γ , or δ group. Positively charged ions such as sodium, calcium, and potassium flow through the channel to achieve equilibrium. The channel is permeable to these ions without showing selectivity. The equilibrium potential of these ion channels can vary, although it typically hovers around -5 mV. Epileptic symptoms have been observed in individuals with mutations in the CHRNA4 gene, responsible for encoding the receptor subunit 4, and the CHRNB2 gene, responsible for encoding the nicotinic acetylcholine receptor 2 subunit. Investigating these mutations' precise impact on neuronal hyperexcitability is crucial for a comprehensive understanding of their role (39,40).

Tabel 1. The Function of Ion Channel and Receptor in Normal and Epileptic Tissue (7)

Ion channels or receptor	Normal brain	Epileptic brain
Voltage-gated Na ⁺ channels	Subthreshold EPSP, upstroke of the action potential	Repetitive action potential firing
Voltage-gated K ⁺ channels	Action potential downstroke	Action potential repolarization
Voltage-gated Ca ⁺ channels	Released transmitter, transports a depolarizing charge from the dendrite to the soma.	A pathogenic intracellular process is activated by excessive transmitter release.
Ca ²⁺ -dependent K ⁺ channels	After hyperpolarization following action potential, sets refractory period	Limit repetitive firing
Non-NMDA receptor	Fast EPSP	Initiates paroxysmal depolarization shift
NMDA receptor	Prolong, slow EPSP	Maintain paroxysmal depolarization shift, Ca ²⁺ activates pathologic process
GABA receptor	IPSP	Limit excitation
Na-K pump	Restore ionic equilibrium	Prevent K ⁺ - induced depolarization

Recent advances in genetics and molecular biology have shown the connection between certain epilepsy disorders and mutations in genes responsible for coding ion channel proteins. These mutations can lead to a condition known as channelopathy, characterized by the malfunction or impairment of ion channels, resulting in heightened neuronal excitability (4,6). Various forms of familial epilepsy arise from genetic anomalies that disrupt the proper functioning of voltage-gated ion channels. These ion channels play a crucial role in regulating membrane potential, depolarization threshold, and synaptic transmission, all of which contribute to maintaining the normal excitability of cells. Mutations affecting these ion channels can lead to functional changes that result in neuronal hyperexcitability, ultimately triggering epileptic seizures (8). Channelopathies have a substantial impact on epileptogenesis, particularly in patients with epilepsy, including those with idiopathic forms. Idiopathic epilepsy has been associated with mutations occurring in genes responsible for encoding various types of ion channels such as potassium, sodium, chloride, and calcium channels, as well as acetylcholine and GABA receptors. These mutations can lead to alterations in ion channel function due to changes in transcriptional and post-translational processes. Consequently, channelopathies have the potential to contribute to the underlying pathology of acquired epilepsy (4). Mutations in key components of the inhibitory system can lead to the breakdown of normal inhibitory processes, thereby contributing to neuronal hyperexcitability in epilepsy. GABA, the principal inhibitory neurotransmitter, acts through ligand-gated chloride ion channels, which facilitate fast inhibitory synaptic transmission. Mutations in specific genes, such as the voltage-gated chloride channel (CLCN2), GABAA receptor subunit $\alpha 1$ (GABRA1), and GABAA receptor subunit $\gamma 2$ (GABRG2), can disrupt the proper functioning of these components. These genetic alterations can result in the dysfunction of GABA conductance and chloride ion flux. As a consequence, the delicate balance between excitatory and inhibitory neurotransmission is disrupted, leading to an increased susceptibility to neuronal hyperexcitability, which is a hallmark of epilepsy. Understanding the impact of these mutations on inhibitory signaling pathways provides crucial insights into the underlying mechanisms of epilepsy (44,45).

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4 Mutations in genes responsible for encoding sodium channel proteins are believed to be a mechanism
5 underlying certain types of inherited epilepsy. In humans, genes like SCN1A-SCN5A and SCN8A-SCN11A code for
6 nine different subtypes of the alpha-subunit of voltage-gated sodium channels, specifically Nav1.1-Nav1.9. These
7 mutations also impact epilepsy-related pathophysiology in the beta1 subunit of voltage-gated sodium channels. When
8 mutations occur in the beta1 subunit, the affected sodium channels remain open for prolonged periods, allowing
9 excessive sodium influx into the cell. Consequently, neurons become hyperexcitable and remain depolarized for
10 extended durations. This leads to the release of large amounts of excitatory neurotransmitters, such as glutamate. As
11 a result, the post-synaptic cells that interact with neighboring glutamatergic neurons experience excessive stimulation,
12 leading to the production of calcium ions, which can be neurotoxic. Mutations in the genes encoding the alpha1
13 voltage-gated sodium channel subunit (SCN1A), which accelerates recovery from an inactive state, can also cause
14 sustained depolarization due to prolonged sodium ion inflow and hyperexcitability. Hereditary epilepsy is linked to
15 mutations in genes producing the voltage-gated sodium channel subunit beta1 (SCN1B) and SCN2A. Generalized
16 epilepsy with febrile seizures involves mutations in sodium channel subunits SCN1A and SCN2A, as well as the
17 GABAA receptor subunit GABRG2. Another form of generalized epilepsy caused by a single gene mutation is benign
18 familial newborn convulsions. These genetic variations affecting sodium channels significantly contribute to the
19 development of epilepsy by disrupting the normal balance of neuronal excitability and neurotransmission (10,12,44–
20 46).

21 Potassium ion channels regulate neuronal excitability by allowing the flow of potassium ions to the
22 extracellular space. This process helps control the resting membrane potential and facilitates rapid repolarization of
23 the action potential. These ion channels consist of four alpha (a)-subunits that form the pores, alongside four beta (b)-
24 subunits for regulation. Among the most significant subset of ion channels are the Kv channels (Kv1–Kv12), which
25 are highly abundant in the central nervous system. Dysfunctions in Kv channels, including calcium-activated
26 potassium ion channels, have been associated with epilepsy. Channelopathies related to epilepsy involve HCN and
27 Kv7 channels. HCN and Kv7 channels, which facilitate cation transport, induce hyperpolarization through voltage-
28 gated ion channels. HCN channels are prominently expressed in the hippocampus, cortex, and thalamus, contributing
29 to localized and widespread epileptic seizures. Kv7 channels also play a role in modulating neuronal hyperexcitability.
30 The HCN channel, however, performs a more intricate role. Genetic deletions in the HCN2 channel subtype do not
31 result in an epilepsy phenotype, whereas deletions in the HCN1 subtype can accelerate epileptogenesis. The absence
32 or inhibition of HCN1 channels increases neuronal hyperexcitability, seizure severity, and the risk of SE-related
33 mortality. Membrane hyperpolarization activates HCN channels, inhibiting I_h, a current that regulates neuronal
34 excitability by controlling the resting membrane potential. Reduced I_h density due to decreased or lost channel
35 expression can lead to neuronal hyperexcitability. Mutations in the KCNQ canal gene, responsible for Kv7 channels,
36 are also associated with epilepsy. Deficiencies in Kv7 subunits, similar to Kv7.2 subunit mutations, can lead to
37 epilepsy. Kv7 channels reduce neuronal hyperexcitability by activating voltage-gated potassium channels. They also
38 inhibit repeated depolarization of action potentials. Mutations in genes encoding potassium channels, such as KCNQ2
39 and KCNQ3, can result in reduced potassium ion flow. This leads to prolonged depolarization and increased neuronal
40 hyperexcitability, as potassium ion influx occurs only after repolarization of the neuronal membrane following
41 depolarization. These insights highlight the critical role of potassium ion channels in maintaining proper neuronal
42 excitability and the consequences of their dysfunction in epilepsy (4,5,12,45,46)

43 The role of calcium ions as second messengers is essential for both healthy brain function and the development
44 of various neurological disorders, including epilepsy. Calcium ions enter cells through voltage-gated calcium
45 channels, which are instrumental in transmitting electrical signals and initiating transduction cascades in diverse cell
46 types. Voltage-gated calcium channels are categorized into two main subtypes: low-voltage-activated (LVA) and high-
47 voltage-activated (HVA) channels. These channels allow calcium ions to flow into the intracellular space, where they
48 are utilized for signal transduction. The composition of these channels involves multiple subunits, including α1, β,
49 α2δ, and γ subunits, within the HVA subtype. The subunits within the HVA subtype, including L, N, P, and Q types,
50 have activation thresholds that generally occur around positive membrane potentials, often up to -20 mV. On the other
51 hand, the LVA channel, or type T, is activated at more positive membrane potentials, reaching up to -70 mV. This
52 particular channel subtype exclusively contains α1 subunits. The transmembrane topology of voltage-gated calcium
53 channels consists of four homologous transmembrane domains, each containing a transmembrane segment and a
54 porous region located between segments S5 and S6. This structural arrangement enables the channel to facilitate the
55 movement of calcium ions across the cell membrane, thereby contributing to various cellular processes and responses.
56 In the context of epilepsy, the dysregulation of calcium ion influx through these channels can disrupt normal brain
57 activity and contribute to the development of seizures and other epileptic phenomena. Understanding the intricate role
58 of voltage-gated calcium channels in neuronal function and their involvement in epilepsy is essential for advancing
59 our knowledge of this complex neurological disorder (46).
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4 Impaired expression of the chloride transporter-like K-Cl cotransporter (KCC2)/NKCC1 complex plays a
5 significant role in epileptogenesis. This transporter system is crucial for regulating the impact of GABA (gamma-
6 aminobutyric acid) on neuronal excitability. GABA, traditionally an inhibitory neurotransmitter, can exert excitatory
7 effects through the K-Cl cotransporter. In mature neurons, GABA typically induces membrane hyperpolarization due
8 to lowered intracellular chloride ion levels. However, during neuronal development, elevated intracellular chloride
9 concentrations can lead to GABA-driven chloride ion efflux and subsequent membrane depolarization. The
10 equilibrium between these opposing effects is tightly controlled by the activity of the K-Cl cotransporter and NKCC1.
11 The balance between KCC2 and NKCC1 activity in neurons dictates whether GABA has a depolarizing or
12 hyperpolarizing effect. In cases of epilepsy, an imbalance in the expression and function of KCC2 and NKCC1 can
13 cause a shift from GABA's traditional inhibitory effect to an excitatory one, contributing to hyperexcitability and
14 seizure activity. Notably, in the hippocampus of rat models with epilepsy, there is evidence of increased NKCC1
15 expression coupled with decreased KCC2 expression. This imbalance likely contributes to the altered neuronal
16 excitability observed in epilepsy. Understanding the intricate interplay between chloride transporters, GABA, and
17 neuronal excitability is essential for unraveling the mechanisms underlying epileptogenesis and potentially identifying
18 therapeutic targets to mitigate the development and progression of epilepsy (12).
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20 **The Role of Neurogenesis in Epileptic Seizures and Epilepsy**

21 Epileptogenesis is a complex process characterized by the formation of new neurons and circuits within the
22 hippocampal region of the brain. After an acute epileptic seizure, individuals with brain injuries can undergo various
23 types of changes. These changes encompass cellular, structural, and neurochemical alterations (4). Granular cells
24 located in the dentate gyrus of the hippocampus exhibit continuous proliferation in nearly all mammalian species.
25 However, epilepsy can disrupt this process of new neuronal growth. Interestingly, neurogenesis tends to increase in
26 the aftermath of epileptogenesis. An epilepsy-related disorder in hippocampal neurogenesis encompasses several
27 factors, such as the formation of granular cells in abnormal locations, heightened proliferation of neural cell
28 progenitors, aberrations in morphological features of the neurons, and neuronal hypertrophy (12).
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30 After a sudden seizure, the hippocampus can undergo a range of structural changes, including the degeneration
31 of CA1-CA3 pyramidal neurons and dentate gyrus hilar neurons, as well as the sprouting and abnormal formation of
32 synapses by mossy fibers. Additionally, the loss of inhibitory GABAergic interneurons occurs. During the process of
33 mossy fiber sprouting, axons originating from granular cells in the dentate gyrus establish synapses in unusual
34 locations, particularly in the inner third molecular layer of the gyrus dentatus or the supragranular region. This
35 phenomenon contributes to the synaptic reorganization of mossy fibers. As mossy cells, significant excitatory neurons
36 in the hilus dentatus that usually project to the supragranular area, undergo neuronal death, this structural
37 rearrangement aids in eliminating synapses. Furthermore, the growth of new neural circuits can give rise to recurrent
38 excitatory circuits in dentate granule cells due to axon sprouting and synaptogenesis in the mossy fiber pathway. This
39 increased excitation can contribute to the development of epileptogenesis. Another theory regarding structural
40 reorganization involves functional disconnections between excitatory neurons and dormant (inhibitory) basket cells.
41 According to this theory, GABAergic interneurons, known as basket cells, survive damage induced by epileptogenesis.
42 However, because most excitatory neurons (mossy cells) perish as a result of epileptic seizures, the inhibitory
43 influence of these basket cells diminishes. As a result, these basket cells become inactive and are unable to provide
44 inhibitory feedback to granular cells. This loss of inhibition resulting from the inactivity of basket cells might
45 contribute to the process of epileptogenesis (4).
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47 Acute epileptic seizures, leading to structural alterations, can also enhance the expression of neurotrophic
48 factors and various proteins within the hippocampus. These include sonic hedgehog, brain-derived neurotrophic factor
49 (BDNF), fibroblast growth factor-2 (FGF-2), and nerve growth factor (NGF). The neurotrophin family of proteins,
50 which encompasses FGF-2, BDNF, and NGF, plays a vital role in influencing neuronal survival, differentiation,
51 development, synaptic plasticity, and excitability. Additionally, vascular endothelial growth factors (VEGF) are
52 responsible for promoting angiogenesis. However, elevated levels of VEGF can disrupt the BBB, potentially leading
53 to brain inflammation. Notably, abnormal cerebral angiogenesis can manifest as increased permeability to
54 immunoglobulin G and the loss of tight junctions. Furthermore, the sonic hedgehog protein, which is secreted,
55 regulates the growth and survival of both neuronal and glial progenitor cells. An elevation in the expression of this
56 protein may contribute to the generation of neurons within the hippocampus (4,17)

57 **The Role of Oxidative Stress in Epileptic Seizure**

58 Oxidative stress plays a critical role in the initiation and progression of epilepsy, stemming from the excessive
59 release of free radicals. Experimental animal models, such as the administration of kainic acid and pilocarpine, induce
60 this condition. In response, antioxidant therapy has gained acceptance as a strategy to mitigate oxidative stress and
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4 potentially serve as a treatment for epilepsy. However, it's important to note that not all types of seizures exhibit the
5 same degree of oxidative damage. The impact of oxidative stress varies among seizure types. Compromised
6 antioxidant systems, mitochondrial dysfunction, redox-active metals, activation of the arachidonic acid pathway, and
7 even aging can contribute to oxidative stress, thereby becoming factors in the development of epileptic seizures.
8 Superoxide radicals are implicated in both excitotoxicity and the mediation of oxidative stress. This understanding
9 has prompted the exploration of antioxidant approaches targeting oxidative stress as potential avenues for treating
10 epileptic seizures. Such methods not only hold promise for managing seizures but also offer potential as
11 neuroprotective strategies in epilepsy cases. By addressing the underlying oxidative stress, these interventions may
12 contribute to improving the management and outcomes of epilepsy (8,12).

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14 The activation of intracellular oxidant systems that rely on calcium ions, such as NADPH oxidase 2 (NOX2),
15 plays a pivotal role in promoting superoxide generation. Notably, NOX2 is regulated by NMDA receptors,
16 highlighting its significance in the context of neuronal hyperexcitability. This phenomenon, observed in the early
17 stages of epilepsy, can lead to chronic epilepsy by inducing enduring neural damage. The interplay between these
18 factors contributes to the complex pathophysiology of epilepsy. Neural networks that govern intricate processes in
19 self-regulatory homeostasis, including the bioenergetic system, become disrupted. This imbalance between excitatory
20 and inhibitory signals, combined with oxidative stress, gives rise to abnormal patterns of activity within neural circuits.
21 These intricate interactions contribute to the intricate nature of epilepsy's progression, as well as its long-term effects
22 on neural function and overall brain health (6).

