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Special Issue Reprint

Recent Advances in Pathophysiology and Therapeutic Approaches in Epilepsy

Edited by
Chandra Prakash, Deepak Sharma and Pavan Kumar

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Editorial

Recent Advances in Pathophysiology and Therapeutic Approaches in Epilepsy

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Epilepsy is a severe neurological disorder involving spontaneous and recurrent seizures, affecting a large number of people worldwide. According to estimates, about 150,000 patients are diagnosed with epilepsy every year, and at present, over 65 million people are affected by this debilitating neurological disorder [1]. The mechanism for the development and progression of epilepsy is diverse and is based on the epilepsy type and associated risk factors [2]. Current anti-seizure medications (ASMs) provide only symptomatic suppression of seizures, irrespective of pathophysiological alterations. Additionally, long-term usage of ASMs may result in adverse effects [3]. Thus, it is crucial to understand biochemical, structural, physiological, and molecular changes in epileptic brains. The search for novel therapeutic targets and treatment options is also warranted for diverse forms of epilepsy.

For this Special Issue, we invited authors with expertise in the pathophysiology and therapeutics of epilepsy to submit relevant, original research articles and review papers. We specifically sought articles addressing the progression of epilepsy in human patients and animal models, as well as those investigating new therapeutic approaches. As a result, twelve papers, including six research articles, five review articles, and one systematic review, were published in this Special Issue.

ASMs can cure the occurrence of seizures in children with epilepsy; however, some continue to have seizures and develop drug-resistant epilepsy (DRE) [4]. The retrospective study published by Liu et al. (contribution 1) found that resection surgery may be the most effective treatment option for DRE in children as it controls seizures and prevents intellectual disability. Nevertheless, patients for whom resection surgery was not an option could benefit from palliative surgery. The authors also suggested that children with DRE should receive treatment as soon as possible, regardless of the surgical treatment plan, to avoid developing brain damage and intellectual disability due to epilepsy.

Neuroinflammation is inherently accompanied by epilepsy, which can be targeted through disease-modifying medications [5]. Regarding this conjecture, Rabidas et al. (contribution 2) comprehensively reviewed the response of flavonoids to the neuroinflammatory process in different forms of experimental epilepsies. They concluded that flavonoids can reduce pro-inflammatory cytokines by modulating mediators like NF-kB and NLRP3 inflammasomes and other signaling molecules. Moreover, flavonoids can increase anti-inflammatory cytokines and decrease inflammatory mediators like COX-2, NOS, etc.; thus, they could be promising therapeutic agents for treating epilepsy.

Epilepsy occurring after a brain trauma is classified as post-traumatic epilepsy (PTE) and accounts for about 20% of total acquired epilepsy cases. Iron-induced epilepsy in rats is a widely used model for studying the pathophysiological mechanisms of PTE and its therapeutic interventions [6]. Research indicates that dehydroepiandrosterone (DHEA), an androgenic hormone, can be of therapeutic interest to epilepsy [7]. A research

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paper published by Prakash et al. (contribution 3) demonstrated that DHEA possesses antiepileptic and neuroprotective properties as it ameliorates astroglial activation, neuronal loss, and dendritic degeneration in the cortex and hippocampus regions of epileptic rats.

The mechanism of epilepsy appears to be connected with alterations in synapses, neurotransmitters, receptors, oxidative stress, mitochondria, cytokines, and apoptosis [8]. A review article by Madireddy and Madireddy (contribution 4) presented the availability of various therapeutic approaches, including ASMs and antioxidants, to treat epilepsy. Even though several ASMs are available, around one-third of patients with epilepsy continue to experience seizures. Individuals suffering from medically refractory epilepsy may experience reduced quality of life, cognitive impairments, and depressive symptoms. Other treatment options include surgery, neuromodulation, and dietary changes.

Patients with DRE with unilateral hemispheric pathology can benefit from surgical treatment, as previous reports show seizure freedom in patients with epilepsy [9]. The group of Del Gaudio et al. (contribution 5) published a case series reporting the safety and success of a modified vertical parasagittal hemispherotomy for hemispheric DRE. They suggested that this modified surgical procedure can help treat hemispheric DRE, as it has a high seizure freedom rate. Additionally, this procedure reduces postoperative hydrocephalus and improves patients' motor and cognitive outcomes.

