Opinion

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Inflammatory pathways of seizure disorders

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Epilepsy refers to a cluster of neurological diseases characterized by seizures. Although many forms of epilepsy have a well-defined immune etiology, in other forms of epilepsy an altered immune response is only suspected. In general, the hypothesis that inflammation contributes to seizures is supported by experimental results. Additionally, antiepileptic maneuvers may act as immunomodulators and anti-inflammatory therapies can treat seizures. Triggers of seizure include a bidirectional communication between the nervous system and organs of immunity. Thus, a crucial cellular interface protecting from immunological seizures is the blood–brain barrier (BBB). Here, we summarize recent advances in the understanding and treatment of epileptic seizures that derive from a non-neurocentric viewpoint and suggest key avenues for future research.

Seizures and epilepsy

A seizure is a paroxysmal event due to an excessive, hypersynchronous (see Glossary) discharge from central nervous system (CNS) neurons or neuronal networks. This abnormal electrical activity causes a range of clinical/behavioral manifestations, ranging from dramatic convulsions often associated with loss of consciousness to experiential phenomena not readily discernible by an observer [1]. The term seizure should be carefully distinguished from epilepsy. Epilepsy is a syndrome of two or more unprovoked or recurrent seizures on more than one occasion. Epilepsy specifically refers to a condition in which a person has recurrent seizures due to a chronic or genetically predetermined underlying process, whereas seizures are symptoms of epilepsy or standalone manifestations of altered brain function also occurring in non-epileptics (due to drug overdose, alcohol withdrawal, etc.). Epileptic patients oscillate unpredictably between the 'ictal' state [seizures present, grossly abnormal electroencephalogram (EEG)] and the 'interictal' state (often no clinical symptoms, slight or no EEG changes).

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The orthodox view of epilepsy centers on neurons as the main culprit of seizures; targeting of neuronal ion channels, GABA, and glutamate receptors has been, for decades, the mainstream pharmacological approach to eradicate seizures. Although the ultimate effectors of seizures are neurons, recent advances in experimental neurology have revealed that inflammation can precipitate seizures or sustain seizure activity [2,3]. Two distinct inflammatory processes have been linked to seizures. Neuroinflammation is present in epileptic brain where it exacerbates seizures or increases their frequency [2,4]. By contrast, systemic inflammation can cause epileptiform neuronal discharge via loss of ionic (e.g., potassium [5–7]) and neurotransmitter (e.g., glutamate [7,8]) homeostasis. Although neuroinflammation directly affects neurovascular and glial function, the effects of systemic inflammation are mediated or facilitated by loss of BBB function [9]. BBB disruption (BBBD) can be triggered by a direct insult to the endothelium [10] or by systemic factors, including activation of circulating [11–15] leukocytes and release of molecular mediators that increase vascular permeability [16,17].

The discovery of the unexpected role of inflammation in epilepsy has changed our view on what factors contribute to seizures and may help elucidate why, in an epileptic brain, seizures occur rather infrequently and are interspersed by long intervals of relatively normal, 'interictal' neuronal activity [11,12,18–35]. In other words, an epileptic patient always has an 'epileptic brain' but rarely does this brain produce symptoms (seizures). With this in mind, it is not surprising that the etiological mechanisms underlying the development of an epileptic brain are not the same as those involved in the generation of seizures. The epileptic brain phenotype is the consequence of developmental, genetic, and molecular factors whereas the transition from interictal-to-ictal neuronal firing may be due to inflammation-driven changes in the neuronal environment and BBBD [36–38]. The pathophysiological rationale for this hypothesis is as follows: (i) 'static' or persistent, inherited, or acquired defects such as expression of abnormal ion channels [39] or malformations of cortical development [40] are unlikely triggers of seldom occurring seizures. These pathophysiological features are instead hallmarks of epilepsy and contribute to the epileptic pathology as a whole (i.e., mental retardation, psychiatric comorbidities, etc.); (ii) The epileptic brain often displays loss- or gain-of-function mutations, such as loss-of-function mutations in the sodium channel