23 24 **The Role of Gene and Protein Regulation in Epileptic Seizure and Epilepsy**

25 Brain injury triggers alterations in gene expression, a process largely governed by transcription factors. Within
26 the context of epileptic seizures, the pathophysiology is believed to involve immediate early genes (IEGs) or inducible
27 transcription factors that play a pivotal role in orchestrating cellular responses. Notably, members of the Jun family
28 (such as c-jun, junB, and junD) and the Fos family (including c-fos, fosB, fra-1, and fra-2) are implicated in this
29 mechanism. Both gene families encode crucial transcription factors, including c-fos and c-jun, which are integral
30 components of the transcription factor activator protein-1. During epileptic seizures, hippocampal neurons have been
31 observed to display heightened mRNA expression of these factors. The precise functions of IEGs in mediating early
32 genes during epileptogenesis remain to be fully elucidated, generating ongoing uncertainty. Broadly, transcription
33 factors from the Fos and Jun families play roles in gene transcription, cell proliferation, regeneration, and cell death
34 within the central nervous system. The expression of IEGs represents a critical step in the biological cascade leading
35 to neuronal apoptosis, ultimately resulting in cell death. Understanding the intricate interplay between these
36 transcription factors and their influence on cellular processes contributes to unraveling the complex molecular
37 landscape underlying epileptic seizures and their aftermath (4).

38 Inducible cyclic adenosine monophosphate (cAMP) early repressor (ICER), another transcription factor,
39 emerges as a significant player in the intricacies of epileptogenesis. A class of proteins collectively known as ICER
40 arises from the cAMP-responsive element modulator (CREM) gene. Additionally, ICER mRNAs are generated by the
41 internal promoter of CREM. The central role of ICER lies in its ability to inhibit the cAMP-responsive element (CRE)-
42 mediated transcription of genes. ICER exerts its influence by suppressing the transcription of genes that rely on CRE-
43 mediated mechanisms. A key aspect of its function is the inhibition of the activity of the transcription factor cAMP-
44 responsive element binding protein (CREB). This unique regulatory action positions ICER as a pivotal player in
45 governing both neuronal plasticity and programmed cell death within the nervous system. Notably, CREB is
46 responsible for activating CRE transcription, which holds paramount importance for maintaining neuronal survival.
47 The impact of ICER on neuronal cultures reveals an apoptotic effect through its overexpression in these contexts.
48 Furthermore, the heightened expression of ICER in neurons following excitatory inputs suggests its involvement in
49 apoptotic processes. Yet, despite ongoing research efforts and some divergent findings, the precise role of ICER in
50 the complex web of epileptogenesis remains elusive. As the scientific community continues to explore this intricate
51 topic, elucidating the specific contributions of ICER to the development of epilepsy holds the promise of enhancing
52 our understanding of the underlying mechanisms and potentially paving the way for innovative therapeutic strategies
53 (4,9,47).

54 A multitude of studies have delved into the impact of epileptic seizures on the expression of neuropeptides
55 within the hippocampus. Among the neuropeptides that have garnered attention in these studies are cortistatin, galanin,
56 neuropeptide Y (NPY), thyrotropin-releasing hormone, cholecystokinin, and neurokinin B (48–50). NPY is localized
57 on GABAergic interneurons within the hippocampus and serves as an inhibitor of excitable neurons. Experimental
58 studies involving models of epileptic seizures have highlighted intriguing findings regarding NPY's expression.
59 Overexpression of NPY and its corresponding mRNA within the hippocampus, as well as aberrant NPY expression
60 in hippocampal granule cells and mossy fibers—cellular regions not typically associated with this neuropeptide—
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4 have been reported in these investigations (51,52). The outcomes of this study underscore an inherent adaptive
5 mechanism that counteracts the hyperexcitable state induced by epileptic episode stimulation. Further support for this
6 notion is provided by investigations showcasing diminished occurrences of spontaneous epileptic seizures and the
7 prevention of epileptogenesis through NPY gene therapy in experimental animal models. Galanin, on the other hand,
8 functions as a modulator of epileptic seizures by activating galanin receptors GalR1 and GalR2. This action enables
9 galanin to sustain a delicate equilibrium between glutamatergic excitation and galaninergic inhibition within the
10 hippocampal gyrus dentatus (4).
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12 **The Role of Thalamocortical Circuit Disruption in Epilepsy, Especially the Absence Type**

13 The disruption of the circuitry connecting the thalamus and the cerebral cortex constitutes the driving
14 mechanism behind absence epilepsy. This circuit plays a pivotal role in orchestrating the rhythmic excitation of the
15 cortex by the thalamus during normal sleep cycles. Within this circuit, three distinct populations of neurons are
16 involved: thalamic relay neurons, thalamic reticular neurons, and cortical pyramidal neurons. Thalamic relay neurons
17 possess the capability to activate cortical pyramidal neurons during wakefulness and rapid eye movement (REM) sleep
18 phases. The underlying electrical activity is likely facilitated by T-type calcium ion channels, which elicit low-
19 threshold depolarization and initiate action potentials through voltage-gated sodium channels. Thalamic reticular
20 neurons, through their inputs, exert control over the activation of thalamocortical circuits by inducing
21 hyperpolarization in relay neurons, thereby generating electrical activity. The mutual inhibition of reticular neurons is
22 achieved through collateral networks stemming from adjacent reticular neurons. Completing this circuit, both cortical
23 pyramidal and thalamic relay neurons project to reticular neurons. Notably, this circuit's dynamics can be modulated
24 by ascending noradrenergic, serotonergic, and dopaminergic inputs to the thalamus, potentially intensifying the
25 electrical activity. During the non-REM sleep phase, depolarized thalamic relay neurons synchronously and
26 rhythmically stimulate the cortex, resulting in the generation of sleep spindles—distinct brain wave patterns.
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28 In contrast to the activity observed during wakefulness, where thalamic relay neurons are depolarized and
29 sensory information is conveyed to the brain in a non-rhythmic manner, the functioning of thalamocortical circuits
30 during sleep follows a distinct pattern. In the context of absence epilepsy, faulty circuitry generates rhythmic activation
31 of the cortex during waking hours, akin to the pattern observed during typical non-REM sleep phases. This aberrant
32 activity gives rise to the characteristic brain wave patterns and clinical manifestations of absence epilepsy. Several
33 potential factors contribute to these circuitry abnormalities, including disruptions in modulatory input from the
34 brainstem, the role of T-type calcium ion channels, alterations in GABA receptor function, or a combination of these
35 factors. However, the precise nature of the circuitry malfunction remains to be definitively identified. An alternate
36 perspective posits that abnormal paroxysmal episodes of rhythmic cortical activation (absence epileptic seizures)
37 could arise from an underlying neural circuit dysfunction that mimics the physiological state of rhythmic cortical
38 activation seen during sleep (10).
39

40 Nowadays, research has showed that the hippocampus circuit plays a crucial role in comprehending the
41 intricacies of epilepsy, particularly the absence type. The hippocampus that works in memory and spatial navigation
42 also has complicated connections with the cortex that contribute to brain activity regulation. The trisynaptic circuit is
43 a critical channel for information processing inside the hippocampus formation. The dentate gyrus, the CA3 area, the
44 CA1 region, and the subiculum are all part of this circuit. At each of these steps, excitatory glutamatergic neurons
45 create synapses, allowing information to be sent sequentially. The circuit's unique design allows for the modification
46 of incoming signals and the encoding of memories. The synchronization of neuronal activity inside the hippocampus
47 is crucial for maintaining optimal brain function. Disruptions in these coordinated rhythms might result in seizure
48 activity. Incorporating the hippocampus into the knowledge of absence epilepsy provides a more comprehensive view
49 of the neuronal networks involved in this condition. The interconnection of the hippocampus and cortex enables not
50 only for the transmission of spatial and contextual information, but also for the control of general brain states,
51 including those linked with seizures. Considering hippocampus circuitry in addition to the thalamocortical circuit
52 gives a more thorough framework for appreciating the complicated web of connections involved in absence epilepsy.
53 The hippocampus may play a role in the rhythmic cortical activity seen during absence seizures, working in tandem
54 with the damaged thalamocortical circuit to generate an environment favourable to aberrant synchronized firing.
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56 **The Role of miRNAs as Biomarkers in Epilepsy and Epileptogenesis**

57 Epigenetic mechanisms that are not linked to changes in DNA sequence but rather to transcription or post-
58 transcriptional regulation can play a role in epileptogenesis. These mechanisms influence various essential biological
59 pathways, some of which are modulated by miRNAs, and are associated with the development of various types of
60 epilepsy (37,53). MicroRNAs (miRNAs) serve as potential regulatory mechanisms and therapeutic targets for epilepsy
61 by modulating mRNA stability and translational processes, thereby inhibiting the expression of numerous proteins.
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4 These miRNAs hold promise as both biomarkers and targets for therapeutic interventions, as they reflect the intricate
5 gene expression regulation associated with epilepsy. Altered expression of specific miRNAs has been observed, such
6 as serum miRNA-4521 in the hippocampus of individuals with temporal lobe epilepsy, as well as changes in brain
7 tissue in experimental animal models of SE (54–57). Following epileptic episodes, alterations were observed in the
8 expression levels of certain miRNAs, including those associated with transcription factors and elements involved in
9 neurotransmitter signaling. Changes in miRNA levels in the bloodstream suggest their potential utility as diagnostic
10 biomarkers. Earlier studies primarily focused on the regulatory impact of miRNAs on their target genes. Some
11 investigations explored the consequences of altered gene expression on individual miRNAs, observing fluctuations in
12 miRNA levels in experimental mouse or rat models, and tracking the progression of epilepsy-related pathology. Recent
13 advances in epigenetic profiling are shedding light on the potential role of miRNAs in the pathophysiology of epilepsy.
14 Methylation status and miRNA expression correlations have been identified in hippocampal samples from both human
15 subjects and mouse models. MiRNAs are abundantly present in the brain and play a crucial role in regulating
16 neurogenesis (37,58,59).

17
18 Numerous types of miRNAs are expressed in response to epileptic conditions. Among them, miR-146a is
19 notable, as it has been observed to exhibit elevated expression in both humans with temporal lobe epilepsy and mice
20 with epilepsy. Sense and antisense oligonucleotides, referred to as agomirs (mimics) and antagomirs, respectively,
21 can efficiently modify the activity of miRNAs. This manipulation of miRNA function can lead to changes in gene
22 expression, offering a promising avenue for the exploration of novel treatment targets for epilepsy (60). Upon initial
23 investigation in individuals with epilepsy, miR-146a, a miRNA known to regulate inflammatory responses, exhibited
24 an increased presence in the hippocampus (37). MicroRNA-134 (miR-134), a widely expressed miRNA in brain
25 neurons, plays a role in controlling the formation of dendritic spines by inhibiting LIM domain kinase 1 (LIMK1) and
26 other targets. This function is particularly relevant as dendritic spine reorganization is a feature observed in
27 experimental human epilepsy and is crucial for intercellular excitatory communication, especially in hyperexcitability
28 disorders like epilepsy. In a study conducted by Reschke et al. (2017), miR-134 was identified as one of the miRNAs
29 exhibiting increased expression in hippocampal regions affected during SE induced by intra-amygdala kainic acid
30 administration in mice. Furthermore, miR-134 was found to be overexpressed in human neocortex resection samples
31 from patients with refractory temporal lobe epilepsy. The upregulation of miR-134 expression was also observed in
32 both in vitro and in vivo models of neuronal stimulation and epileptic seizures. Remarkably, intracerebroventricular
33 administration of cholesterol-tagged locked nucleic acid (LNA) antagomirs (Ant-134) effectively reduced miR-134
34 levels for an extended period of at least one month in mice experiencing epileptic seizures resistant to the convulsant
35 effects of kainic acid and pilocarpine hydrochloride. This intervention highlights the potential of targeting miR-134
36 for therapeutic purposes in epilepsy (61).

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38 MiRNAs such as miR-146a, miR-134, miR-132, miR-128, miR-34a, miR-324-5p, miR-124, miR-155, miR-
39 142-5p, miR-21-5p, miR-187, and miR-106b-5p have all been implicated in the process of epileptogenesis. Among
40 these, miR-106b-5p stands out as a particularly accurate predictor of epileptogenesis, as suggested by Tiwari et al.
41 (2018). Studies conducted by Cava et al. (2018) have also shown that miR-106b-5p is upregulated in epileptic
42 conditions. The expression of circulating miRNAs shows dynamic variations in connection with the pathological
43 process of epilepsy. Some miRNAs can be rapidly released following an epileptic seizure, while others may not be
44 expressed until the condition progresses to a chronic stage. Over the past five years, research by Wang et al. (2015)
45 has led to the discovery of around 100 distinct miRNAs in both epileptic patients and animal models. This extensive
46 research provides compelling evidence for the significant changes in miRNA expression associated with epilepsy.
47 Although several miRNAs have been linked to epilepsy, further investigations are necessary to determine whether
48 these dysregulated miRNAs can serve as predictive markers for epilepsy risk, diagnostic tools, or indicators of disease
49 progression. Wang et al. (2015) conducted a study involving 117 epilepsy patients and 112 controls, utilizing serum
50 samples from epilepsy patients. The study revealed that Let-7d-5p, miR-106b-5p, miR-130a-3p, and miR-146a-5p
51 exhibited increased expression compared to controls, while miR-15a-5p and miR-194-5p showed decreased
52 expression ($p < 0.0001$). Notably, miR-106b-5p in serum demonstrated the highest diagnostic value for epilepsy, with
53 a sensitivity of 80.3% and specificity of 81.2% (56).

54
55 In contrast to therapy-responsive groups, a study conducted by Alsharafi et al. (2015) highlighted that another
56 investigation identified altered miRNA expression patterns in drug-resistant individuals. Among the findings, 12
57 miRNAs exhibited decreased expression, while three miRNAs showed increased expression in this drug-resistant
58 group. In comparison to both the treatment-responsive group and the control group, certain miRNAs confirmed
59 through quantitative real-time polymerase chain reaction (qRT-PCR) analysis, including miR-194-5p, miR-301a-3p,
60 miR-30b-5p, miR-342-5p, and miR-4446-3p, displayed statistically significant dysregulation in the drug-resistant
61 group. In this context, miR-301a-3p emerged as a standout diagnostic biomarker for drug-resistant epilepsy among
62 these five miRNAs, demonstrating high levels of both sensitivity and specificity. Notably, it was observed that patients
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4 with temporal lobe epilepsy exhibited lower expression levels of the miRNAs miR-301a-3p and miR-106b-5p within
5 their hippocampal tissue (62)

6 In a study conducted by An et al. in 2016, the roles of eight circulating miRNAs associated with epilepsy were
7 investigated in relation to various cellular processes linked to epilepsy (63). Through in silico analysis, the study
8 revealed distinct pathways influenced by these miRNAs. Firstly, the "immune system" pathway was highlighted,
9 particularly in the context of neuro-inflammation involving microglia. MiRNAs including miR-15a-5p (with
10 decreased expression), miR-106b-5p, miR-146, and miR-451 were found to impact this pathway, resulting in the
11 upregulation of several genes involved in immune response. MiR-106b, which is also associated with inflammation
12 in Alzheimer's disease, exhibited increased expression in epilepsy. Secondly, the "cell cycle" pathway was identified
13 through in silico analysis, encompassing processes related to neurogenesis, cell cycle regulation, and cell proliferation.
14 This pathway indicated that these miRNAs, specifically miR-15a-5p, miR-34a, miR-106b-5p, and miR-146, likely
15 influence genes involved in neuronal cell differentiation and proliferation. Notably, miR-106b-5p displayed
16 heightened expression in the early stages of an epilepsy mouse model induced by electrical stimulation. This suggests
17 that this miRNA could potentially contribute to the interruption of neuronal cell cycle progression and subsequent
18 neuronal death. Thirdly, the study identified confirmed targets of miR-15a-5p (with reduced expression), miR-106b-
19 5p, miR-146, and miR-45 that are implicated in both pro- and anti-apoptotic signaling pathways. This suggests a
20 complex role for these miRNAs in regulating apoptosis, which is a significant process in the pathophysiology of
21 epilepsy. Overall, this study sheds light on the intricate involvement of specific miRNAs in various cellular processes
22 related to epilepsy, further enhancing our understanding of the molecular mechanisms underlying this disorder (37)

23 24 **Conclusion**

25 The advancement of molecular science has provided valuable insights into the understanding of epilepsy. The
26 pathophysiology of epileptogenesis, the process leading to epilepsy, is intricate and involves the interplay of various
27 factors such as neurotransmitters, ion channels, receptors, neurogenesis, oxidative stress, inflammation, apoptosis,
28 hyperactivation of the mTOR signaling pathway, and the regulation of numerous genes and proteins. Epileptic seizures
29 arise from a sudden imbalance between excitatory and inhibitory neurotransmitters. Inflammation and oxidative stress
30 can occur both prior to and following epileptic convulsions. Disruption of calcium ion homeostasis due to
31 excitotoxicity can lead to cell death through apoptosis or necrosis pathways. The process of epileptogenesis, marked
32 by diverse structural, neurochemical, and cellular changes, is also influenced by hippocampal neurogenesis. Further
33 exacerbating brain damage and increasing the risk of recurrent epileptic seizures are glial cell alterations and
34 modifications to the BBB. Recent research has highlighted the role of specific microRNAs (miRNAs) such as miR-
35 146a, miR-134, miR-132, miR-128, miR-34a, miR-324-5p, miR-124, miR-155, miR-142-5p, miR-21-5p, miR-187,
36 and miR-106b-5p in epileptic disorders. However, a deeper investigation is needed to fully understand the individual
37 contributions of each miRNA to epilepsy. Encouragingly, emerging evidence supports the potential of utilizing
38 microRNA-induced silencing as a therapeutic target in epilepsy. Addressing the challenges in pinpointing the target
39 and cell specificity of miRNAs and developing safe and effective strategies to modulate miRNAs will be pivotal in
40 translating these promising findings into clinical applications for the treatment of epilepsy or the prevention of
41 epileptogenesis.