Status epilepticus (SE) is a medical emergency characterized by seizures that are prolonged or happen quickly, one after the other, with no recovery time [10]. Daytime-restricted feeding (DRF) is a period of intermittent fasting that exerts anticonvulsant properties through metabolic activation, epigenetic mechanisms [11], anti-inflammatory, and neuroprotective effects [12]. A research paper published by Mercado-Gómez et al. (contribution 6) reported that DRF reduces oxidative stress via Nrf2-mediated upregulation of antioxidant enzymes in a pilocarpine-induced acute seizure model. Furthermore, they demonstrated that DRE activates Nrf2 in astrocytes and can be used as a possible adjuvant treatment for SE.

When ASMs fail to control seizures on SE, they proceed to refractory SE, which further leads to super-refractory SE (srSE) if the seizures prolong longer than 24 h despite anesthesia [13]. A systematic review by Stavropoulos et al. (contribution 7) discussed how srSE in children may benefit from neuromodulation treatments such as deep brain stimulation, vagus nerve stimulation, and electroconvulsive therapy. Additionally, they recommended that, to avoid long-term neurologic complications, neuromodulation treatments should be considered at earlier stages of epilepsy.

The most common type of self-limited focal epilepsy is called self-limited focal epilepsy with centrotemporal spikes (SeLECTS). It is responsible for 6–7% of childhood epilepsy cases and affects children who had normal brain MRIs before seizure onset [14]. SeLECTS is an excellent model for examining the effects of interictal epileptic discharges (IEDs) on cognition due to its frequent and regular IEDs, low requirement for ASMs, and infrequent seizures [15]. A retrospective study that explored the relationship between IEDs and cognitive function was conducted by Dontaine et al. (contribution 8) on the SeLECTS cohort. The study showed that visuospatial skills for neuropsychological evaluations and qualitative EEG assessing the diffusion of focal spike waves to other brain regions should be included with standard quantitative EEG indices.

Transition readiness refers to the degree to which patients and members of their support system (parents, healthcare providers, etc.) can successfully transition from child-centered to adult-oriented healthcare [16]. Vacca et al. (contribution 9) proposed a comprehensive framework encompassing clinical and psychological aspects associated with the transition from childhood to adult medical care in patients with epilepsy. They concluded that increasing awareness of transition readiness is crucial to helping patients with epilepsy and their parents develop self-management skills. Anticipating the transition period may be useful in preventing problematic sleep patterns and fostering independence in health care management. Thus, the parents of patients with epilepsy and other rare

disorders should be examined for their mental health, which can have an impact on their children's well-being.

The development and progression of seizures involve multiple brain regions; thus, epilepsy can be considered a "network disease" [17,18]. A better understanding of epilepsy networks can help clinicians plan how to disrupt these networks and enhance surgical results. A review paper by Hines and Wu (contribution 10) demonstrated that exploring epilepsy networks enhances the understanding of pathophysiological developments and surgical outcomes. The removal of nodal networks implicated in epilepsy lowers the risk of seizure recurrence. The neuromodulation of various targets reduces seizure frequency while allowing exploration of important brain circuitry and white matter pathways. Finally, invasive diagnostics, including EEG, can provide critical information about epilepsy networks that helps guide future surgical decisions.

Another review article by Boleti et al. (contribution 11) discusses that though surgery is the most effective option for achieving long-term seizure freedom, it is only an option for patients who are unable to control their seizures with medication. Alongside medication intervention, non-pharmacological strategies can also be used; these include invasive and non-invasive neuromodulation therapies, a ketogenic diet, etc. Overall, a deeper understanding of the pathophysiology and etiology of epilepsy is necessary to identify new targets and clarify those already recognized, allowing for the development of newer treatments with fewer side effects and an even smaller influence on comorbidities.

