Epilepsy: Don’t get too excited

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Abstract
Epilepsy is a brain disorder experienced by 65 million people worldwide. It is characterized by the overstimulation or hypersynchrony of neurons that leads to epileptic seizures. Physical symptoms include weakness, inability to communicate clearly, and excessive exhaustion. Epilepsy can be categorized based on two factors: if the disorder is idiopathic or symptomatic, and if the disorder is generalized or partial. Idiopathic epilepsy is genetic-based, while symptomatic epilepsy has no known cause or is developed from a traumatic brain injury. If the epilepsy is generalized it occurs in multiple parts of the brain, while partial is specific to a particular brain region. A variety of ions, neurotransmitters, and extracellular components affect the level of excitation experienced by the cell, leading to the different types of epilepsy. In this review, we cover the dysfunctions in the central nervous system that can lead to the development of idiopathic generalized and idiopathic partial epilepsy. Additionally, we investigate the recent research and insights into these mechanisms and determine possible therapeutic targets.

Introduction
Dating back at least 4 millennia, epilepsy is a disease with a long history. Early theories of epilepsy correctly identified the brain as the site of the problem, but wrongly believed it was caused by too much phlegm in the brain or the result of evil influences (Gross, 1992). It wasn’t until the Enlightenment and advances in anatomy, chemistry, and physiology that epilepsy was seen in a modern light (Gross, 1992). In addition to these advances, Hippocrates is credited with giving the first formal description identifying it using a scientific approach, forming the base of what we know about epilepsy today (Magiorkinis, 2012).

Epilepsy affects an estimated 65 million people worldwide, with infants and people over 60 being the most at risk (Epilepsy Foundation). Epilepsy can be described as the state resulting from an abnormal and excessive neuronal discharge within the central nervous system (Epilepsy Foundation). It is possible for the seizure to occur in any area of the brain and it may or may not spread to other parts (Penfield & Erickson, 1941). This hyperexcitability of neurons causes chronic, unpredictable seizures and can even lead to other neurological problems (Epilepsy Foundation).

While there are two major known causes of epilepsy, traumatic brain injury (TBI) and chemical imbalances, in over half of the cases of epilepsy, the cause is unknown (Fischer, 2015). The type of injury that leads to a seizure is age-dependent. For example, seizures in children are often linked to birth traumas or infections, such as meningitis, or high fevers, while seizures in the elderly are likely due to tumors or strokes (Fischer, 2015). This review will focus on epilepsies caused by chemical imbalances and the mechanisms of dysfunction of different receptors, such as receptors for glutamate, GABA, potassium, sodium, and calcium.

A. Healthy Neuron

B. Epileptic Neuron

Figure 1. Neuronal Excitability in an Epileptic Model

A) In a healthy neuron, receptors are working properly and allow the correct amount of ion flow in and out of the cell. This results in the appropriate amount of stimulation in the cell. B) In an epileptic neuron, the K+ channels do not allow enough K+ into the cell, while the Ca2+, Na+, GABA, and glutamate receptors allow too many ions into the cell, causing hyper-excitability.

GABA Receptors
GABA, gamma-Aminobutyric acid, is the main inhibitory neurotransmitter of the central nervous system that counterbalances neuronal excitation (Treiman, 2001). GABA is formed in the GABAergic axon terminals and then it is released into the synapse. Once in the synapse, it acts via two receptors GABA A and GABA B (Belelli et al., 2009). GABA A receptors are ligand-gated channels that induce a rapid inhibitory effect by increasing the amount of chloride conductance into the neuron, causing the neuron to be hyperpolarized (Treiman, 2001). GABA B receptors also hyperpolarize the neuron, but they do it by increasing the conductance of potassium. GABA B receptors also decrease calcium conductance and have a slow inhibitory effect (Treiman, 2001).