42 43 **Abbreviations**

44	AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
45	ATP	Adenosine Triphosphate
46	BBB	Blood-Brain Barrier
47	cAMP	Cyclic Adenosine Monophosphate
48	ESAM	Endothelial Selective Adhesion Molecules
49	FCD	Focal Cortical Dysplasia
50	GABA	γ -Amino Butyric Acid
51	HCN	Hyperpolarization-Activated Cyclic Nucleotide-Gated Channels
52	HMGB1	High-Mobility Group Box 1
53	ICE	Interleukin-1 β Converting Enzyme
54	ICER	Inducible cAMP Early Repressor
55	IL-1	Interleukin-1
56	IL-6	Interleukin-6
57	IL-IRI	Interleukin-1 Receptor Type I
58	JAMs	Junctional Adhesion Molecules
59	LD	Lafora Disease

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LVA	Low-Voltage-Activated
mTOR	Mammalian Target of Rapamycin
NKCC1	Na-K-2Cl
NMDA	N-Methyl-D-Aspartate
NMDAR	N-Methyl-D-Aspartate Receptors
PDS	Paroxysmal Depolarization Shifts
Pgp	P-glycoprotein
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase- α
PTEN	Phosphatase and Tensin Homolog
SE	Status Epilepticus
STRADA	STE20-Related Kinase Adaptor- α
TNFR1	Tumor Necrosis Factor Receptor 1
TSC	Tuberous Sclerosis Complex

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Authors' contributions

All authors participated in the writing of the manuscript. KTS prepared the figures and conceptualized the content scope of the article. SHWrote, Reviewed, and Edited the Manuscript. DCT proofread and edited the final manuscript. The author(s) read and approved the final manuscript.

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Ethics approval and consent to participate

Ethics approval and consent to participate is not applicable in this study.

Consent for publication

Consent for publication is not applicable in this study.

Competing interests

All authors declare that there is no competing interests

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REVIEW

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Biomolecular mechanisms of epileptic seizures and epilepsy: a review

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Abstract

Epilepsy is a recurring neurological disease caused by the abnormal electrical activity in the brain. This disease has caused about 50 new cases in 100,000 populations every year with the clinical manifestations of awareness loss, bruising, and mobility abnormalities. Due to the lack understanding of the pathophysiology behind the illness, a wide variety of medications are available to treat epilepsy. Epileptogenesis is the process by which a normally functioning brain undergoes alterations leading to the development of epilepsy, involving various factors. This is related to the inflammation which is driven by cytokines like IL-1 and tumor necrosis factor- α (TNF- α) leads to neuronal hyperexcitability. Pro-inflammatory cytokines from activated microglia and astrocytes in epileptic tissue initiate an inflammatory cascade, heightening neuronal excitability and triggering epileptiform activity. The blood-brain barrier (BBB) maintains central nervous system integrity through its tight endothelial connections, but inflammation impact BBB structure and function which leads to immune cell infiltration. The mammalian target of rapamycin (mTOR) pathway's excessive activation influences epileptogenesis, impacting neuronal excitability, and synapse formation, with genetic mutations contributing to epilepsy syndromes and the modulation of autophagy playing a role in seizure onset. The apoptotic pathway contribute to cell death through glutamate receptor-mediated excitotoxicity, involving pro-apoptotic proteins like p53 and mitochondrial dysfunction, leading to the activation of caspases and the disruption of calcium homeostasis. Ionic imbalances within neural networks contribute to the complexity of epileptic seizures, involving alterations in voltage-gated sodium and potassium channels, and the formation of diverse ion channel subtypes. Epileptogenesis triggers molecular changes in hippocampus, including altered neurogenesis and enhanced expression of neurotrophic factors and proteins. Oxidative stress leads to cellular damage, disrupted antioxidant systems, and mitochondrial dysfunction, making it a key player in epileptogenesis and potential neuroprotective interventions. Thalamocortical circuitry disruption is central to absence epilepsy, the normal circuit becomes faulty and results in characteristic brain wave patterns.

Keywords Epileptic seizures, Epilepsy, Epileptogenesis, Molecular mechanism

Introduction

Epilepsy is one of the most prevalent neurological conditions that is caused by the abnormal electrical activity in the brain. It is a chronic, paroxysmal, and recurring disease marked by neurobiological, cognitive, psychological, and social factors that collectively contribute to recurrent seizures, alongside enduring risk factors for epileptic episodes [1, 2]. Epilepsy may significantly reduce quality of life, as it limits daily activities, social contacts, and even work chances. Moreover, this disease has caused about

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50 new cases in 100,000 populations every year. About 75% of these cases start in childhood indicating the increased vulnerability of brain development to experience seizures [1, 2]. Clinical signs of epilepsy include loss of awareness, mobility abnormalities, sensation (vision, hearing, and taste), mood or cognitive capacities, bruising, and the danger of mortality. A study showed that medication will control the seizures of around 60% to 70% of persons with epilepsy. The general likelihood of epilepsy recurring after experiencing a solitary seizure ranges from 27-71%. Roughly 70% of children will enter a phase where they experience a span of at least 2 years without encountering a seizure. Due to the lack of a complete understanding of the pathophysiology behind the illness, a wide variety of medications are available to treat epilepsy. This circumstance results in a variety of distinct pharmaceutical agents to treat it. Epilepsy characterized by epileptic seizures starts in the gray matter of cortex or subcortex. Epileptic seizures results from an abrupt imbalance between excitation and inhibition in the network of cortical neurons. It starts when neurons exhibit high, coordinated, and sustained neuronal excitability activity.

The term hypersynchrony and hyperexcitability are two different words found in the epilepsy pathophysiology with a related significance among them. The abnormal reactivity of neurons to stimulation input is referred to as hyperexcitability. This condition happens when neurons react too strongly when they receive signals from other cells causing excessive firing of nerve cells in the brain. Neurons commonly start more than two or one electrical activity, which is known as hypersynchrony. This involves several neighboring neurons in aberrant electrical bursts. In this case, a small number of neurons first undergo abnormal stimulation. The normal inhibitory ion exchange and membrane conductance of the synaptic gap are altered, which raises neuronal excitability and synchronization and results in an epileptic seizure. Hyperexcitability is typically brought on by modifications to intrinsic neuronal components and synapse function. According to developments in molecular genetics, several distinct types of human epilepsy are considered to be brought on by mutations in ion channel genes [3–8]. This article aims to comprehensively delve into the intricate molecular underpinnings that give rise to epileptic seizures and the overarching epileptic condition, thereby contributing to a more profound understanding of the etiological complexities inherent to epileptogenesis.

Epileptogenesis

Epileptogenesis refers to the process through which a normal brain develops alterations that contribute to the formation of epilepsy, alluding brain transformation that

was previously functioning normally into a brain that experiences seizures. Thus far, the definition of epileptogenesis remains a topic of ongoing discussion and lacks a universally accepted characterization. This considered involving two processes, including the development of epileptiform processes after brain injury and the process leading to the first spontaneous epileptic seizure. Research shows that neurological alterations during the latent period persist long after epilepsy has been identified and aid in the disease's development, it means that the mechanisms involved in both starting and developing epilepsy are counted as part of epileptogenesis. The subsequent development or changes that occur following epileptogenesis is called epileptic maturation. The processes of starting epilepsy and what happens afterward are different from each other, it involves the establishment of enduring alterations in neural circuitry and excitability, also often characterized by an increased propensity for recurrent seizures and the manifestation of clinical epileptic symptoms [4]. Prior to the occurrence of the epileptic seizure, the gradual changes of epileptogenesis occurs in neuronal excitability led to changes in interconnections and structure. This include the neurodegeneration, neurogenesis, gliosis, axonal damage or sprouting, dendritic plasticity, breakdown of the BBB, infiltration of inflammatory cells into the brain parenchyma, re-organization of the extracellular matrix, and re-organization of the molecular architecture of individual neurons [9].

Excitability also occurs in the membrane or metabolic components changes of individual neurons. It includes the itogenesis from specific neurons, neuronal surroundings, and neuronal populations. This results from the suboptimal environmental control, extracellular ion concentration, and neurotransmitters. A neuron can develop into a population of hyperexcitable neurons through anatomical and physiological modifications [5, 10]. By blocking synaptic and voltage-gated inhibitory conductance or activating synapse and voltage-gated excitatory conductance, epileptic activity can be acutely produced. However, it differs from acute seizures in persistent epileptic seizures which are unexpected and less often. In chronic epilepsy, a second mechanism is required to account for the timing of the episodic change from regular activity to an epileptic seizure.

The biological processes, structural changes, and functional alterations play a crucial role in epileptogenesis. The fundamental physiology of an epileptic seizure includes the instability of the cell membrane or the surrounding or neighboring supporting cells. A contributing factor to cell membrane instability involves the deficiency of adenosine triphosphate (ATP) acetate associated with ion transport or anomalies in

potassium ion conductance. Some examples of neurotransmitters that can boost excitability and expand neuronal activity are glutamate, aspartate, acetylcholine, norepinephrine, histamine, corticotropin-releasing factor, purines, peptides, cytokines, and steroid hormones. At the same time, dopamine and γ -amino butyric acid (GABA) restrict neuronal activity and growth. During an epileptic seizure, there is an increased blood flow requirement to the brain, facilitating the transportation of carbon dioxide and metabolic substrates essential for neuronal activity. Various forms of epilepsy are associated with mutations in a multitude of genes. It encompasses several types that are correlated with genes encoding protein subunits of voltage-sensitive and ligand-activated ion channels [4, 8].

Epileptic seizures and epilepsy arise from a multitude of causes and sustained through a process of positive reinforcement, where an initial imbalance between neural inhibition and excitation triggers further imbalances. The initial triggering activity not only initiates the seizure but also fosters ongoing neural excitation while simultaneously inhibiting the natural restraining mechanisms. This self-perpetuating cycle is facilitated by positive feedback, effectively prolonging the epileptic seizure once it has been initiated [11]. Epileptogenesis is influenced by factors including oxidative stress, neurochemical alterations in the brain due to neurotransmitters and ion channels, fluctuations in ion concentration, variations in cell surface receptors, and the presence of inflammation. Some targets in epileptogenesis include the mammalian target of rapamycin (mTOR), P-glycoprotein (P-gp), and mutations in voltage-gated ion channels like hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and Kv7 channels (or M-channels), Na-K-2Cl cotransporter isoform 1 (NKCC1) [8, 12].

The role of inflammation in epileptic seizure and epilepsy

Inflammation indeed plays a significant role in the progression of epileptogenesis. It reduces the threshold for the initiation of epileptic seizures, and it becomes further exacerbated under conditions of SE [8]. Death of neuronal cells, malfunction of synapses, and the onset of hyperexcitability are all symptoms of brain injury brought on by concussions, hypoxia, or febrile convulsions. The release of cytokines, chemokines, lipid mediators, and proteases into the neuronal environment indicates that brain trauma has caused an organized cascade of biological processes. Depending on the part of the brain that is affected, dysregulation of these mediators and the expression of their receptors might result in clinical symptoms that include permanent neuronal injury [13] (Fig. 1).

Brain inflammation in people with epilepsy

Epileptogenesis is closely linked to high degree of inflammation in the neural tissue's milieu both acutely and persistently. The central nervous system may be the source of the inflammatory response with possible damage to the BBB. This inflammation can spread from the central nervous system into the systemic circulation, and exacerbations of pre-existing chronic neuroinflammation may also occur as a result of acute neuroinflammation [13]. Small amounts of the cytokine interleukin-1 (IL-1) are produced by activated astrocytes and microglia and expressed in the human central nervous system, these levels are increased during pathogenic processes. These cytokines cause astrocytes to consume less and release more glutamate, resulting in neuronal hyperexcitability. IL-1 is thought to cause epileptic seizures by increasing N-methyl-D-aspartate (NMDA) receptor expression in post-synaptic cells through activation of the GluN2B subunit of the NMDA receptor [13–15].

Active astrocytes and microglia emit the pro-inflammatory cytokine tumor necrosis factor- α (TNF- α). Several research shows that TNF- α controls N-cadherin which is essential for the development and structure of excitatory and inhibitory synapses. TNF- α also boosts glutaminase production at gap junctions, which in turn causes microglia to release more glutamate. TNF- α also increase the the expression of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which amplifies glutamatergic transmission and results in excessive calcium ion influx and neurotoxicity. It also triggers GABA receptor endocytosis which reduces the inhibitory excitability, and results in significant alterations in excitability [13–15]. Another multifunctional cytokine that will increase along with the increment of TNF- α , IL-1 β , and interferon- γ (IFN- γ) is the interleukin-6 (IL-6) which controls inflammation as immunological responses. The production of IL-6 in the central nervous system remains in low level. However, it can be boosted by the activation of astrocytes and microglia. The increasing levels of IL-6 expression reduces long-term potentiation (LTP), inhibits hippocampus neurogenesis, and increases gliosis, which lead to epileptogenesis [13–15]. The innate and acquired immune systems are activated in patients with epilepsy. Analysis of brain samples from patients with refractory epilepsy demonstrated heightened expression of IL-1 β and high-mobility group box 1 (HMGB1), along with their corresponding receptors, namely interleukin-1 receptor type I (IL-1RI) and TLR4, in both glia cells and neurons. This clarifies that patients with epilepsy experience activation of inflammatory mechanisms [8, 13].