Interictal spikes are inherently associated with behavioral and cognitive deficits in epilepsy as well as other psychiatric diseases [19]. However, most animal models exhibit both spikes and seizures, making it difficult to determine the precise role of interictal spikes in seizures and behavior. In this context, a contribution made by Eslami et al. (contribution 12) profoundly reviewed the investigations conducted on an interictal spiking model developed by tetanus toxin. Furthermore, they discussed that, although ASMs can decrease seizures, there is a scarcity of treatments targeting spike activity. The potential therapeutic targets can be identified for epileptic-spiking brain regions on the tetanus toxin model, which enables us to evaluate promising, novel treatments for clinical translation.

In summary, the articles collected in this Special Issue show the current research in the area of pathophysiological and therapeutic advancements in epilepsy. We believe that this Special Issue will significantly expand our current understanding of this field and help us develop novel treatment approaches for patients with epilepsy.

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Conflicts of Interest: The author declares no conflicts of interest.

List of Contributions

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Review

Modeling the Interictal Epileptic State for Therapeutic Development with Tetanus Toxin

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Abstract: Focal forms of epilepsy can result from a wide range of insults and can vary from focal symptoms to generalized convulsions. Most drugs that have been developed for epilepsy focus on the prevention of seizures. On Electroencephalography (EEG), seizures are characterized by a repetitive buildup of epileptic waveforms that can spread across the brain. Brain regions that produce seizures generate far more frequent 'interictal' spikes seen between seizures, and in animal models, these spikes occur prior to the development of seizures. Interictal spiking by itself has been shown to have significant adverse clinical effects on cognition and behavior in both patients and animal models. While the exact relationships between interictal spiking and seizures are not well defined, interictal spikes serve as an important biomarker that, for some forms of epilepsy, can serve as a surrogate biomarker and as a druggable target. While there are many animal models of seizures for drug development, here we review models of interictal spiking, focusing on tetanus toxin, to study the relationship between interictal spiking, seizures, cognition, and behavior. Studies on human cortical regions with frequent interictal spiking have identified potential therapeutic targets; therefore, having a highly consistent model of spiking will be invaluable not only for unraveling the initial stages of the pathological cascade leading to seizure development but also for testing novel therapeutics. This review offers a succinct overview of the use of tetanus toxin animal models for studying and therapeutic development for interictal spiking.

Keywords: animal epilepsy models; interictal spiking; therapeutic epileptiform activities

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1. Introduction

Epilepsy is a common neurological condition characterized by spontaneous seizures and associated with many behavioral comorbidities [1]. Focal epilepsy stands out as a prominent subtype among epilepsies due to its localization within specific brain regions, making it suitable for surgical resections. During seizure episodes, epileptic activity may initially remain confined to this localized area or propagate to involve a larger brain volume, potentially extending across both hemispheres and resulting in a secondary generalized seizure [2,3]. However, seizures are relatively infrequent occurrences, posing challenges in identifying epileptic foci based on seizure onset. Emerging evidence indicates that abnormal brain networks extend beyond seizure events, manifesting more frequently in interictal periods [4,5]. Among these interictal events, interictal spikes are characterized as brief electrographic transients lasting less than 200 milliseconds, featuring a short sharp wave followed by a sustained slow wave [5,6]. In focal epilepsy, interictal spiking does not follow a regular pattern and typically occurs at a frequency of less than 3 Hz. This contrasts with the spike-and-wave complexes seen in genetically generalized epilepsies. Interictal spikes are detected in over 90% of individuals with epilepsy during repeated electroencephalogram (EEG) monitoring [7]. There is evidence suggesting that, following a brain injury, spikes may often emerge before seizures, implying a potential role in facilitating epileptogenesis. This notion gains further support from studies demonstrating that the surgical removal

of areas exhibiting high spike activity, along with seizure-generating regions, correlates with improved surgical outcomes [8]. Our understanding of the dynamic network of these events and their relationship to seizures remains limited. While interictal spikes commonly originate from synchronously firing neurons near or at seizure onset zones, they are also observed in cortical regions distant from seizure initiation. Furthermore, beyond their role as biomarkers of epileptic brain regions, interictal spikes may exert an independent impact on behavior. They are found across a spectrum of neuropsychiatric disorders, including anxiety, attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), obsessive–compulsive disorder, depression, and schizoaffective disorder, even in the absence of seizures [9–14].