GABA A receptors are responsible for a type of receptor-mediating inhibition called tonic inhibition, which occurs because of the activation of the extrasynaptic receptors due to low concentrations of ambient GABA (Belelli et al., 2009). GABA spillover from the synaptic cleft activates the postsynaptic GABA A receptors to generate tonic inhibition, which corresponds with the expression of relatively rare subunits, such as δ4, δ6, and δ subunits (Belelli et al., 2009). For the α1βδ, the presence of α4 and δ6 increases GABA sensitivity. Slower and less extensive desensitization is also seen (Belelli et al., 2009). In thalamo-cortical neurons of the ventrobasal thalamus, an increase in tonic inhibition is seen before the onset of seizures, suggesting that it may be a contributor to seizure genesis (Belelli et al., 2009). Because seizures can be caused by over-excitation or under-inhibition, dysfunction of GABA receptors (as seen in figure 1), is believed to have a large role in the mechanisms underlying epilepsy.

Potassium Receptors
Potassium channels are important for cellular signaling processes, such as regulating neurotransmitter release and neuronal excitability (Shieh et al., 2000). They are assembled as a tetramer with cytoplasmic N- and C-termini and six transmembrane segments that form a core domain. Segments 1-4 are symmetrically arranged around the pore to create the voltage-sensing domain (Miceli et al., 2013). Over 50 genes that code for various K+ channels have been identified and mutations in these genes lead to dysfunction of the channel, causing disease (Shieh et al., 2000). For example, mutations in the K7.2 gene, which encodes for voltage-dependent K+ channel subunits, leads to neonatal epilepsies (Miceli et al., 2013).

Two mutations have been identified, among others that lead to neonatal epilepsies. The R213W mutation causes benign familial neonatal seizures, while the R213Q mutation leads to neonatal epileptic encephalopathy (Miceli et al., 2013). Both of these two mutations destabilize the channel, while it is in its open state. This causes a marked increase in the channel’s voltage sensitivity, by reducing stability of the channel’s voltage sensing domain configuration (Miceli et al., 2013).

NMDA Receptors
N-methyl-D-aspartate, or NMDA, receptors are glutamate-gated cation channels that play a critical role in the development of the central nervous system and processes that form the basis of learning, memory, and neuroplasticity (Blanke et al., 2009). They regulate the plasticity of synapses, dendrites, and neurons by allowing specific calcium-dependent signaling cascades to be activated (Blanke et al., 2009). In the brain, excitatory synaptic transmission depends on the release of L-glutamate from pre-synaptic terminals that move across the synaptic cleft and bind to post-synaptic NMDA receptors (Blanke et al., 2009). This functionality is demonstrated in figure 1. During baseline activity, excitatory synaptic inputs do not result in calcium influx due to the receptor’s voltage dependence (Blanke et al., 2009). The overstimulation of these receptors may result in excitotoxicity.

NMDA receptor induced excitotoxicity, which is dependent on the influx of calcium through NMDA ionotropic glutamate receptors, is considered to be a contributor to the cell death that is associated with epilepsy (Parsons & Raymond, 2014). Some recent studies have shown that the activation of NMDA receptors inside the synapse may contribute to cell survival (Parsons & Raymond, 2014). This idea led to the “localization hypothesis”, which states that the activation of synaptic NMDA receptors promotes cell survival, while activation of the receptors outside of the synapse promotes the signaling of cell death (Parsons & Raymond, 2014). The NMDA receptor is composed of two subunits, GluN1 and GluN2, both consisting of two different variants. The two variants of the GluN2 subunit is GluN2A and GluN2B (Parsons & Raymond, 2014). Many studies show that the NMDA receptors containing a larger GluN2B subunit may preferentially signal for cell death (Parsons & Raymond, 2014).

In experiments done with epileptic rats, the rats showed an increase in GluN2B content outside of the synapse and a decrease inside the synapse (Parsons & Raymond, 2014). As these extrasympathetic NMDA receptors respond to the release of glial glutamate, it is possible that their activation plays a critical role in the synchronized neuronal activity associated with epileptic seizures (Parsons & Raymond, 2014).