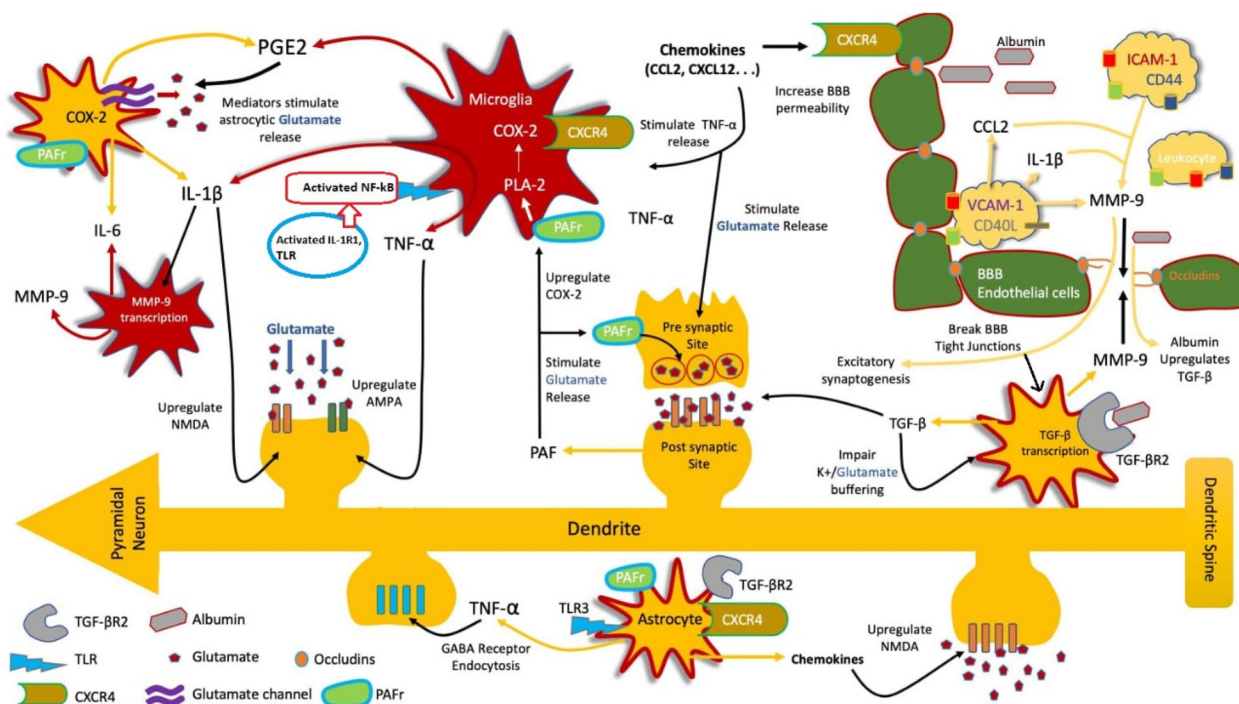


Fig. 1 The role of inflammation in epileptic seizure and epilepsy. A cascade of events unfolds after brain injury that triggers central inflammation and alters normal neuronal connections in the hippocampus. Meanwhile, systemic inflammatory diseases cause inflammation in the peripheral tissues, accelerating the accumulation of inflammatory agents. This dual inflammation, both in the peripheral and central systems, contributes to the weakening of the protective BBB by enhancing the levels of inflammatory agents. This compromised barrier permits the infiltration of immune cells, initiating heightened neuronal activity and further amplifying the production of inflammatory agents. The uncontrolled inflammation in both peripheral and central regions, coupled with the compromised blood-brain barrier, sets the stage for structural changes in synaptic connections within the hippocampus. These processes culminate in the progression of epilepsy. Adapted from Rana and Musto [13]

Targets and sources of cytokines and inflammatory mediators in epileptic tissue

Microglia and astrocytes as glial cells and non-neuronal parts of the central nervous system are the source of pro-inflammatory cytokines in epileptic tissue. In epileptogenesis, glial cells control inflammatory and immunological responses, it causes a rapid inflammatory response in epileptic seizures caused by chemo seizures or electrical stimulation. Within the first 30 minutes of an epileptic seizure, activated astrocytes and microglia trigger heightened levels of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α. This process initiates an inflammatory cascade that involves neurons and BBB endothelial cells, ultimately leading to the activation of the acquired immune system. This cascade includes the activation of NF-κB, COX-2, complement system, chemokines, and acute phase proteins. These cytokines have the potential to influence susceptibility to stimuli that trigger epileptic seizures, as well as contribute to the increased IL-1β levels resulting from epileptic convulsions. The initiation of this signaling cascade exacerbates epileptic seizures. The conversion of IL-1β into its active form is facilitated by the enzyme IL-1β converting enzyme (ICE/caspase-1).

Considering this, exploring ICE/caspase-1 inhibitors as potential targets for antiseizure medications holds promise [4, 8, 12].

Rapid HMGB1 release from neurons, microglia, and astrocytes after pro-convulsant-induced brain injury was also shown to significantly influence the onset of brain inflammation and lower the threshold for epileptic seizures. Toll like receptor (TLR) signaling also plays a significant role in astrocytes and neurons. Injured or stressed cells emit danger signals to warn the surrounding environment of impending or ongoing damage, including in the HMGB1. The innate immune response and accompanying inflammatory pathways are activated in the tissue due to the interaction between HMGB1 and its cognate TLR4 [4, 8, 12]

The role of cytokines on neuronal excitability

Inflammatory cytokines play a role in changing neuronal excitability, producing toxic mediators, and making the BBB more impermeable. The proconvulsant effects are produced by NMDA receptors and IL-1β. Specifically, IL-1β can phosphorylate the NR2B subunit of the NMDA receptor via IL-1RI-dependent activation of Src kinases

and neuronal sphingomyelinases. This phosphorylation amplifies NMDA-mediated calcium ion influx into neurons, thereby inducing neuronal hyperexcitability. Activated astrocytes release glutamate, which serves as the primary source, initiating a gradual influx of ions into neurons. This process involves NMDA receptors that incorporate the NR2B subunit. This influx of ions has a direct impact on excitatory events, exerting a substantial influence on neural synchronization and potentially giving rise to action potentials within neurons.

An essential biological mechanism that intensifies epileptic seizures involves the protein IL-1 β . Furthermore, IL-1 β has the capacity to reduce glutamate absorption by astrocytes, elevate glutamate release from glial cells through TNF- α , and induce hyperexcitability. The presence of IL-1 β also leads to an inhibition of glutamate uptake by nitric oxide. Similar to IL-1 β , TNF- α might boost the expression of AMPA receptors, facilitating the entry of calcium ions into neurons, while concurrently diminishing the expression of GABA receptors, thereby weakening the potency of inhibitory synapses. The signaling cascade mechanism governing glutamate exocytosis from astrocytes involves the participation of tumor necrosis factor (TNF- α). This process engages tumor necrosis factor receptor 1 (TNFR1) activation within astrocytes, thereby amplifying the signaling cascade responsible for glutamate release. This mechanism operates through the stimulation of CXCR4-dependent signaling in both astrocytes and microglia. Furthermore, it works synergistically to facilitate the release of TNF- α [4, 8, 12].

Inflammatory cytokines can lead to neuronal death through apoptosis, operating independently from excitotoxicity. This effect is potentially facilitated by the release of neurotoxic mediators. Additionally, glutamatergic excitotoxicity, which involves AMPA and NMDA receptors, can also contribute to neuronal demise. Inflammatory responses can also change the permeability of BBB. When BBB is damaged, astrocytes take up serum albumin. Within astrocytes, this albumin binds to TGF- β receptors, initiating a sequence of events that culminate in neuronal hyperexcitability and epileptiform activity. The impairment of the BBB leads to increased abnormal neuronal depolarization processes. The breach in the BBB allows leukocytes to enter the brain parenchyma and proteins to leak from the serum. These exogenous inflammatory mediators collectively reduce the threshold for epileptic seizures. This scenario underscores the significant impact of inflammatory processes on epileptogenesis within the brain [4, 8, 12]. The pathophysiology of epileptic seizures involves the adherence of inflammatory cells. After a pilocarpine hydrochloride-induced epileptic seizure, there is an increase in the production of vascular

cell adhesion molecules (VCAM) and an increase in leukocyte adherence to endothelial cells in cerebral blood arteries, which is mediated by leukocyte mucin P-selectin glycoprotein ligand-1 and leukocyte integrins. Consequently, escalated leukocyte extravasation, brain inflammation, BBB leakage, increased neuronal excitation, and ultimately, the onset of epileptogenesis ensue [4].

Peripheral inflammation has been correlated with the stimulation of gene transcription through NF- κ B, an unconventional intracellular signaling pathway activated by pro-inflammatory mediators within epileptogenic tissue. The recognition of this distinct mechanism provides insights into the genesis of epileptic seizures driven by neuronal hyperexcitability. This pathway regulates a subset of interactions between neuronal and glial elements that contribute to the reduction in the threshold for epileptic seizures [8, 16]. When pro-inflammatory molecules activate other kinase families, such as mitogen-activated protein kinase (MAPK), protein kinase A (PKA), and protein kinase C (PKC), voltage-dependent calcium, sodium, and potassium ion channels undergo rapid post-translational changes that have a significant effect on neuronal excitability. Increased extracellular glutamate levels are caused by IL-1 promoting glutamate release by glial cells and decrease glutamate absorption by astrocytes through the generation of TNF- α . Furthermore, the amplified glutamatergic transmission, a consequence of IL-1 β produced by both neurons and astrocytes, yields pro-convulsant effects. Recent findings emphasize the critical role of astrocytic glutamate release in shaping the initiation and severity of epileptic convulsions. Additionally, IL-1 impedes inhibitory transmission by blocking GABA-mediated chloride ion influx. The presence of inflammatory compounds in the brain could also incite enduring transcriptional processes, thereby activating genes integral to the plasticity mechanisms underlying epileptogenesis [8, 16].

The role of P2X7 receptors in epilepsy

The P2X7 receptor is a cation-permeable ATP-gated non-selective ionotropic receptor expressed only by neurons and glia cells in the brain. Neuronal excitability in the hippocampus was shown to be modulated by activation of this receptor, which is also linked to microglia activation and neuro-inflammatory reactions. These receptors play a role in the neurological system disorders, such as epilepsy. P2X receptors are ligand-gated ion channels that ATP activates, and activating these receptors transfers signals between neurons and glia cells. Potassium ion concentration in the extracellular space rises during an epileptic episode. The Pannexin1 (Panx1) channels of hippocampal neurons and astrocytes activate in response to this alteration, releasing

ATP. Neuronal hyperactivity brought on by the activation of ATP receptors leads to the onset of an epileptic seizure. Adenosine, as opposed to ATP, exhibits anti-epileptic action via P1A1 receptors. These purinergic receptors are inhibited from releasing glutamate when they are activated. Several P2X family receptors, including P2X2, P2X4, and P2X7, are damaged in the hippocampus during status epilepticus (SE). Following SE, a reduction in P2X2 receptor expression was also observed. In contrast, P2X7 receptor expression was higher in glial and neuronal injury regions. After SE, neuronal death is reduced by a lack of P2X4 receptors [12].

The structural, molecular, and functional alterations of BBB in epileptogenesis and epilepsy

BBB, which is impermeable to a wide range of chemicals, toxins, and cells due to the presence of non-fenestrated endothelial cells forming inter-endothelial tight connections, serves as a protective shield for the central nervous system, preventing any immune system reactions. The BBB is composed of both chemical and structural barriers. It comprises neovascular units such as the basement membrane, endothelial cells that line the blood arteries, neurons, astrocyte processes, tight junctions, and pericytes. These components collectively form the structural barrier of the BBB. Nectrin 1, functioning as a laminin-like protein responsible for regulating glial cell motility, is secreted by endothelial cells in response to signals from astrocytes, which concurrently produce the sonic hedgehog protein. In a concerted effort to fortify the BBB and diminish the infiltration of leukocytes, netrin 1 plays a crucial role in enhancing endothelial junctional proteins. These include occludins, claudins, junctional adhesion molecules (JAMs), and endothelial selective adhesion molecules (ESAM). This ensemble of proteins collectively widens the intercellular gaps, constituting the foundational elements of tight junctions and intercellular barriers. Members of the immunoglobulin superfamily, such as ESAM and JAMs, play a pivotal role in both the formation and upkeep of tight junctions. The basement membrane, constituting a foundational structure, embeds components like type 4 collagen, fibrillin, laminin, fibronectin, extracellular matrix proteins, and parasite matrix proteins, which collectively give rise to the basal capillaries or basement membrane. Within the brain capillaries, tight junctions are safeguarded by efflux transporters, forming a chemical barrier. These transporters, operating at the junctions between endothelial cells, necessitate the presence of ATP. Prominent among these are P-gp and breast cancer resistance protein (BCRP), functioning as flux transporters. Their active mechanism involves pumping potentially harmful lipophilic compounds back into the bloodstream, thereby restricting

the assimilation of certain medicinal substances into the brain parenchyma. Furthermore, the chemical barrier of the BBB derives additional strength from the drug-metabolizing enzyme cytochrome P450 (CYP450), which is localized within the endothelial cells of brain capillaries. Studies by Rana & Musto [13] and Löscher & Friedman [17] have underscored these aspects. During epileptic seizures, the release of glutamate, facilitated by NMDA and COX-2 receptors, is accountable for the escalation in P-gp activity [12].

Seizures or the processes underlying them have the potential to impact the BBB, brain capillaries, and the neovascular unit in diverse ways. This can encompass thickening of the basement membrane, alterations in the constituent endothelial cells of the BBB, modifications in the neurons comprising the neovascular unit, and changes in the astrocyte processes forming the glia limitans—a secondary barrier. Epileptogenic brain injury can lead to the leakage of blood albumin and immune cells into the brain parenchyma. Notably, the escalated expression of efflux transporters such as P-gp and BCRP on the apical surface of endothelial cells, along with heightened activity of the enzyme CYP450 on these cells, has been associated with BBB damage. Concurrently, the presence of persistently activated microglia clusters may contribute to the disruption of the BBB. Furthermore, post brain injury, astrocytes undergo alterations in both morphology and function. These changes can collectively lead to a complex interplay of factors influencing the integrity and functionality of the BBB and related systems [17].

The mechanism by which epileptic convulsions impact the BBB remains uncertain. Observations in epilepsy patients and experimental animal models suggest that BBB failure can occur before, during, or after an epileptic seizure. Epileptic convulsions induce temporary changes in both the structural and functional aspects of the BBB. Within 30 min of an epileptic episode, there is a rapid increase in permeability that persists for hours. Elevated levels of glutamate during epileptic episodes disrupt the BBB. The expression and activity of MMP-2 and MMP-9 at the BBB also rise during seizures, contributing to BBB dysfunction. These matrix metalloproteinases influence the integrity of the BBB by altering the extracellular matrix surrounding brain capillaries and degrading the tight junction proteins that line the endothelium. The extracellular matrix, in turn, affects processes like AMPA receptor mobilization, paired-pulse depression, L-type voltage-dependent calcium channel activity, and the long-term potentiation (LTP) process. Changes in the expression of hyaluronic acid, a crucial component of the neuronal extracellular matrix, can lead to dysregulation of calcium ions and neuronal hyperexcitability. Inducing epileptic seizures can also permit ions to enter the

parasite, thereby modifying its function and the constituents of the BBB both *in vitro* and *in vivo*. These defense system breaches, causing epileptic seizures, can establish a positive feedback loop. In this loop, seizures resulting from these breaches lead to a continuation of epileptic episodes, ultimately culminating in epilepsy. The breach of the cerebral blood barrier defense can trigger epileptic episodes and contribute to the development of epilepsy [6, 14, 17].

The BBB serves as a crucial barrier to maintain the peripheral immune cells in a healthy state, preventing their entry into the central nervous system. However, when breached, the BBB can allow both innate and acquired peripheral immune cells, including monocytes, neutrophils, B cells, and T cells, to infiltrate the central nervous system. These immune cells then carry out specific functions that could be either neuroprotective, neurotoxic, or even a combination of both. A significant facet of neuro-inflammation involves the entrapment of these immune cells within the central nervous system through the BBB. This orchestrated process hinges on adhesion interactions between lymphocytes and capillary endothelium. When immune cells come into contact with the activated endothelium, selectins facilitate loose binding, causing the leukocytes to roll along the endothelial surface. Additionally, chemokines present on the luminal surface of the endothelium trigger the activation of leukocytes, leading to the engagement of leukocyte integrins and subsequent halting of leukocyte movement on the endothelial surface. Once the appropriate adhesion occurs, immune cells can traverse into the perivascular space through either of two transmigration processes: the trans-cellular route, where they navigate through the endothelial cell body, or the paracellular route, utilizing the endothelial cleft. Upon infiltrating the perivascular space, these immune cells interact with resident perivascular immune cells before being guided by chemoattractants like chemokines and cytokines toward the brain parenchyma. Employing an enzyme-mediated mechanism, these infiltrating lymphocytes breach the glia limitans and access the brain parenchyma. Once within the brain parenchyma, they become stimulated to produce cytokines, contributing to the cascade of immune responses and inflammation. The BBB serves as a sentinel that regulates the entry of peripheral immune cells into the central nervous system. Breaching the BBB leads to a series of orchestrated events involving adhesion, activation, and migration of immune cells, ultimately culminating in their interaction with brain parenchymal cells and the release of cytokines [17].