The hallmark of epilepsy is the sudden, synchronous firing of numerous neurons, leading to various pathological psychomotor manifestations. The mechanisms underlying epileptic activity are complex and involve multiple cellular and molecular processes. One contributing factor is the dysregulation of extracellular potassium levels, which can influence neuronal excitability and seizure propagation. For instance, the astrocytic syncytium helps regulate extracellular potassium, mitigating its impact on neural circuits. Understanding these and other mechanisms is crucial for developing more effective treatments for epilepsy [15]. At present, our clinical inventory for managing epilepsy primarily revolves around medications aimed at suppressing seizures, but they do not offer curative solutions. Despite considerable endeavors, current anti-seizure drugs exhibit restricted effectiveness in halting the development of epilepsy, along with considerable, often life-long side effects [16]. Furthermore, the widespread occurrence of pharmacoresistance and the enduring presence of comorbidities following seizure management emphasize the crucial need for a deeper and more comprehensive understanding of the underlying mechanisms. Although certain anti-seizure drugs have demonstrated efficacy in suppressing spikes, there is currently a lack of medications specifically designed to target spike activity. This enhanced understanding is crucial for driving the development of innovative medications and therapeutic strategies capable of effectively addressing both seizure activity and the associated comorbid conditions [17].

Reliable animal models are critical to facilitate the transition from experimentation to human clinical trials and provide opportunities to enhance our understanding of the underlying pathophysiology and mechanisms of focal forms of epilepsy and interictal spiking. Animal models additionally provide insight into the pharmacokinetics, side effects, potency, efficacy, and tolerance of potential therapeutic agents under investigation. In rodents, chronic epilepsy can be effectively induced through various methods, including the administration of kainic acid (KA), pilocarpine, kindling, and intracerebral injection of tetanus toxin (TeNT). Both the pilocarpine and KA models involve the administration (either systemic or intracerebral) of chemoconvulsant agents to trigger status epilepticus (SE) [18]. These models exhibit a high mortality rate, particularly during the SE period, and result in significant neuronal loss across the cortex [19,20]. In contrast, the kindling model employs repeated electrical stimulation to induce seizures. However, it is challenging to evoke spontaneous seizures with this model, often necessitating electrical stimulation to precipitate seizures [21]. Kindling also induces neuronal damage, leading to a loss of up to 50% of hippocampal neurons [22]. Another acute model of focal cortical seizures involves administering repeated doses of 4-aminopyridine (4-AP). This approach induces repetitive seizures that originate at the injection site and spread to the contralateral hemisphere, leading to tonic-clonic seizures that escalate in severity over time. Although the seizures provoked by 4-AP are severe, their effects are transient [23]. Conversely, the TeNT model can be employed to replicate either temporal lobe epilepsy or neocortical epilepsy. This is achieved by administering an intracerebral injection of TeNT to induce seizures without triggering status epilepticus. Additionally, this model has the capability to induce chronic interictal spiking following a single neocortical TeNT injection without any seizure development. Similar to the 4-AP model, contralateral spread to the opposite hemisphere is also seen with TeNT. One notable advantage of the TeNT model lies in its ability to

maintain cortical cytoarchitecture. Neurons remain intact following toxin injection, thereby reducing the potential interference of neuron loss and neuroinflammation associated with cell death [24–27].

Here, we present the development of the TeNT animal model for studying and developing therapeutics for interictal spiking. Additionally, we explore novel therapeutic approaches for interictal spiking in preclinical studies.

2. Interictal Spiking Has Highly Reproducible Propagation Patterns and Adversely Affects Cognition and Behavior

Preclinical investigations have revealed that interictal spiking often appears prior to the development of subsequent seizure activity following brain injury [28]. Spike rates can also serve as valuable biomarkers, aiding in seizure prediction. However, it remains unclear whether spikes directly promote or inhibit seizures. Long-term electroencephalography recordings reveal a significant correlation between the probability distributions of spikes and seizures, indicating interconnected processes. A surprising decrease in spike rate prior to seizures suggests spikes may not directly trigger seizures. Nevertheless, the parallel distributions imply potential interactions, with spikes potentially inhibiting seizures or signaling impending seizure activity [29].