**Sodium Receptors**

The main receptor for sodium that contributes to the excitatory nature of a neuron is the Voltage Gated Sodium Channel (VGNC). This receptor’s function is to initiate and propagate action potentials in the neuron. It is comprised of one α subunit and four β subunits (Frank et al., 2006). The α subunit serves as the voltage sensor and the ion pore, allowing the receptor to sense a change in electrical signaling and convey it into chemical signals, via the ion pore. There are six α-helical transmembrane domains of the α subunit, where the fifth and sixth form the ion pore (Squire, 2013). The other subunits, termed β subunits, modify the voltage dependence and kinetics of the receptor (Squire, 2013). These subunits are constructed of cell adhesion molecules (CAMs), which function in connecting the receptor to the neuron’s cytoskeleton and to the extracellular membrane (Frank et al., 2006).

In an epileptic brain, any dysfunction in the VGNC could alter the excitability of a neuron due to the receptor’s key function in propagation of an action potential. This dysfunction is demonstrated in the healthy and epileptic neuron in figure 1. Since there are many subunits and domains that can be mechanically dysfunctional, we will focus on one prime example: the Nav1.1 domain. When there is a loss of function mutation in SCN1A, the gene that codes for this domain, we see epilepsy in the mouse model (Kanai et al., 2004). This is because when the receptor can no longer initiate or propagate an action potential in GABAAergic interneurons specifically, this may over-excite the neuron and lead to the inhibitory effect of these neurons. This will cause over-excitation due to the lack of balance of stimulation with inhibition (Kanai et al., 2004). Though this is a common example of how dysfunction of the VGNC can lead to epilepsy, there are many more examples involving different subunits and domains.

**Calcium Receptors**

Calcium is one of the most important ions in a neuron. It serves to depolarize the cell enough to propagate an action potential. Hence the voltage gated calcium channels (VGCC) are a key component of the cell and of normal neurological function. These receptors can alter gene transcription, neurotransmitter release, neural growth, and activation of other enzymes (Simms et al., 2014). Prolonged activation of the receptor can lead to excitotoxicity in the cell, as shown in figure 1. These receptors are located on the active zone of the presynaptic terminal, allowing for quick vesicular release of neurotransmitters upon activation (Squire, 2013).

There are a variety of subunits of this receptor that each have differing functions. The Cav2 subunits of the VGCC allow for vesicular release of neurotransmitters by being bound to the SNARE complex via synprint, a protein on the receptor (Squire, 2013). Thus, activation of this subunit promotes binding and leads to vesicular release through the SNARE complex. Overstimulation of this process could lead to an excess of neurotransmitter release and therefore hyper-stimulation of neurons (Simms et al., 2014).

Another subunit called the Cavβ subunit regulates the amount of a hormone called thyroid that is being released (Simms et al., 2014). This subunit’s normal function is to suppress the amount of the hormone by downregulation. It does so by binding to the transcription factor Pax6 upon activation. This binding initiates the transcription factor and allows for regulation of hormone synthesis (Simms et al., 2014). Hormone concentration, namely thyroid hormone, has been directly implicated in epileptic susceptibility due to its ability to increase excitability of the neurons (Pen- nell, 2009). Therefore, any alteration in this pathway could lead to epileptic brain activity.

The final example of a VGCC subunit that could lead to epilepsy is the Cav3 subunit, which is coded for by the C456S gene, and its normal function is to regulate neuronal excitability (Simms et al., 2014). Any mutation in this subunit is directly implicated in an increase in neuronal firing due to a reduction of the rebound-bursting threshold in the neuron (Eckle et al., 2014). Additionally, researchers found that elimination of these Cav3 subunits prevents epileptic tendencies in a mouse model (Eckle et al., 2014). In summary, proper functionality of all subunits and domains of the VGCC is critical to maintain a normal level of excitation in the central nervous system. All of the above dysfunctions, as well as others not discussed, can increase neuronal excitability, leading to epilepsy.