Albumin extravasation plays a significant role in both epileptogenesis and the migration of immune cells

across a compromised BBB. The initiation of acute epileptic seizures can be attributed to BBB impairment, and the progression from epileptic episodes to epilepsy is facilitated by the osmotic disruption of the BBB. Once extravasated, albumin enters the brain parenchyma and binds to various cellular components, including neuronal cells, astrocytes, and microglia. This binding triggers a sequence of events, including the entry of albumin into astrocytes through the TGF- β receptors. Consequently, the expression of critical components such as the Kir 4.1 channel, the water channel aquaporin 4 (AQP4), and glutamate transporters is reduced. This alteration results in a decreased ratio of extracellular potassium to glutamate, creating an environment that facilitates NMDA receptors in triggering epileptiform activity and amplifying neuronal hyperexcitability. The impact of TGF- β signaling extends further, encompassing changes in the extracellular matrix, excitatory synaptogenesis, pathological plasticity, and inflammation-related transcriptional alterations. These cumulative effects collectively contribute to the lowering of the threshold for epileptic seizures during the process of epileptogenesis. In essence, albumin extravasation sets in motion a complex cascade of events that involve multiple cellular components and signaling pathways. This interplay ultimately heightens neuronal excitability and paves the way for epileptogenesis and the progression towards epilepsy [17]. Albumin binding to TGF-RII receptors on astrocytes triggers the activation of the TGF- β signaling pathway, leading to the production of TGF- β . This, in turn, activates astrocytes, causing a disruption in the balance between cellular potassium and glutamate levels. Additionally, the ALK5/TGF- β pathway is responsible for inducing excitatory synaptogenesis [13]. In models of epileptogenesis, augmenting TGF- β signaling has been shown to have a preventive effect on the development of epilepsy. Furthermore, another study established a connection between compromised BBBs and irregular neuronal activity [17].

Damage to the BBB triggers gliosis and activation of astrocytes. After inducing SE, astrocytes positive for glial fibrillary acidic protein (GFAP) become activated within a span of 24 to 48 h. Reactive gliosis becomes evident around three to four months following the initial insult. The inability of these reactive astrocytes to effectively manage extracellular glutamate leads to hyperexcitability and neuronal damage. Furthermore, these activated astrocytes release C-C motif chemokine ligands 2, 3, and 5, fostering interactions with other inflammatory cells and giving rise to the production of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α . This cascade contributes to heightened neuronal excitability, the onset of seizures, cell death, and the propagation of neuro-inflammation [13].

The role of mTOR pathway in epileptic seizure and epilepsy

The excessive activation of the mTOR signaling pathway directly influences the progression of epileptogenesis and neuronal excitability. This process involves the phosphorylation of molecules within this pathway. Additionally, the mTOR pathway plays a role in governing the expression of glutamate receptors. Recent research further underscores the potential for disrupted neural signaling, as mTOR contributes to both synapse formation and the mechanisms underlying plasticity [12]. Moreover, mTOR exerts control over the expression of diverse ion channels and neurotransmitter receptors, as well as influencing synaptic plasticity, neuronal morphology, and synaptic transmission [18].

In prevalent causes of hereditary epilepsy such as focal cortical dysplasia (FCD) and tuberous sclerosis complex (TSC), the involvement of the mTOR pathway in epileptogenesis is notably evident. Beyond these conditions, the mTOR pathway plays a role in the development of both inherited and acquired epilepsies, encompassing conditions like Lafora disease (LD), temporal lobe epilepsy, brain injuries, and experimental epileptic states induced by chemically triggered convulsions. Additionally, the mTOR pathway is implicated in systemic lupus erythematosus, an inflammatory disorder that stands as a prominent contributor to epileptic seizures [19]. Genetic mutations impacting various components of the mTOR pathway, such as *TSC1* and *TSC2*, *STE20*-related kinase adaptor- α (*STRADA*), phosphatase and tensin homologue (*PTEN*), AKT isoform predominant in the brain (*AKT3*), phosphatidylinositol-4,5-bisphosphate 3-kinase- α (*PIK3CA*), and mTOR itself, result in the hyperactivity of the mTOR pathway. This hyperactivity, in turn, triggers the initiation of epilepsy syndromes [20–24]. Conversely, the inhibition of mTOR can forestall the onset of epilepsy. The modifications in autophagy driven by mTOR are interconnected with the process of epileptogenesis. Following mTOR hyperactivation, autophagy experiences suppression, and the commencement of spontaneous epileptic seizures is associated with the breakdown of autophagy. Autophagy governs diverse processes including axon guidance, synapse development, dendritic spine architecture and pruning, vesicular release, and synaptic plasticity. It also exercises control over the proliferation and migration of both interneuronal and neuronal cortical progenitors.

Evidence points towards mTOR-mediated autophagy failure giving rise to aberrations in the clustering of GABA_A receptors at synapses, thereby leading to an imbalance between excitation and inhibition—an essential factor in epileptogenesis. Perturbations with irregular activity of the dopamine system additionally

influence the functioning of mTOR-mediated autophagy, contributing to epileptogenesis. This intricate interplay arises from bidirectional communication and shared biochemical pathways, involving cross-reactions between the immune and neurological systems. The connection between mTOR and cell-clearing mechanisms responsible for regulating the metabolic processes of lymphocytes and other immune cells, as well as the antigen-processing capabilities of antigen-presenting cells (APCs) in both the central and peripheral nervous systems, further bolsters this notion. Changes in mTOR-associated cell-clearing processes can disrupt and misalign the communication between the immune and neurological systems [19]. In parallel with its impact on autophagy, epilepsy also exerts effects on the ubiquitin-proteasome system (UPS), a regulatory network governing neuronal excitability, synapse plasticity, and neuro-inflammation/immunity. Disturbances in the UPS within the context of epilepsy are associated with mTOR hyperactivation, akin to the phenomenon observed with autophagy disruption. Recent investigations have elucidated the role of mTOR in orchestrating the convergence of autophagy and UPS morphological pathways, as well as their collective cell-clearing mechanisms. These findings underscore that mTOR-mediated autophagy and UPS activities collectively contribute to epilepsy by influencing brain plasticity and promoting neuroprotection against excitotoxicity. This interplay sheds light on the multifaceted mechanisms through which epilepsy shapes neurological processes and underscores the significance of mTOR regulation in these contexts [19].

Autophagy operates at the interface of the GABA, dopamine, and glutamate systems, contributing to the molecular mechanisms underlying epileptogenesis and the neuronal changes prompted by epileptic convulsions. In instances of autophagy failure, heightened p62 levels conceal the surface presentation of GABA_A receptors, leading to a reduction in GABA signaling. Furthermore, the degradation of NMDAR and AMPAR glutamate receptors no longer takes place, resulting in irregular glutamate signaling and an influx of calcium ions. This disarray disrupts the delicate equilibrium between excitatory and inhibitory neurotransmission pivotal to epileptogenesis. On the dopaminergic front, the role of autophagy extends to curtailing dopamine release through the disintegration of synaptic vesicles at the dopamine terminal. In cases where autophagy falters, anomalous dopamine release ensues, accompanied by the stimulation of dopamine D1 receptors. This, in turn, exacerbates autophagy suppression through mTOR hyperactivation. This detrimental cycle leads to the accumulation of damaged cellular components, which

combine with glutamate-associated excitotoxicity, ultimately culminating in neuronal demise [19] (Fig. 2).

The role of apoptotic pathway in epileptic seizure and epilepsy

Beyond its pivotal role in neuronal development, differentiation, and synaptic plasticity [26, 27], glutamate receptor-mediated excitotoxicity is also recognized as a major mechanism contributing to cell death in various disorders of the central nervous system (CNS).

These include conditions like brain injury, neurodegenerative diseases, and epilepsy [28]. Elevated levels of glutamate receptor activity have the potential to induce cell damage and subsequent death through mechanisms involving apoptosis or autophagy. As a consequence of heightened glutamate receptor activity, the expression of pro-apoptotic proteins such as p53 is triggered [29–31]. This activation of pro-apoptotic proteins is often prompted by immediate excitotoxic injury [32, 33].

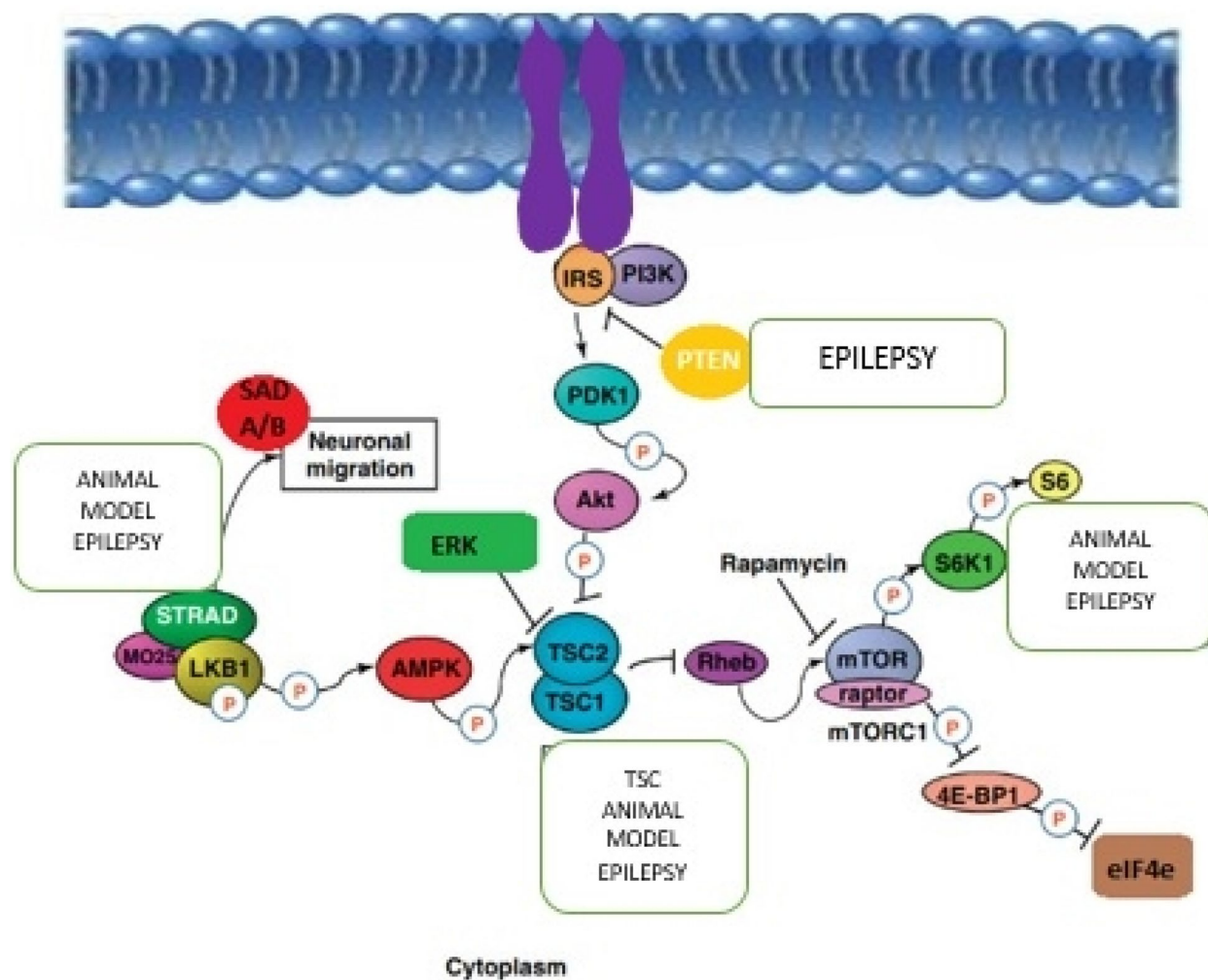


Fig. 2 The role of mTOR pathway in epileptogenesis. Dysmorphic neurons and balloon cells, which lack dendrites or axons, undergo alterations in neurotransmitter receptor subunit expression and uptake site distribution in conditions like TSC, FCD, and hemimegalencephaly (HME). Notably, changes are prominent in GluR, NMDAR, and mGluR subtypes. The direct causation of these alterations—whether they arise from heightened mTOR signaling, tissue cytoarchitecture changes, or adaptations stemming from recurrent seizures—remains uncertain. The accompanying diagram illustrates various locations where dysregulation of mTORC1 (influenced by both phosphoactivation and phosphoinhibition effects) is implicated in human neurological disorders collectively referred to as “mTORopathies,” which encompass epilepsy. Components such as PTEN, STRADA, TSC1/TSC2, and S6K1 might play roles in this context. Experimental animal models involving these proteins also exhibit compromised seizure susceptibility and/or deficits in social and behavioral functions. Rapamycin and other mTOR inhibitors emerge as primary potential therapeutic agents for modulating mTORC1 signaling. These interventions hold promise for regulating the mTOR pathway, offering prospects for addressing the underlying mechanisms of mTOR-related neurological disorders, including epilepsy [21, 25]. Adapted from Crino [21]

Excessive activation of glutamatergic receptors or their analogs leads to a phenomenon known as excitotoxicity, which ultimately results in cell death. When these receptors experience an overstimulation, an excessive influx of calcium ions (Ca^{2+}) occurs, with a significant portion becoming sequestered within mitochondria. Consequently, cellular metabolism becomes impaired, giving rise to the generation of free radicals. This excessive calcium influx also triggers the activation of enzymes like proteases, phospholipases, endonucleases, and nitric oxide synthase. Furthermore, the process inhibits protein synthesis, exacerbating the cascade of detrimental events within the affected cells [34]. Disruption of calcium homeostasis leads to an accumulation of calcium within the mitochondrial matrix. This accumulation has the effect of depolarizing the mitochondrial membrane through the activation of mitochondrial transition pores, which normally function to redistribute calcium back to the cytosol. This process unfolds in two primary steps. Initially, the elevation in positive ion concentration within the mitochondrial matrix diminishes the chemosmotic potential across the membrane. This reduction in potential subsequently slows down the rate at which ATP synthesis occurs. In the second step, irreversible damage takes place at the mitochondrial membrane. This disruption in calcium regulation and the ensuing impact on mitochondrial function represent critical components of the excitotoxic cascade [35, 36].

The complexity of neuronal cell death associated with neurodegeneration is not mitigated by neuronal cell death triggered by epileptic seizures. However, the precise nature of neuronal cell demise, whether through necrosis or apoptosis, remains a subject of debate. While necrosis is frequently cited as the principal cause of neuronal cell death following epileptic seizures and is widely considered the predominant mechanism, the discussion persists. Biochemical investigations have unveiled the involvement of the B-cell lymphoma 2 (Bcl-2) family and caspases in the cell death process during epileptic seizures. Consequently, the potential contribution of apoptosis cannot be dismissed. Indicative of apoptosis, DNA fragmentation and activation of endonucleases have been identified in the cell death program. Additionally, there is evidence of p53 accumulation in the nucleus in tandem with increasing concentrations of cell death receptors and ligands. These indications point to the involvement of apoptotic pathways in contributing to cell death [37, 38] (Fig. 3).

The *p53* gene emerges as the initial apoptotic regulator disrupted by an epileptic episode. Subsequent to an epileptic seizure, the process involves the binding of p53 to DNA. This is accompanied by heightened expression of Bcl-2-associated X (Bax) protein, which in turn leads

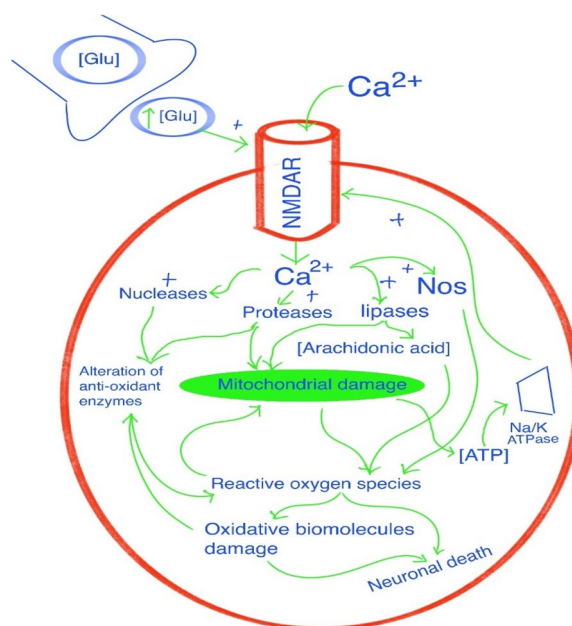


Fig. 3 The mechanism of excitotoxicity. The sustained activation of N-methyl-D aspartate receptors (NMDAR) due to excessively high concentrations of glutamate (Glu) results in a substantial influx of calcium into the cell. This influx, in turn, triggers the activation of lytic enzymes and nitric oxide synthase (NOS). Concurrently, heightened levels of arachidonic acid and mitochondrial impairment foster the generation of reactive oxygen species, initiating a cascade of biomolecular damage. Ultimately, these processes lead to the activation of apoptotic death programs within cells. The impact of energy shortages compounds the degenerative process. Energy deficits prolong membrane depolarization by impairing the energy supply to the sodium/potassium pump (Na/K ATPase). This, in turn, maintains NMDAR in an active state, rendering the cell more susceptible to the typical glutamatergic inputs from the cerebral cortex. Adapted from Lorigados et al [38]

to increased levels of both mRNA and protein. Protection against excitotoxicity induced by kainic acid was observed when inhibitors of p53 production were administered. Studies involving p53-deficient mice's neurons indicated heightened resilience to apoptosis induced by excitotoxicity and epileptic seizures. Given the multifaceted roles of p53, it remains unclear precisely how p53 is altered in epileptic seizures that trigger cell death. However, the prevailing evidence predominantly points toward caspases, Bcl-2, and p53 as integral components of the pathways underlying neuronal cell death induced by epileptic seizures [38].