Spikes are not isolated but propagate throughout the neocortex and hippocampus with highly reproducible patterns [30]. From human intracranial grid recordings, we found that interictal spikes spread throughout the epileptic neocortex in highly stereotypical patterns, revealing consistent and personalized propagation patterns within each patient. Spikes were observed to traverse various frequency bands, providing novel insights into cortical structure and spike dynamics. Local propagation was predominant, with sporadic long-distance transmission hindered by the central sulcus [30]. Surprisingly, brain regions with the highest spike occurrence did not consistently initiate the spikes but received propagating spikes from multiple brain areas [5]. This was true both in the human neocortex and in the hippocampus, where patients with foramen ovale electrodes had interictal spike networks that were remarkably consistent over time and across various frequency bands within the temporal lobe [31]. These networks often showed 'reverberations' between nearby brain regions and were closely associated with seizure onset zones and structural lesions. Additionally, only a small subset of mesial temporal spikes were associated with cortical spikes but lacked a distinct pattern of propagation [31].

Interictal spikes, commonly observed in patients diagnosed with epilepsy, are often linked to cognitive and behavioral comorbidities. Research suggests that approximately 50% of spikes may induce temporary cognitive impairment [32,33]. In adults with epilepsy, spikes have been shown to hinder memory retention and word retrieval, delay reaction times, and increase the risk of accidents in virtual driving simulations. Notably, individuals with temporal lobe epilepsy often exhibit a negative correlation between spikes and executive functioning, particularly verbal fluency [34–37]. Furthermore, a higher spike frequency has been associated with lower intelligence quotient scores, indicating a potential biomarker for cognitive impairment in adult epilepsy patients [38]. In children, spikes can impair arithmetic skills and reduce attention and processing speeds [39]. Centrotemporal spikes originating near the hippocampus have been found to impede both short- and long-term declarative memory abilities [40]. Additionally, children with left-sided spikes may experience more pronounced impairments in reading performance compared to those with right-sided spikes, suggesting a predictive value of spike location for functional deficits [41]. However, persistent spiking-induced neuronal synchrony can disrupt cortical function in distant regions connected to the spike-onset zone, potentially resulting in widespread deficits despite localized spiking [42].

A number of animal models have corroborated these human findings, showing that interictal spikes are associated with impairments in short-term memory, spatial memory, and object recognition [43,44]. In a rodent model of temporal lobe epilepsy, spikes were specifically found to impair memory retrieval without affecting memory encoding or

maintenance [45]. Spikes induced during early life can have lasting effects into adulthood, leading to impaired performance on spatial memory tasks, reduced long-term potentiation, and persistent deficits in sociability and attention [46]. In our studies, we refined a chronic rat model that predominantly induces interictal spiking following the injection of TeNT into the rat somatosensory cortex. Animals exhibiting interictal spiking are hyperactive compared to control animals. Furthermore, the level and type of locomotor activity correlate with the intensity and location of their spiking [47,48]. In a recent investigation, we established that treatment of animals with MAPK inhibitors following TeNT injection into the somatosensory cortex resulted in reduced interictal spikes, alleviated microglial activation, mitigated the loss of inhibitory neurons, and led to improved cognition [49]. Interestingly, a significant decrease in high-amplitude, short-duration spikes in animals treated with MAPK inhibitors demonstrated enhanced spatial memory performance on the Barnes maze.