**Extracellular Glial Regulation**

Glia cells are supportive cells found in the brain that do not conduct electrical signals the way neurons do, but instead support the neuron’s functionality via a variety of mechanisms (Miller, 2005). Glial cells outnumber neurons 10:1 in the brain, functioning both as the support system but also as the immune system of the central nervous system (Miller, 2005). One important mechanism glia perform is to take up the excess neurotransmitter that remains in the synapse after the postsynaptic terminal has received the signal (Squire, 2013). This mechanism is essential for the prevention of hyperexcitation of the cells (Wetherington et al., 2008) and when it fails there is an influx of postsynaptic glutamate, as demonstrated in figure 1. The receptors primarily involved in the uptake of glutamate are EAAT1 and EAAT2. These are located on the astroglia and their function is to ensure that extracellular glutamate levels remain relatively low (Wetherington et al., 2008). Glia also have adenosine kinase (ADK), which regulates the concentrations of extracellular adenosine levels by phosphorylating it to a monophosphate version of itself. Inhibition of this enzyme in epileptic models suppresses seizure activity (Wetherington et al., 2008). Thus, we can infer that higher levels of extracellular adenosine kinase promote normal brain function. Therefore, any dysfunction in EAAT1/EAAT2 or overactivation of ADK can promote epileptic nature in neurons.

Glia also have an important role in maintaining the integrity of the blood brain barrier (BBB) and this is compromised in an epileptic model. Normally, Glia wrap their end-feet around endothelial cells and this allows them to help form and maintain the tight junctions that are hallmarks of the BBB (Squire, 2013). Additionally, the vasculature of the brain undergoes conformational changes in an epileptic model. The brain exhibits increased angiogenesis (blood vessel proliferation) as well as increased permeability of the BBB (Wetherington et al., 2008). The change in permeability arises due to the release of VEGF and proinflammatory molecules, which are released during an epileptic episode. Failure to extinguish these factors, in a timely manner, results in increased permeability of the BBB (Wetherington et al., 2008).

In conclusion, glia play a very crucial role in maintaining a healthy environment for neurons. Alterations in their ability to function properly, such as those previously discussed, will consequently alter neuronal excitability.

**Therapy**

Figure 2 outlines many possible areas for therapeutic targets. Because dysfunction of ion receptors and channels are often the cause of epileptic seizures, they are the main targets for therapeutic drugs. For ex-
ample, drugs that increase the amount of synaptic GABA have shown to be an effective antiepileptic drug (Treiman, 2001). One way they do this is by enhancing binding affinity of GABA for the GABA receptor, which increases the frequency of the opening of chloride channels. This increases the inhibitory affect of GABA on the neuron, decreasing excitability (Squire, 2013). Similarly, barbiturates prolong the length of time the chloride channels are open and therefore decrease excitability (Treiman, 2001). Another area for target is the postsynaptic glial uptake of synaptic glutamate. As seen in figure 2, the promotion of uptake of glutamate will decrease the amount of postsynaptic excitation, leading to amelioration of epilepsy. By decreasing the amount of glutamate released by the presynaptic terminal, you will get a similar effect. Additionally, by decreasing the amount of sodium flow into the neuron, hyperexcitation is limited and normal neuronal signaling is promoted. Some of these therapies are currently approved for clinical use, such as barbiturates. Though we only discuss drug therapies due to the focus of this article, there are more invasive therapies such as vagus nerve stimulation and deep brain stimulation. Though there are many possible treatments for an epileptic patient, there is no ultimate cure for the disease.

Conclusion

Epilepsy is a common disease experienced worldwide. There are many different types and causes of epilepsy. Although there is no cure, there are multiple treatments that are currently on the market. Some of these treatments target the dysfunctional ion flow in the neurons that cause over excitation, while others target the faulty glial reuptake of glutamate in the synapse. Epilepsy is caused by the hyperexcitability or hyper-synchronicity of neurons. Overstimulation of the neurons not only manifests as physical symptoms, mainly seizures, but it can also lead to excitotoxicity. Proper stimulation of the neuron leads to proper function, which is essential for a healthy brain.

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