In mammals, the regulation of the apoptotic process revolves around two principal gene families: caspases and the Bcl-2 family of proteins. The caspase family, composed of caspases 2, 8, 9, and 10 as initiators, and caspases 3, 6, and 7 as executors, serves pivotal roles within the apoptotic pathway. On the other hand, the Bcl-2 family of proteins exerts crucial control over whether a

cell will survive or undergo apoptosis. This family can be categorized into three distinct types of proteins, each of which contributes uniquely to the apoptotic process. The first group encompasses anti-apoptotic proteins featuring four Bcl-2 homology (BH) domains: BH1-4. The second category, effector proteins Bax and Bak include BH1-4 domains and. The third set is marked by the presence of the BH3-only domain (Bid, Bad, Bim, Bik, etc). Among these, the BH3-only proteins and effector proteins of the Bcl-2 BH family act as pro-apoptotic factors. The anti-apoptotic Bcl-2 family proteins such as Bcl-2 and Bcl-XL bind to the pro-apoptotic protein Bax/Bak, inhibiting their capacity to form mitochondrial membrane pores. Conversely, pro-apoptotic proteins like Bid, Bad, Bim, and Bik detach Bax/Bak from the Bcl-2-Bax/Bak or Bcl-XL-Bax/Bak complex, thereby initiating Bax translocation to the mitochondrial membrane and facilitating pore formation. Upon release from the anti-apoptotic protein connection, Bax/Bak dimerizes and remains in the active mitochondrial membrane. Subsequently, the creation of mitochondrial pores leads to the release of crucial proteins like cytochrome C, pivotal for triggering apoptosis through the mitochondrial pathway. Alongside caspases and Bcl-2 family proteins, other prominent players, including p53, TNF, Fas ligand, and NF-κB, play significant roles in orchestrating the cell death pathway [4].

The process of excitotoxicity involves the hyperactivation of glutamatergic receptors and their analogs, leading to cell death (Fig. 4). The excessive influx of calcium ions into the cell, as a result of this hyperactivation, accumulates within mitochondria. This accumulation triggers a chain of events including metabolic dysfunction, the generation of free radicals, activation of enzymes like proteases, phospholipases, endonucleases, and nitric oxide synthase, as well as the inhibition of protein synthesis. Additionally, disruptions in calcium ion homeostasis

stem from malfunctions in the calcium ion pump, sodium and calcium ion exchange mechanism, and calcium-balancing proteins. When this delicate balance is perturbed, surplus calcium ions amass in the mitochondrial matrix. There are two primary mechanisms through which this buildup occurs. Firstly, an elevated concentration of positive ions within the mitochondrial matrix reduces the chemo-osmotic potential across the membrane, slowing down the rate of ATP synthesis. Secondly, the activation of the mitochondrial transition pore aims to transport calcium ions back to the cytosol. However, if the returned calcium ions are not effectively cleared, it results in irreversible membrane depolarization. The heightened concentration of calcium ions in the mitochondrial matrix further fosters the generation of free radicals. This, in turn, leads to lipid membrane peroxidation, nitric oxide production, and the activation of enzymes involved in the breakdown of proteins, phospholipids, and nucleic acids. Notably, nitric oxide can also act as a retrograde messenger, amplifying glutamate release from the terminal pre-synapse, thereby exacerbating its excitotoxic effects [38].

Ionic environment modifications causing epileptic seizures and epilepsy

While certain forms of epileptic seizures might originate in subcortical regions, the majority of such seizures stem from the cortical and hippocampal structures. Various factors, including the site of disruption, the timing and duration of the electrical bursts, and the associated clinical manifestations, collectively contribute to the complexity of epileptic seizures. Beyond instigating abnormal activity within individual neurons, alterations in excitability, intrinsic to epileptogenesis, encompass networks of hyperexcitable cells. These cells function cohesively, demonstrating a high degree of coordination via both functional and potentially dysfunctional pathways [39].

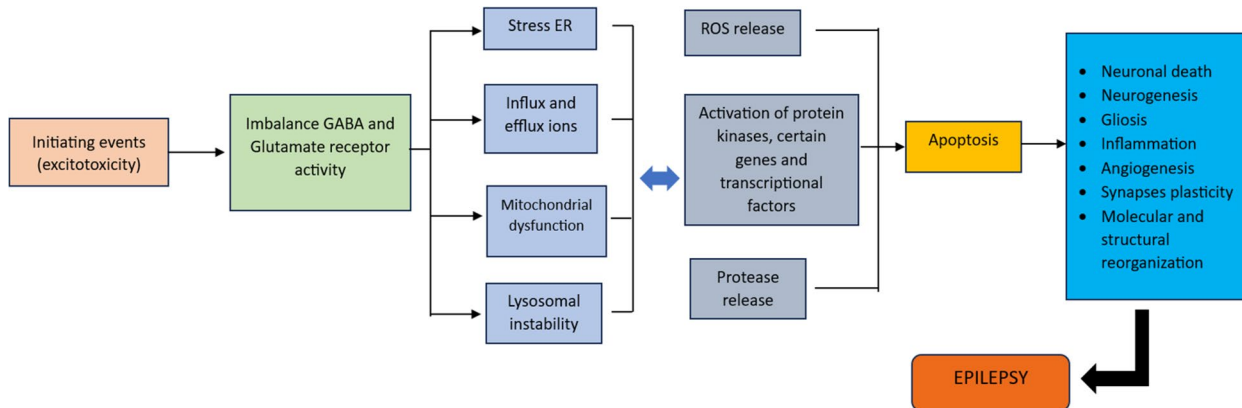


Fig. 4 The mechanism scheme of excitotoxicity induces epilepsy. Adapted from Lorigados et al [38]. ER: endoplasmic Reticulum, ROS: reactive oxidative stress

Upon membrane depolarization, voltage-gated sodium channels are activated, leading to a pivotal shift in the action potential phase. Concurrently, various voltage-gated potassium channels, integral to the repolarization phase, initiate their activity. Following their activation, sodium ion channels transition into an inactive state due to the ball-and-chain mechanism, which closes the channel pore, effectively halting further sodium ion passage. Notably, as the action potential reaches its zenith, the majority of sodium ion channels are already inactive, with an exception being the persistent sodium channels (I_{NaP}) that remain open. Even this subtle continuation of sodium ion conduction by I_{NaP} channels suffices to propel the membrane past the action potential threshold. This mechanism forms the basis of the intrinsic electrical impulse observed in layer V pyramidal neurons. Consequently, this inherent electrical discharge extends throughout axon branches, fostering synaptic connections with proximate neurons and preserving their synchronized activity [39].

Ion channels are integral proteins that form pores within the lipid membrane of a cell. These pores facilitate the selective passage of specific ions, thereby contributing to the maintain the negatively charged resting membrane potential of cells. These membrane proteins, termed hetero-oligomeric ion channels, consist of two to six subunits. Each subunit features transmembrane segments and differing domain counts, culminating in the creation of diverse channel subtypes. The assembly of these subunits generates a range of channels, each equipped with distinct functionalities. Ion channels are classified as either voltage-gated or ligand-gated, contingent upon how they respond to alterations in the membrane potential. Ligands such as GABA and the neurotransmitter acetylcholine can activate ion channels, serving as prime examples. Cation channels play a pivotal role in initiating the action potential, thus contributing to neuronal excitability. Conversely, anion channels are instrumental in executing inhibitory processes that counteract neuronal stimulation. Disturbances in ion charge equilibrium resulting from anomalies in anion or cation channel function, known as channelopathies, have the potential to trigger epileptogenesis [4, 39, 40].

Sodium ion channels

The movement of sodium ions through the inactivation of fast channel propels the action potential phase upward. This phenomenon is intertwined with the inactivation of the slower sodium ion channel component, a process pivotal in governing membrane excitability. The presence of toxins that hinder the rapid inactivation of sodium ion channels can additionally amplify the inactivation of the slow component. This dynamic shift leads

pyramidal neurons in the neocortex to transition from regular spiking to burst firing. This alteration can be epileptogenic in two distinct ways. Firstly, it can trigger paroxysmal depolarizing shifts (PDS) inducing electrical bursts within the affected neurons. Secondly, it can enhance synchronization stemming from intrinsic electrical bursts in the pyramidal neurons of the neocortex's layer V. As a consequence, a substantial cohort of neurons becomes engaged, thereby laying the foundation for an epileptogenic process to take root [39, 40].

Potassium ion channels

Calcium ion influx triggers the activation of potassium channels, playing a pivotal role in the subsequent repolarization of the cell membrane following excitation. In fact, the calcium ion-dependent flow of potassium ions maintains its distinct contribution to post-action potential hyperpolarization in cells intrinsically stimulated, such as pyramidal neurons within the CA area. This potassium ion movement encompasses the M current, named due to its inhibition by muscarinic acetylcholine receptor activation—an integral mechanism for regulating neuronal excitability. The M current holds significance in governing membrane excitability below the threshold and the ability to respond to synaptic input. Its activation relies on a slow conductance that occurs between -60 and -20 mV. Blocking the M current by muscarinic receptor binding induces a shift in the neuronal membrane towards greater depolarization. Consequently, this alteration can lead to the occurrence of repetitive action potentials interspersed with periods of burst activity. In the context of benign neonatal familial convulsions, genetic factors linked to M current deficiency play a pathogenetic role. This M current is governed by two potassium ion channel subunits, namely KCNQ2 and KCNQ3, and mutations within the genes encoding these subunits—located at chromosome positions 20q13 and 8q24, respectively—contribute to M current disorders that are associated with the phenotype of benign neonatal familial convulsions [39, 40].

Sodium ion channels

The movement of sodium ions through ion channels, facilitated by the fast channel's inactivation, leads to a rise in the action potential phase. This phenomenon is intricately linked to the inactivation of the slow sodium ion channel component, which also exerts a significant influence on membrane excitability. Toxins that impede the fast inactivation of sodium ion channels can additionally intensify the inactivation of the slow component, inducing a shift in pyramidal neurons within the neocortex from regular spiking to burst firing. This alteration can exhibit a proepileptogenic impact through two distinct

pathways. Firstly, it can give rise to PDS-inducing electrical bursts within the affected neurons. Secondly, it has the potential to amplify synchronization resulting from intrinsic electrical bursts in pyramidal neurons within the layer V of neocortex. Consequently, this action can set the stage for an epileptogenic process to ensue, involving a substantial ensemble of neurons [39, 40].

Potassium ion channels

Calcium ion influx initiates the activation of potassium channels, which are vital for the subsequent repolarization of the cell membrane following excitation. Remarkably, the flow of potassium ions governed by calcium ions maintains its unique contribution, distinct from the post-action potential hyperpolarization observed in intrinsically stimulated cells, such as pyramidal neurons within the CA area. Within the realm of potassium ion movement, the M current plays a pivotal role. This name originates from its inhibition by muscarinic acetylcholine receptor activation—a pivotal mechanism for modulating neuronal excitability. The M current is especially adept at controlling membrane excitability beneath the threshold and facilitating responsiveness to synaptic input. This is accomplished through its reliance on activating a slow conductance spanning the range of -60 to -20 mV. In the event that the M current is obstructed due to muscarinic receptor binding, the neuronal membrane experiences an increase in depolarization. This phenomenon can lead to the generation of repetitive action potentials characterized by interposing gaps and burst activity in proximity. The genetic factors associated with M current deficiency are evident in cases of benign neonatal familial convulsions, where such factors have a pathogenetic influence. Regulation of the M current hinges on two subunits of potassium ion channels—KCNQ2 and KCNQ3. Mutations within the genes coding for these subunits (located at chromosomal positions 20q13 and 8q24, respectively) result in M current disorders that are intimately linked to the phenotype of benign neonatal familial convulsions [39, 40].

Calcium ion channels

Calcium ion channels play a crucial role in transient and persistent cell membrane depolarization, driven by their threshold and kinetic activation properties. However, understanding the involvement of calcium ion flux in epileptogenesis poses several challenges. Firstly, due to its essential role in protein synthesis and metabolism, the majority of calcium ions are stored within the endoplasmic reticulum. Manipulating calcium ion concentrations artificially, as often done in experimental setups, can trigger the release of stored calcium ions from their compartments, thereby introducing complex

effects on cell excitability that are further compounded by metabolic influences. Secondly, when assessing the impact of calcium ion concentration on membrane excitability, it's important to consider its role in mediating vesicle fusion in nerve terminals. This fusion process is vital for neurotransmitter release and synaptic function, adding another layer of complexity to the relationship between calcium ions and excitability. Thirdly, intentional reduction of calcium ion concentration can disrupt various types of calcium-dependent potassium currents that contribute to cell excitability. This disruption can lead to indirect and unpredictable outcomes that directly influence calcium ion movement through the cell membrane. This interconnectedness makes it challenging to isolate the precise effects of altered calcium ion concentrations on cellular excitability. Comprehending the role of calcium ion flux in epileptogenesis is intricate due to its multifaceted functions. Manipulating calcium ion concentrations can trigger cascading effects on cell excitability, neurotransmission, and potassium currents, making it challenging to draw definitive conclusions about its contribution to the epileptogenic process [39, 40].

Excitatory amino acid receptors

In the cerebral cortex, the two primary excitatory amino acid neurotransmitters are glutamate and aspartate. These neurotransmitters exert their effects through distinct receptor subtypes. The specific level of ion permeability and the corresponding rates of ion flow are governed by the composition of their respective subunits [41, 42]. Two distinct types of receptors exist: NMDA and AMPA (with kainate and quisqualate subtypes). The ionophores associated with these receptor types allow the permeation of sodium ions, while the NMDA receptor type also permits calcium ion entry. NMDA receptors are blocked by magnesium ions, which remains dependent on the membrane's polarization state. To activate this channel, sufficient depolarization of the membrane must first occur to remove the magnesium blockage. Glutamate binds to metabotropic receptors as well, which serve as second messengers in addition to ionotropic receptors. The processes involving sodium and calcium ions in the AMPA and NMDA components of excitatory postsynaptic potentials (EPSPs) are potent enough to sustain membrane depolarization, significantly extending and amplifying EPSPs. Anomalously formed synaptic connections, resulting from sprouting mossy fibers, can lead to the creation of additional excitatory feedback circuits. This feedback loop augments conductance facilitated by NMDA receptors [39, 43].

GABA receptors

Chloride and potassium ionophores are attributed to the GABAA and GABAB subtypes, respectively, among the primary classes of ionotropic GABA receptors. While it has been proposed that the third receptor subtype, GABAC, may serve physiological functions, this remains uncertain. GABA binding to its receptors induces membrane hyperpolarization and generates inhibitory postsynaptic potentials (IPSPs), accomplished through the entry of chloride ions and the exit of potassium ions. GABA-mediated IPSPs have a notable ability to quell neuronal electrical bursts, primarily due to their connection with reduced input membrane resistance. Local inhibitory circuits comprised of Golgi type 2 GABAergic neurons regulate the main population of pyramidal neurons in the cerebral cortex. In individuals with refractory temporal lobe epilepsy, reduced GABAergic neuron density and impaired GABAergic circuit function have been observed in brain tissue. This underscores the role of GABA-inhibitory abnormalities in the progression of epilepsy [39].

Acetylcholine receptors

Nicotinic acetylcholine receptors hold particular significance due to their presence in the central nervous system. These receptors form pentameric structures composed of three subunits: one subunit from the α group ($\alpha 1 - \alpha 8$) and another subunit from the β , γ , or δ group. Positively charged ions such as sodium, calcium, and potassium flow through the channel to achieve equilibrium. The channel is permeable to these ions without showing selectivity. The equilibrium potential of these ion channels can vary, although it typically hovers around -5 mV. Epileptic symptoms have been observed in individuals with mutations in the *CHRNA4* gene, responsible for encoding the receptor subunit 4, and the *CHRN2*

gene, responsible for encoding the nicotinic acetylcholine receptor 2 subunit. Investigating the precise impact of these mutations on neuronal hyperexcitability is crucial for a comprehensive understanding of their role [39, 40] (Table 1).