3. Tetanus Toxin: An Ideal Model to Study the Development of Focal Epilepsy and for Drug Development

The TeNT model holds several advantages over other experimental models of chronic focal epilepsy because of its ability to induce spontaneous, chronic seizures and epileptiform activity in numerous higher regions of the brain without causing substantial neuronal loss, tissue damage, or other significant alterations to neurological structures [24,50,51]. Chronic, focal neocortical epilepsy can be produced by direct injection of TeNT into the gray matter of specific brain regions, resulting in spontaneous recurrent seizures and epileptiform activities [52]. Seizures can be induced in a dose-dependent manner with TeNT and are typified by myoclonic movements of the forelimbs [53]. The toxin appears to exert its effects by binding to neuronal cells through an interaction in the plasma membrane. Its light chain functions as a zinc protease that selectively targets vesicle-associated membrane protein (VAMP; synaptobrevin), while the heavy chain facilitates its uptake into neurons. Since VAMP is crucial for synaptic transmission, its proteolysis by TeNT disrupts synaptic function [54–56]. The toxin moves by anterograde axonal transport to synapses, where it acts to presynaptically block the release of inhibitory neurotransmitters, most notably gamma-aminobutyric acid (GABA) and glycine [57]. This action reduces inhibitory control over motor and sensory neurons, leading to increased neuronal excitability. The imbalance between excitatory neurotransmitters (e.g., glutamate) and inhibitory neurotransmitters (e.g., GABA) may result in epileptic seizures [58–60]. Additionally, tetanus toxin can activate microglia, triggering inflammation that further disrupts inhibitory control and enhances the potential for epileptic activity [49].

While these are acute effects of the toxin, TeNT's long-lasting effects are what enable it to be useful in studying chronic epilepsy development following a single injection in a specific brain region. The earliest study that used TeNT demonstrated that it was an effective agent capable of producing a chronically active, focal discharging lesion in the cortical brain regions of dogs. Major recurrent convulsions and abnormal spiking first appeared in the tetanus-injected dogs between two and seven days post-injection and persisted, in some cases, for over a month [61]. Subsequent studies repeatedly demonstrated the successful application of TeNT to numerous telencephalic regions, including the cat motor cortex [62] and rat hippocampus [24]. Injection of TeNT into the rat's hippocampus resulted in alterations in brain function and the occurrence of spontaneous seizures in the absence of status epilepticus. Approximately one month after administration, spike-wave activity, characterized by frequencies ranging from 3 to 20 Hz, was observed in the EEG [52]. Following the injection of a small amount of TeNT into the rat neocortex, excessive synchronization of neuronal activity occurred. This manifested as spontaneous paroxysmal field potentials and/or evoked all-or-none population burst discharges in parietal and temporal areas of both the injected and contralateral hemispheres, starting as early as 16 h after injection and persisting up to 7 months [63]. In another mouse model, unilateral injection of TeNT into the visual cortex was monitored using two electrodes placed into either side

of the visual cortex for one-hour local field potential (LFP) recordings. Recordings were performed three days after the surgery and continued for up to 45 days. Utilizing nonlinear time series analysis methods, it was demonstrated that TeNT injection into one hemisphere significantly influenced the local electrical activity of neural populations in both the injected and the opposite hemisphere [64,65].

We have established and characterized a TeNT model of chronic, focal interictal spiking in rats optimized for long-term video EEG recordings and behavioral analyses (see Figure 1). This model involves the injection of TeNT into the somatosensory cortex of rats and results in a prolonged latent period spanning several weeks before the onset of spontaneous spikes and seizures. During this latent period, there is a gradual development and progression of interictal spiking (from 10 spikes per hour on day 5, increasing to 180 spikes per hour after 30 days), providing an opportunity to investigate the development and clinical consequences of neocortical spiking in the absence of seizures. Unlike other animal models of epilepsy that have significant and often diffuse brain injuries, this model offers a unique vantage point to view the specific effects of both spike and subsequent seizure development. This model nicely replicates the latency period observed in human focal epilepsy before the onset of spontaneous seizures [50].