Glossary

Cryptogenic, idiopathic, and symptomatic epilepsy: a cryptogenic or idiopathic disease is a disease with unknown etiology. In the case of epilepsy, these terms refer to patients where no genetic or metabolic disorder is identified and imaging (MRI) of the cortex and hippocampus does not reveal detectable abnormalities. The term symptomatic epilepsy is, by contrast, used to define an epileptic disorder due to a structural or metabolic condition, genetic or acquired, that has been demonstrated to be associated with a substantially increased risk of developing epilepsy. Lesional epilepsy is the antonym of cryptogenic and refers to patients with a distinct abnormality visible in MRI scans.

Hyperexcitable and hypersynchronous: epileptic seizures are characterized by increased neuronal excitability and hypersynchronous activity in the cortical network. The term 'hyperexcitable' refers to a neuron or to a neuronal network characterized by an increased probability of firing an action potential or a series of action potentials in response to a stimulus that normally elicits a subthreshold response or a single spike. Neuronal networks can oscillate between a resting and firing state activity in response to either intrinsic (pacemaker) properties or as a result of the activity of many neurons. In individual neurons, oscillations can appear either as oscillations in membrane potential or as rhythmic patterns of action potentials. Synchronized activity of large numbers of neurons occurs during epileptic seizures. The summation of electrical signals from this large assembly of neurons is the basis of the EEG appearance during a seizure, which is characterized by large amplitude (voltage) signals.

Ictogenic process, epileptogenesis: seizures are symptoms of epilepsy, a cluster of neurological diseases. Ictogenesis refers to the events leading to the development of a seizure, including the prodromic features named 'auras' and EEG changes that predict seizure onset ('ripples', 'slowing', etc.). Epileptogenesis refers to the events occurring during the often silent (no seizures) period between an insult (e.g., traumatic brain injury) and the development of a first seizure. The epileptogenic process may last days to years.

Immunological synapses: in analogy to the chemical synapse in neurons, the immunological synapse refers to the microenvironment hosting the interface between an antigen-presenting cell and a lymphocyte such as an effector T cell or NK cell. These immune–immune cell interactions are modulated by the presence of closely associated adrenergic or cholinergic nerve terminals.

Inflammatory reflex: this term refers to the neuronal circuits responsible for the control of systemic inflammation. In analogy to the regulation of heart rate by adrenergic and cholinergic nerves, the inflammatory reflex has a 'motor' component that either increases or decreases the activity of systemic inflammatory organs and cells. The inflammatory reflex is regulated by cytokines and other mediators of the immune response. The best understood inflammatory reflex consists of the anti-inflammatory effects of parasympathetic nicotinic synapses on organs such as the spleen. As in many cholinergic systems, opposing effects are achieved by muscarinic receptor activation.

Interictal, ictal EEG: the EEG associated with epileptic seizures (referred to as 'ictal', from *ictus*, Latin for 'stroke' or 'blow') is characterized by an abrupt change of the signal. Focal seizures are typically characterized by the appearance of local low-voltage fast activities progressively replaced by slower quasi-rhythmic activities often spreading to the neighboring regions. Between seizures, the EEG may appear normal or feature interictal epileptic abnormalities (e.g., spikes, sharp waves, slow waves) isolated or in brief discharges.

Seizure threshold: this term is used to describe how susceptible one is to seizures at a given time. Both internal and external factors and stimuli contribute towards this threshold. As described in this review, ions, transmitters, inflammatory mediators, and body temperature are examples of internal factors that alter the epileptogenic threshold. External stimuli may be sensory, electrical, or chemical. These are often used to trigger experimental seizures (kainic acid, electrical stimulation of the amygdala). A complex interaction between external and internal factors explains why precipitating events of comparable potency may or not trigger seizures.