Recent advances in genetics and molecular biology have shown the connection between certain epilepsy disorders and mutations in genes responsible for coding ion channel proteins. These mutations can lead to a condition known as channelopathy, characterized by the malfunction or impairment of ion channels, resulting in heightened neuronal excitability [4, 6]. Various forms of familial epilepsy arise from genetic anomalies that disrupt the proper functioning of voltage-gated ion channels. These ion channels play a crucial role in regulating membrane potential, depolarization threshold, and synaptic transmission, all of which contribute to maintaining the normal excitability of cells. Mutations affecting these ion channels can lead to functional changes that result in neuronal hyperexcitability, ultimately triggering epileptic seizures [8]. Channelopathies have a substantial impact on epileptogenesis, particularly in patients with epilepsy, including those with idiopathic forms. Idiopathic epilepsy has been associated with mutations occurring in genes responsible for encoding various types of ion channels such as potassium, sodium, chloride, and calcium channels, as well as acetylcholine and GABA receptors. These mutations can lead to alterations in ion channel function due to changes in transcriptional and post-translational processes. Consequently, channelopathies have the potential to contribute to the underlying pathology of acquired epilepsy [4]. Mutations in key components of the inhibitory system can lead to the breakdown of normal inhibitory processes, thereby contributing to neuronal hyperexcitability in epilepsy. GABA, the principal inhibitory neurotransmitter, acts through ligand-gated

Table 1 The function of ion channel and receptor in normal and epileptic tissue [7]

Ion channels or receptor	Normal brain	Epileptic brain
Voltage-gated Na ⁺ channels	Subthreshold EPSP, upstroke of the action potential	Repetitive action potential firing
Voltage-gated K ⁺ channels	Action potential downstroke	Action potential repolarization
Voltage-gated Ca ²⁺ channels	Released transmitter, transports a depolarizing charge from the dendrite to the soma	A pathogenic intracellular process is activated by excessive transmitter release
Ca ²⁺ -dependent K ⁺ channels	After hyperpolarization following action potential, sets refractory period	Limit repetitive firing
Non-NMDA receptor	Fast EPSP	Initiates paroxysmal depolarization shift
NMDA receptor	Prolong, slow EPSP	Maintain paroxysmal depolarization shift, Ca ²⁺ activates pathologic process
GABA receptor	IPSP	Limit excitation
Na ⁺ - K ⁺ pump	Restore ionic equilibrium	Prevent K ⁺ - induced depolarization

EPSP excitatory postsynaptic potential, IPSP inhibitory postsynaptic potentials

chloride ion channels, which facilitate fast inhibitory synaptic transmission. Mutations in specific genes, such as the voltage-gated chloride channel (*CLCN2*), GABAA receptor subunit $\alpha 1$ (*GABRA1*), and GABAA receptor subunit $\gamma 2$ (*GABRG2*), can disrupt the proper functioning of these components. These genetic alterations can result in the dysfunction of GABA conductance and chloride ion flux. As a consequence, the delicate balance between excitatory and inhibitory neurotransmission is disrupted, leading to an increased susceptibility to neuronal hyperexcitability, which is a hallmark of epilepsy. Understanding the impact of these mutations on inhibitory signaling pathways provides crucial insights into the underlying mechanisms of epilepsy [8, 44].

Mutations in genes responsible for encoding sodium channel proteins are believed to be a mechanism underlying certain types of inherited epilepsy. In humans, genes like *SCN1A-SCN5A* and *SCN8A-SCN11A* code for nine different subtypes of the alpha-subunit of voltage-gated sodium channels, specifically Nav1.1-Nav1.9. These mutations also impact epilepsy-related pathophysiology in the beta1 subunit of voltage-gated sodium channels. When mutations occur in the beta1 subunit, the affected sodium channels remain open for prolonged periods, allowing excessive sodium influx into the cell. Consequently, neurons become hyperexcitable and remain depolarized for extended durations. This leads to the release of large amounts of excitatory neurotransmitters, such as glutamate. As a result, the post-synaptic cells that interact with neighboring glutamatergic neurons experience excessive stimulation, leading to the production of calcium ions, which can be neurotoxic. Mutations in the genes encoding the alpha1 voltage-gated sodium channel subunit (*SCN1A*), which accelerates recovery from an inactive state, can also cause sustained depolarization due to prolonged sodium ion inflow and hyperexcitability. Hereditary epilepsy is linked to mutations in genes producing the voltage-gated sodium channel subunit beta1 (*SCN1B*) and *SCN2A*. Generalized epilepsy with febrile seizures involves mutations in sodium channel subunits *SCN1A* and *SCN2A*, as well as the GABAA receptor subunit *GABRG2*. Another form of generalized epilepsy caused by a single gene mutation is benign familial newborn convulsions. These genetic variations affecting sodium channels significantly contribute to the development of epilepsy by disrupting the normal balance of neuronal excitability and neurotransmission [8, 10, 12, 44, 45].

Potassium ion channels regulate neuronal excitability by allowing the flow of potassium ions to the extracellular space. This process helps control the resting membrane potential and facilitates rapid repolarization of the action potential. These ion channels consist of four alpha

subunits that form the pores, alongside four beta subunits for regulation. Among the most significant subset of ion channels are the Kv channels (Kv1-Kv12), which are highly abundant in the central nervous system. Dysfunctions in Kv channels, including calcium-activated potassium ion channels, have been associated with epilepsy. Channelopathies related to epilepsy involve HCN and Kv7 channels. HCN and Kv7 channels, which facilitate cation transport, induce hyperpolarization through voltage-gated ion channels. HCN channels are prominently expressed in the hippocampus, cortex, and thalamus, contributing to localized and widespread epileptic seizures. Kv7 channels also play a role in modulating neuronal hyperexcitability. The HCN channel, however, performs a more intricate role. Genetic deletions in the HCN2 channel subtype do not result in an epilepsy phenotype, whereas deletions in the HCN subtype1 (HCN1) can accelerate epileptogenesis. The absence or inhibition of HCN1 channels increases neuronal hyperexcitability, seizure severity, and the risk of SE-related mortality. Membrane hyperpolarization activates HCN channels, inhibiting I_h , a current that regulates neuronal excitability by controlling the resting membrane potential. Reduced I_h density due to decreased or lost channel expression can lead to neuronal hyperexcitability. Mutations in the *KCNQ* canal gene, responsible for Kv7 channels, are also associated with epilepsy. Deficiencies in Kv7 subunits, similar to Kv7.2 subunit mutations, can lead to epilepsy. Kv7 channels reduce neuronal hyperexcitability by activating voltage-gated potassium channels. They also inhibit repeated depolarization of action potentials. Mutations in genes encoding potassium channels, such as *KCNQ2* and *KCNQ3*, can result in reduced potassium ion flow. This leads to prolonged depolarization and increased neuronal hyperexcitability, as potassium ion influx occurs only after repolarization of the neuronal membrane following depolarization. These insights highlight the critical role of potassium ion channels in maintaining proper neuronal excitability and the consequences of their dysfunction in epilepsy [4, 5, 8, 12, 45].

The role of calcium ions as second messengers is essential for both healthy brain function and the development of various neurological disorders, including epilepsy. Calcium ions enter cells through voltage-gated calcium channels, which are instrumental in transmitting electrical signals and initiating transduction cascades in diverse cell types. Voltage-gated calcium channels are categorized into two main subtypes: low-voltage-activated (LVA) and high-voltage-activated (HVA) channels. These channels allow calcium ions to flow into the intracellular space, where they are utilized for signal transduction. The composition of these channels involves multiple subunits, including $\alpha 1$, β , $\alpha 2$, δ , and γ subunits,

within the HVA subtype. The subunits within the HVA subtype, including L-, N-, and P/Q-types, have activation thresholds that generally occur around positive membrane potentials, often up to -20 mV. On the other hand, the LVA channel, or type T, is activated at more positive membrane potentials, reaching up to -70 mV. This particular channel subtype exclusively contains $\alpha 1$ subunits. The transmembrane topology of voltage-gated calcium channels consists of four homologous transmembrane domains, each containing a transmembrane segment and a porous region located between segments S5 and S6. This structural arrangement enables the channel to facilitate the movement of calcium ions across the cell membrane, thereby contributing to various cellular processes and responses. In the context of epilepsy, the dysregulation of calcium ion influx through these channels can disrupt normal brain activity and contribute to the development of seizures and other epileptic phenomena. Understanding the intricate role of voltage-gated calcium channels in neuronal function and their involvement in epilepsy is essential for advancing our knowledge of this complex neurological disorder [45].

Impaired expression of the chloride transporter-like K-Cl cotransporter isoform 2 (KCC2)/NKCC1 complex plays a significant role in epileptogenesis. This transporter system is crucial for regulating the impact of GABA (gamma-aminobutyric acid) on neuronal excitability. GABA, traditionally an inhibitory neurotransmitter, can exert excitatory effects through the K-Cl cotransporter. In mature neurons, GABA typically induces membrane hyperpolarization due to lowered intracellular chloride ion levels. However, during neuronal development, elevated intracellular chloride concentrations can lead to GABA-driven chloride ion efflux and subsequent membrane depolarization. The equilibrium between these opposing effects is tightly controlled by the activity of the K-Cl cotransporter and NKCC1. The balance between KCC2 and NKCC1 activity in neurons dictates whether GABA has a depolarizing or hyperpolarizing effect. In cases of epilepsy, the imbalance in the expression and function of KCC2 and NKCC1 can cause a shift from the traditional inhibitory effect of GABA to an excitatory one, contributing to hyperexcitability and seizure activity. Notably, in the hippocampus of rat models with epilepsy, there is evidence of increased NKCC1 expression coupled with decreased KCC2 expression. This imbalance likely contributes to the altered neuronal excitability observed in epilepsy. Understanding the intricate interplay between chloride transporters, GABA, and neuronal excitability is essential for unraveling the mechanisms underlying epileptogenesis and potentially identifying therapeutic targets to mitigate the development and progression of epilepsy [12].

The role of neurogenesis in epileptic seizures and epilepsy

Epileptogenesis is a complex process characterized by the formation of new neurons and circuits within the hippocampal region of the brain. After an acute epileptic seizure, individuals with brain injuries can undergo various types of changes. These changes encompass cellular, structural, and neurochemical alterations [4]. Granular cells located in the dentate gyrus of the hippocampus exhibit continuous proliferation in nearly all mammalian species. However, epilepsy can disrupt this process of new neuronal growth. Interestingly, neurogenesis tends to increase in the aftermath of epileptogenesis. An epilepsy-related disorder in hippocampal neurogenesis encompasses several factors, such as the formation of granular cells in abnormal locations, heightened proliferation of neural cell progenitors, aberrations in morphological features of the neurons, and neuronal hypertrophy [12].

After a sudden seizure, the hippocampus can undergo a range of structural changes, including the degeneration of CA1-CA3 pyramidal neurons and dentate gyrus hilar neurons, as well as the sprouting and abnormal formation of synapses by mossy fibers. Additionally, the loss of inhibitory GABAergic interneurons occurs. During the process of mossy fiber sprouting, axons originating from granular cells in the dentate gyrus establish synapses in unusual locations, particularly in the inner third molecular layer of the gyrus dentatus or the supragranular region. This phenomenon contributes to the synaptic reorganization of mossy fibers. As mossy cells, significant excitatory neurons in the hilus dentatus that usually project to the supragranular area, undergo neuronal death, this structural rearrangement aids in eliminating synapses. Furthermore, the growth of new neural circuits can give rise to recurrent excitatory circuits in dentate granule cells due to axon sprouting and synaptogenesis in the mossy fiber pathway. This increased excitation can contribute to the development of epileptogenesis. Another theory regarding structural reorganization involves functional disconnections between excitatory neurons and dormant (inhibitory) basket cells. According to this theory, GABAergic interneurons, known as basket cells, survive damage induced by epileptogenesis. However, because most excitatory neurons (mossy cells) perish as a result of epileptic seizures, the inhibitory influence of these basket cells diminishes. As a result, these basket cells become inactive and are unable to provide inhibitory feedback to granular cells. This loss of inhibition resulting from the inactivity of basket cells might contribute to the process of epileptogenesis [4].

Acute epileptic seizures, leading to structural alterations, can also enhance the expression of neurotrophic

factors and various proteins within the hippocampus. These include sonic hedgehog, brain-derived neurotrophic factor (BDNF), fibroblast growth factor-2 (FGF-2), and nerve growth factor (NGF). The neurotrophin family of proteins, which encompasses FGF-2, BDNF, and NGF, plays a vital role in influencing neuronal survival, differentiation, development, synaptic plasticity, and excitability. Additionally, vascular endothelial growth factors (VEGF) are responsible for promoting angiogenesis. However, elevated levels of VEGF can disrupt the BBB, potentially leading to brain inflammation. Notably, abnormal cerebral angiogenesis can manifest as increased permeability to immunoglobulin G and the loss of tight junctions. Furthermore, the sonic hedgehog protein, which is secreted, regulates the growth and survival of both neuronal and glial progenitor cells. An elevation in the expression of this protein may contribute to the generation of neurons within the hippocampus [4, 17].

The role of oxidative stress in epileptic seizure

Oxidative stress plays a critical role in the initiation and progression of epilepsy, stemming from the excessive release of free radicals. Experimental animal models, such as the administration of kainic acid and pilocarpine, induce this condition. In response, antioxidant therapy has gained acceptance as a strategy to mitigate oxidative stress and potentially serve as a treatment for epilepsy. However, it's important to note that not all types of seizures exhibit the same degree of oxidative damage. The impact of oxidative stress varies among seizure types. Compromised antioxidant systems, mitochondrial dysfunction, redox-active metals, activation of the arachidonic acid pathway, and even aging can contribute to oxidative stress, thereby becoming factors in the development of epileptic seizures. Superoxide radicals are implicated in both excitotoxicity and the mediation of oxidative stress. This understanding has prompted the exploration of antioxidant approaches targeting oxidative stress as potential avenues for treating epileptic seizures. Such methods not only hold promise for managing seizures but also offer potential as neuroprotective strategies in epilepsy cases. By addressing the underlying oxidative stress, these interventions may contribute to improving the management and outcomes of epilepsy [8, 12].

The activation of intracellular oxidant systems that rely on calcium ions, such as NADPH oxidase 2 (NOX2), plays a pivotal role in promoting superoxide generation. Notably, NOX2 is regulated by NMDA receptors, highlighting its significance in the context of neuronal hyperexcitability. This phenomenon, observed in the early stages of epilepsy, can lead to chronic epilepsy by inducing enduring neural damage. The interplay between these

factors contributes to the complex pathophysiology of epilepsy. Neural networks that govern intricate processes in self-regulatory homeostasis, including the bioenergetic system, become disrupted. This imbalance between excitatory and inhibitory signals, combined with oxidative stress, gives rise to abnormal patterns of activity within neural circuits. These complex interactions contribute to the intricate nature of the progression of epilepsy, as well as its long-term effects on neural function and overall brain health [6].

The role of gene and protein regulation in epileptic seizure and epilepsy

Brain injury triggers alterations in gene expression, a process largely governed by transcription factors. Within the context of epileptic seizures, the pathophysiology is believed to involve immediate early genes (IEGs) or inducible transcription factors that play a pivotal role in orchestrating cellular responses. Notably, members of the Jun family (such as c-jun, junB, and junD) and the Fos family (including c-fos, fosB, fra-1, and fra-2) are implicated in this mechanism. Both gene families encode crucial transcription factors, including c-fos and c-jun, which are integral components of the transcription factor activator protein-1. During epileptic seizures, hippocampal neurons have been observed to display heightened mRNA expression of these factors. The precise functions of IEGs in mediating early genes during epileptogenesis remain to be fully elucidated, generating ongoing uncertainty. Broadly, transcription factors from the Fos and Jun families play roles in gene transcription, cell proliferation, regeneration, and cell death within the central nervous system. The expression of IEGs represents a critical step in the biological cascade leading to neuronal apoptosis, ultimately resulting in cell death. Understanding the intricate interplay between these transcription factors and their influence on cellular processes contributes to unraveling the complex molecular landscape underlying epileptic seizures and their aftermath [4].