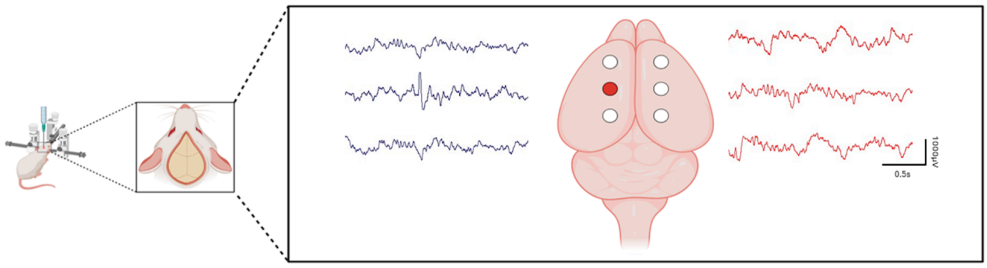

Figure 1. Electrode placement and spike localization in a rat model of interictal spiking generated from a single TeNT injection into the somatosensory cortex. Schematic of the stereotactic surgery illustrates the placement of six recording electrodes. Sample EEG traces demonstrate the localization of interictal spikes. The field is most prominent over the injection site but is also detectable to a lesser extent in adjacent leads. The electrode positioned over the injection site is marked by a red circle. Scale bars: 0.5 s (horizontal) × 1000 μV (vertical).

Depending on the cortical injection site, the TeNT model can be used to generate either spikes alone or spikes and seizures [50,66]. Using the exact same protocol for injecting TeNT and epidural electrode implantation, we compared the effects of somatosensory and motor cortex injection sites. Unlike the somatosensory cortex-injected animals, which only displayed spiking activity without the presence of spontaneous seizures, the motor cortex-injected animals developed both spikes and spontaneous seizures. These results confirm the observations from previous studies using rat models involving TeNT injections into the somatosensory cortex and motor cortex [48]. While both somatosensory cortex- and motor cortex-injected animals exhibited abnormal spiking, it occurred in different brain regions. Contralateral spiking was present in both somatosensory cortex and motor cortex-injected animals. However, a primary spiking focus was more likely to develop contralaterally to the injection site in motor cortex-injected animals [48]. Not surprisingly, animals with spiking-only or spiking-plus seizures also displayed marked differences in behavior. While somatosensory cortex-injected animals showed hyperactive behaviors, those with motor cortex injections and seizures were hypoactive. In both models, spikes were not restricted to the injection site, but spiking in specific brain regions correlated with specific locomotor behaviors.

In summary, depending on the location of the injection site and the amount of TeNT injected, the TeNT model offers wide-ranging opportunities to study the development of both interictal spikes and seizures. An important take-home message from these studies is that while the toxin has early effects that appear to be localized to the injection site, the

downstream epileptogenic process can involve both this site and other ipsilateral as well as contralateral network sites throughout the brain.

4. Identification of Novel Therapeutics against Interictal Spiking from Human Epileptic Tissues

Studies on the human interictal spiking cortex of patients undergoing surgery for refractory seizures offer a unique opportunity to identify novel therapeutic targets for spiking from human tissues removed to control seizures [28]. Differential gene expression from human epileptic, spiking, and non-spiking brain regions has generated a 'pipeline' of novel therapeutic targets that selectively target interictal spiking.

Ontological analysis of these differentially expressed genes pointed to the activation of the mitogen-activated protein kinase pathway (MAPK) in the superficial layers of the neocortex (layers 1–3) in the high-spiking human cortex [67], suggesting inhibiting MAPK signaling as a potential therapeutic intervention [28,66,67]. In addition, small patches of an endogenous MAPK inhibitor gene called dual specificity phosphatase 4 (DUSP4) were expressed in the same layers 2/3 of the epileptic neocortex and were associated with a significant reduction in MAPK genes at these sites. Consistently, in vitro studies on the human neuronal-like cell line (Sh-SY5Y) demonstrated that DUSP4 acts as a potent and transient MAPK antagonist, is induced rapidly after repeated depolarizations, and is dependent on MAPK signaling. Overall, these findings suggest that DUSP4 functions as an activity-dependent, negative feedback inhibitor of MAPK signaling expressed in focal brain regions, potentially serving as a localized, endogenous inhibitor for the propagation of epileptic signaling [68]. Bioinformatic and genomic studies have also revealed a group of long non-coding RNAs (lncRNAs) co-regulated with MAPK genes in the human epileptic neocortex. Some of these lncRNAs were directly regulated by MAPK signaling, while other lncRNAs induced in spiking regions specifically downregulated the expression of their antisense coding genes [69]. Building upon these findings, our investigations extended into experimental validation studies using the TeNT model [49].