Status epilepticus and super-refractory status epilepticus: according to the 'Glossary of Descriptive Terminology for Ictal Semiology' of the International League Against Epilepsy (ILAE), the term status epilepticus (SE) refers to a seizure that shows no clinical signs of arresting after a duration encompassing the great majority of seizures of that type in most patients or recurrent seizures without resumption of baseline CNS function interictally. The most common SE is generalized tonic–clonic SE, a potentially fatal condition associated with neuronal injury and respiratory and metabolic dysfunction. Although the ILAE does not define the minimum duration for a seizure to be defined as status, the operational definitions propose to start treatment when seizure activity continues beyond 5 minutes. Refractory SE is defined as SE that has not responded to first-line therapy with a benzodiazepine or second-line therapy, and which requires the application of general anesthesia. Super-refractory SE is defined as SE that has continued or recurred despite 24 hours of general anesthesia.

$Na_V1.1$, which cause severe myoclonic epilepsy of infancy (SMEI, or Dravet syndrome [39]); (iii) on an epileptic brain background, the transition from interictal to ictal neuronal firing is influenced by changes in ionic homeostasis (e.g., diminished action potential repolarization due to elevated extracellular potassium concentration ($[K^+]_{out}$), acute or transient antibody-mediated 'loss-of-function' (i.e., antibodies against voltage-gated potassium channels, glutamic acid decarboxylase, and glutamate receptors [41]), or altered glutamate uptake by astrocytes [39,42]. This hypothesis builds on several experimental reports linking BBBD, neuroinflammation, and epilepsy but also suggests a systemic inflammatory explanation for the neurovascular changes that trigger seizures. Thus, here we argue that although abnormal neuronal excitability and synchronization 'cause' seizures, the mechanisms responsible for abnormal neuronal firing may involve non-neuronal players such as the BBB.

Inflammatory mechanisms involved in BBB disruption

If BBBD is responsible for loss of CNS homeostasis and abnormal neuronal firing, how and when do BBB cells lose their physiological function? Owing to its intravascular location, the BBB is prone to incursions by circulating inflammatory signals [9,27,43–46]. These attacks could be facilitated by increased expression of adhesion molecules on endothelial cells seen in epileptic brain [47]. In addition to leukocyte–endothelial interactions, BBBD may also result from other factors. These are summarized in Figure 1 and Table 1 and reviewed in the following paragraphs.

Animal models of seizures typically rely on chemical or electric methodologies to induce an acute status epilepticus (SE) that may evolve into chronic seizures [14,24,48]. In these models, proinflammatory events leading to seizures have been shown to occur in the brain and peripherally (Figure 1 and Table 1). However, findings derived from experimental models have produced contrasting findings (e.g., [11,13,15,24,48,49]).

Targeting seizure-induced brain inflammation reduces seizure severity and number [2,50], whereas extravasation of proinflammatory molecules into the brain across a leaky BBB or their *ex novo* expression by brain cells is ictogenic or can exacerbate abnormal ictal activity [44,51]. The molecular players involved in seizure-related inflammation are often the same as those that participate in systemic inflammation. For example, altered brain expression of cyclooxygenase-2 (COX-2) and prostaglandin during seizures affects neuronal excitability [52] with a mechanism similar to inflammation-derived peripheral pain. Seizure-dependent neuronal COX-2 tilts the scale in favor of early neuroprotection but causes a delayed neurodegeneration of pyramidal cells (Table 1). The high-mobility group box (HMGB) proteins are immune activators that have multiple functions in the regulation of immunity and inflammation [53]. Blocking Toll-like receptor-4 (TLR-4) and HMGB-1 signaling decreases kainate-induced seizures [54].

Neurons are not the only brain cells to display an inflammatory phenotype in epileptic brain because other brain cells contribute to the seizure-related immune response (Table 1) [37]. Adhesion molecules (P- and E-selectin) are upregulated in response to electrographic seizures at the luminal side of the endothelium forming the BBB [47], chemokines and their receptors [chemokine (C–X–C