Inducible cyclic adenosine monophosphate (cAMP) early repressor (ICER), another transcription factor, emerges as a significant player in the intricacies of epileptogenesis. A class of proteins collectively known as ICER arises from the cAMP-responsive element modulator (CREM) gene. Additionally, ICER mRNAs are generated by the internal promoter of CREM. The central role of ICER lies in its ability to inhibit the cAMP-responsive element (CRE)-mediated transcription of genes. ICER exerts its influence by suppressing the transcription of genes that rely on CRE-mediated mechanisms. A key aspect of its function is the inhibition of the activity of the transcription factor cAMP-responsive element binding protein (CREB). This unique regulatory action

positions ICER as a pivotal player in governing both neuronal plasticity and programmed cell death within the nervous system. Notably, CREB is responsible for activating CRE transcription, which holds paramount importance for maintaining neuronal survival. The impact of ICER on neuronal cultures reveals an apoptotic effect through its overexpression in these contexts. Furthermore, the heightened expression of ICER in neurons following excitatory inputs suggests its involvement in apoptotic processes. Yet, despite ongoing research efforts and some divergent findings, the precise role of ICER in the complex web of epileptogenesis remains elusive. As the scientific community continues to explore this intricate topic, elucidating the specific contributions of ICER to the development of epilepsy holds the promise of enhancing our understanding of the underlying mechanisms and potentially paving the way for innovative therapeutic strategies [4, 9, 46].

A multitude of studies have delved into the impact of epileptic seizures on the expression of neuropeptides within the hippocampus. Among the neuropeptides that have garnered attention in these studies are cortistatin, galanin, neuropeptide Y (NPY), thyrotropin-releasing hormone, cholecystokinin, and neurokinin B [47–49]. NPY is localized on GABAergic interneurons within the hippocampus and serves as an inhibitor of excitable neurons. Experimental studies involving models of epileptic seizures have highlighted intriguing findings regarding NPY's expression. Overexpression of NPY and its corresponding mRNA within the hippocampus, as well as aberrant NPY expression in hippocampal granule cells and mossy fibers—cellular regions not typically associated with this neuropeptide—have been reported in these investigations [50, 51]. The outcomes of this study underscore an inherent adaptive mechanism that counteracts the hyperexcitable state induced by epileptic episode stimulation. Further support for this notion is provided by investigations showcasing diminished occurrences of spontaneous epileptic seizures and the prevention of epileptogenesis through NPY gene therapy in experimental animal models. Galanin, on the other hand, functions as a modulator of epileptic seizures by activating galanin receptors GalR1 and GalR2. This action enables galanin to sustain a delicate equilibrium between glutamatergic excitation and galaninergic inhibition within the hippocampal gyrus dentatus [4].

The role of thalamocortical circuit disruption in epilepsy, especially the absence type

The disruption of the circuitry connecting the thalamus and the cerebral cortex constitutes the driving mechanism behind absence epilepsy. This circuit plays a pivotal role in orchestrating the rhythmic excitation of

the cortex by the thalamus during normal sleep cycles. Within this circuit, three distinct populations of neurons are involved: thalamic relay neurons, thalamic reticular neurons, and cortical pyramidal neurons. Thalamic relay neurons possess the capability to activate cortical pyramidal neurons during wakefulness and rapid eye movement (REM) sleep phases. The underlying electrical activity is likely facilitated by T-type calcium ion channels, which elicit low-threshold depolarization and initiate action potentials through voltage-gated sodium channels. Thalamic reticular neurons, through their inputs, exert control over the activation of thalamocortical circuits by inducing hyperpolarization in relay neurons, thereby generating electrical activity. The mutual inhibition of reticular neurons is achieved through collateral networks stemming from adjacent reticular neurons. Completing this circuit, both cortical pyramidal and thalamic relay neurons project to reticular neurons. Notably, this circuit's dynamics can be modulated by ascending noradrenergic, serotonergic, and dopaminergic inputs to the thalamus, potentially intensifying the electrical activity. During the non-REM sleep phase, depolarized thalamic relay neurons synchronously and rhythmically stimulate the cortex, resulting in the generation of sleep spindles—distinct brain wave patterns.

In contrast to the activity observed during wakefulness, where thalamic relay neurons are depolarized and sensory information is conveyed to the brain in a non-rhythmic manner, the functioning of thalamocortical circuits during sleep follows a distinct pattern. In the context of absence epilepsy, faulty circuitry generates rhythmic activation of the cortex during waking hours, akin to the pattern observed during typical non-REM sleep phases. This aberrant activity gives rise to the characteristic brain wave patterns and clinical manifestations of absence epilepsy. Several potential factors contribute to these circuitry abnormalities, including disruptions in modulatory input from the brainstem, the role of T-type calcium ion channels, alterations in GABA receptor function, or a combination of these factors. However, the precise nature of the circuitry malfunction remains to be definitively identified. An alternate perspective posits that abnormal paroxysmal episodes of rhythmic cortical activation (absence epileptic seizures) could arise from an underlying neural circuit dysfunction that mimics the physiological state of rhythmic cortical activation seen during sleep [10].

Nowadays, research has showed that the hippocampus circuit plays a crucial role in comprehending the intricacies of epilepsy, particularly the absence type. The hippocampus that works in memory and spatial navigation also has complicated connections with the cortex that contribute to brain activity regulation. The trisynaptic

circuit is a critical channel for information processing inside the hippocampus formation. The dentate gyrus, the CA3 area, the CA1 region, and the subiculum are all part of this circuit. At each of these steps, excitatory glutamatergic neurons create synapses, allowing information to be sent sequentially. The circuit's unique design allows for the modification of incoming signals and the encoding of memories. The synchronization of neuronal activity inside the hippocampus is crucial for maintaining optimal brain function. Disruptions in these coordinated rhythms might result in seizure activity. Incorporating the hippocampus into the knowledge of absence epilepsy provides a more comprehensive view of the neuronal networks involved in this condition. The interconnection of the hippocampus and cortex enables not only for the transmission of spatial and contextual information, but also for the control of general brain states, including those linked with seizures. Considering hippocampus circuitry in addition to the thalamocortical circuit gives a more thorough framework for appreciating the complicated web of connections involved in absence epilepsy. The hippocampus may play a role in the rhythmic cortical activity seen during absence seizures, working in tandem with the damaged thalamocortical circuit to generate an environment favourable to aberrant synchronized firing.

The role of miRNAs as biomarkers in epilepsy and epileptogenesis

Epigenetic mechanisms that are not linked to changes in DNA sequence but rather to transcription or post-transcriptional regulation can play a role in epileptogenesis. These mechanisms influence various essential biological pathways, some of which are modulated by miRNAs, and are associated with the development of various types of epilepsy [37, 52]. MicroRNAs (miRNAs) serve as potential regulatory mechanisms and therapeutic targets for epilepsy by modulating mRNA stability and translational processes, thereby inhibiting the expression of numerous proteins. These miRNAs hold promise as both biomarkers and targets for therapeutic interventions, as they reflect the intricate gene expression regulation associated with epilepsy. Altered expression of specific miRNAs has been observed, such as serum miRNA-4521 in the hippocampus of individuals with temporal lobe epilepsy, as well as changes in brain tissue in experimental animal models of SE [53–56]. Following epileptic episodes, alterations were observed in the expression levels of certain miRNAs, including those associated with transcription factors and elements involved in neurotransmitter signaling. Changes in miRNA levels in the bloodstream suggest their potential utility as diagnostic biomarkers. Earlier studies primarily focused on the regulatory impact of miRNAs on their target genes. Some investigations

explored the consequences of altered gene expression on individual miRNAs, observing fluctuations in miRNA levels in experimental mouse or rat models, and tracking the progression of epilepsy-related pathology. Recent advances in epigenetic profiling are shedding light on the potential role of miRNAs in the pathophysiology of epilepsy. Methylation status and miRNA expression correlations have been identified in hippocampal samples from both human subjects and mouse models. MiRNAs are abundantly present in the brain and play a crucial role in regulating neurogenesis [37, 57, 58].

Numerous types of miRNAs are expressed in response to epileptic conditions. Among them, miR-146a is notable, as it has been observed to exhibit elevated expression in both humans with temporal lobe epilepsy and mice with epilepsy. Sense and antisense oligonucleotides, referred to as agomirs (mimics) and antagomirs, respectively, can efficiently modify the activity of miRNAs. This manipulation of miRNA function can lead to changes in gene expression, offering a promising avenue for the exploration of novel treatment targets for epilepsy [59]. Upon initial investigation in individuals with epilepsy, miR-146a, a miRNA known to regulate inflammatory responses, exhibited an increased presence in the hippocampus [37]. MicroRNA-134 (miR-134), a widely expressed miRNA in brain neurons, plays a role in controlling the formation of dendritic spines by inhibiting LIM domain kinase 1 (LIMK1) and other targets. This function is particularly relevant as dendritic spine reorganization is a feature observed in experimental human epilepsy and is crucial for intercellular excitatory communication, especially in hyperexcitability disorders like epilepsy. In a study conducted by Reschke et al. [60], miR-134 was identified as one of the miRNAs exhibiting increased expression in hippocampal regions affected during SE induced by intra-amygdala kainic acid administration in mice. Furthermore, miR-134 was found to be overexpressed in human neocortex resection samples from patients with refractory temporal lobe epilepsy. The upregulation of miR-134 expression was also observed in both *in vitro* and *in vivo* models of neuronal stimulation and epileptic seizures. Remarkably, intracerebroventricular administration of cholesterol-tagged locked nucleic acid (LNA) antagomirs (Ant-134) effectively reduced miR-134 levels for an extended period of at least one month in mice experiencing epileptic seizures resistant to the convulsant effects of kainic acid and pilocarpine hydrochloride. This intervention highlights the potential of targeting miR-134 for therapeutic purposes in epilepsy [60].

MiRNAs such as miR-146a, miR-134, miR-132, miR-128, miR-34a, miR-324-5p, miR-124, miR-155, miR-142-5p, miR-21-5p, miR-187, and miR-106b-5p have

all been implicated in the process of epileptogenesis. Among these, miR-106b-5p stands out as a particularly accurate predictor of epileptogenesis, as suggested by Tiwari et al. [59]. Studies conducted by Cava et al. [37] have also shown that miR-106b-5p is upregulated in epileptic conditions. The expression of circulating miRNAs shows dynamic variations in connection with the pathological process of epilepsy. Some miRNAs can be rapidly released following an epileptic seizure, while others may not be expressed until the condition progresses to a chronic stage. Over the past five years, research by Wang et al. [55] has led to the discovery of around 100 distinct miRNAs in both epileptic patients and animal models. This extensive research provides compelling evidence for the significant changes in miRNA expression associated with epilepsy. Although several miRNAs have been linked to epilepsy, further investigations are necessary to determine whether these dysregulated miRNAs can serve as predictive markers for epilepsy risk, diagnostic tools, or indicators of disease progression. Wang et al. [55] conducted a study involving 117 epilepsy patients and 112 controls, utilizing serum samples from epilepsy patients. The study revealed that Let-7d-5p, miR-106b-5p, miR-130a-3p, and miR-146a-5p exhibited increased expression compared to controls, while miR-15a-5p and miR-194-5p showed decreased expression ($p < 0.0001$). Notably, miR-106b-5p in serum demonstrated the highest diagnostic value for epilepsy, with a sensitivity of 80.3% and specificity of 81.2% [55].

In contrast to therapy-responsive groups, a study conducted by Alsharafi et al. [61] highlighted that another investigation identified altered miRNA expression patterns in drug-resistant individuals. Among the findings, 12 miRNAs exhibited decreased expression, while three miRNAs showed increased expression in this drug-resistant group. In comparison to both the treatment-responsive group and the control group, certain miRNAs confirmed through quantitative real-time polymerase chain reaction (qRT-PCR) analysis, including miR-194-5p, miR-301a-3p, miR-30b-5p, miR-342-5p, and miR-4446-3p, displayed statistically significant dysregulation in the drug-resistant group. In this context, miR-301a-3p emerged as a stand-out diagnostic biomarker for drug-resistant epilepsy among these five miRNAs, demonstrating high levels of both sensitivity and specificity. Notably, it was observed that patients with temporal lobe epilepsy exhibited lower expression levels of the miRNAs miR-301a-3p and miR-106b-5p within their hippocampal tissue [61].

In a study conducted by An et al. in 2016, the roles of eight circulating miRNAs associated with epilepsy were investigated in relation to various cellular processes linked to epilepsy [62]. Through *in silico* analysis, the

study revealed distinct pathways influenced by these miRNAs. Firstly, the “immune system” pathway was highlighted, particularly in the context of neuro-inflammation involving microglia. MiRNAs including miR-15a-5p (with decreased expression), miR-106b-5p, miR-146, and miR-451 were found to impact this pathway, resulting in the upregulation of several genes involved in immune response. MiR-106b, which is also associated with inflammation in Alzheimer’s disease, exhibited increased expression in epilepsy. Secondly, the “cell cycle” pathway was identified through *in silico* analysis, encompassing processes related to neurogenesis, cell cycle regulation, and cell proliferation. This pathway indicated that these miRNAs, specifically miR-15a-5p, miR-34a, miR-106b-5p, and miR-146, likely influence genes involved in neuronal cell differentiation and proliferation. Notably, miR-106b-5p displayed heightened expression in the early stages of an epilepsy mouse model induced by electrical stimulation. This suggests that this miRNA could potentially contribute to the interruption of neuronal cell cycle progression and subsequent neuronal death. Thirdly, the study identified confirmed targets of miR-15a-5p (with reduced expression), miR-106b-5p, miR-146, and miR-45 that are implicated in both pro- and anti-apoptotic signaling pathways. This suggests a complex role for these miRNAs in regulating apoptosis, which is a significant process in the pathophysiology of epilepsy. Overall, this study sheds light on the intricate involvement of specific miRNAs in various cellular processes related to epilepsy, further enhancing our understanding of the molecular mechanisms underlying this disorder [37].

Conclusions

The advancement of molecular science has provided valuable insights into the understanding of epilepsy. The pathophysiology of epileptogenesis, the process leading to epilepsy, is intricate and involves the interplay of various factors such as neurotransmitters, ion channels, receptors, neurogenesis, oxidative stress, inflammation, apoptosis, hyperactivation of the mTOR signaling pathway, and the regulation of numerous genes and proteins. Epileptic seizures arise from a sudden imbalance between excitatory and inhibitory neurotransmitters. Inflammation and oxidative stress can occur both prior to and following epileptic convulsions. Disruption of calcium ion homeostasis due to excitotoxicity can lead to cell death through apoptosis or necrosis pathways. The process of epileptogenesis, marked by diverse structural, neurochemical, and cellular changes, is also influenced by hippocampal neurogenesis. Further exacerbating brain damage and increasing the risk of recurrent epileptic seizures

are glial cell alterations and modifications to the BBB. Recent research has highlighted the role of specific microRNAs (miRNAs) such as miR-146a, miR-134, miR-132, miR-128, miR-34a, miR-324-5p, miR-124, miR-155, miR-142-5p, miR-21-5p, miR-187, and miR-106b-5p in epileptic disorders. However, a deeper investigation is needed to fully understand the individual contributions of each miRNA to epilepsy. Encouragingly, emerging evidence supports the potential of utilizing microRNA-induced silencing as a therapeutic target in epilepsy. Addressing the challenges in pinpointing the target and cell specificity of miRNAs and developing safe and effective strategies to modulate miRNAs will be pivotal in translating these promising findings into clinical applications for the treatment of epilepsy or the prevention of epileptogenesis.

Abbreviations

AMPA	α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
cAMP	Cyclic adenosine monophosphate
ESAM	Endothelial selective adhesion molecules
FCD	Focal cortical dysplasia
GABA	γ -Amino butyric acid
HCN	Hyperpolarization-activated cyclic nucleotide-gated
HMGB1	High-mobility group box 1
ICE	Interleukin-1 β converting enzyme
ICER	Inducible cAMP early repressor
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-1RI	Interleukin-1 receptor type I
JAMs	Junctional adhesion molecules
LD	Lafora disease
LVA	Low-voltage-activated
mTOR	Mammalian target of rapamycin
NKCC1	Na-K-2Cl cotransporter isoform 1
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptors
PDS	Paroxysmal depolarization shifts
P-gp	P-glycoprotein
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase- α
PTEN	Phosphatase and tensin homolog
SE	Status epilepticus
STRADA	STE20-related kinase adaptor- α
TNFR1	Tumor necrosis factor receptor 1
TSC	Tuberous sclerosis complex

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Authors' contributions

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