5. Testing Potential Therapeutics That Target Interictal Spiking Using the TeNT Model

The key to the successful translation of potential therapeutics to patients requires animal models that closely parallel the human condition. We have found that the TeNT model of interictal spiking closely parallels human neocortical epilepsy [49,66]. The TeNT model shows a gradual development of spiking with activation of MAPK/CREB signaling pathways in the same neuronal lamina (layers 2/3) as well as many of the downstream genes found in human cortical spiking brain regions. Using this model, a highly specific MAPK inhibitor effectively prevented the onset of epileptic discharges following TeNT injection into the neocortex, with no discernible side effects on brain activity. This experimental outcome underscores the pivotal involvement of the MAPK signaling pathway in the genesis of epileptic activity and highlights the potential of MAPK inhibition as a promising therapeutic intervention [66].

The effects of narcotics on seizure or spiking activity are evident both in the hemisphere where the seizures or spiking are induced and in the contralateral hemisphere. In a more recent study using a different MAPK inhibitor, we demonstrated that a one-week administration of CI-1040, targeting MAP2K, reduced spike frequency and was associated with improved behavior when given either directly after the TeNT administration or two weeks later [49]. In addition to reducing interictal spike occurrence, the treatment prevented the loss of inhibitory neurons and other neuronal and microglial changes seen both in human cortical spiking regions and in the TeNT model. Importantly, animals treated with CI-1040 exhibited a significant decrease in high-amplitude, short-duration spikes, which positively correlated with improved spatial memory performance on the Barnes maze [49].

6. Conclusions

The TeNT animal epilepsy model can generate a diverse repertoire of conditions that lead to the development of spike and seizure networks. Many of the changes seen in this animal model closely parallel the human condition and help us enhance our understanding of epilepsy and develop new therapeutics. Interictal spikes have been linked to cognitive and behavioral impairments in patients with epilepsy and those with non-epileptic psychiatric disorders. However, understanding the specific role of interictal spikes has been challenging due to most animal models exhibiting both spikes and seizures, hindering the study of spiking's relationship with behavior in a seizure-free context. To address this, our laboratory has developed a rat model of interictal spiking wherein rats display high levels of spiking activity without seizures. This model utilizes tetanus toxin injected into the somatosensory cortex to induce consistent and reproducible spikes. Although some anti-seizure drugs have demonstrated the ability to suppress spikes, there is currently a lack of drugs that explicitly target spike activity. Identification of potential therapeutic targets from differential gene expression profiles originating from human epileptic brain-spiking brain regions paired with a highly comparable TeNT model can enable the ability to test promising new therapeutics for clinical translation.

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How the Spreading and Intensity of Interictal Epileptic Activity Are Associated with Visuo-Spatial Skills in Children with Self-Limited Focal Epilepsy with Centro-Temporal Spikes

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Abstract: This paper investigates brain–behaviour associations between interictal epileptic discharges and cognitive performance in a population of children with self-limited focal epilepsy with centro-temporal spikes (SeLECTS). Sixteen patients with SeLECTS underwent an extensive neuropsychological assessment, including verbal short-term and episodic memory, non-verbal short-term memory, attentional abilities and executive function. Two quantitative EEG indices were analysed, i.e., the Spike Wave Index (SWI) and the Spike Wave Frequency (SWF), and one qualitative EEG index, i.e., the EEG score, was used to evaluate the spreading of focal SW to other parts of the brain. We investigated associations between EEG indices and neuropsychological performance with non-parametric Spearman correlation analyses, including correction for multiple comparisons. The results showed a significant negative correlation between (i) the awake EEG score and the Block Tapping Test, a visuo-spatial short-term memory task, and (ii) the sleep SWI and the Tower of London, a visuo-spatial planning task ($p_{corr} < 0.05$). These findings suggest that, in addition to the usual quantitative EEG indices, the EEG analysis should include the qualitative EEG score evaluating the spreading of focal SW to other parts of the brain and that neuropsychological assessment should include visuo-spatial skills.

Keywords: IED and cognition; visuo-spatial skills; EEG score

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1. Introduction

The link between interictal epileptic discharges (IED) and cognition has mostly been studied within the framework of self-limited focal epilepsy (SeLFE), more specifically in self-limited focal epilepsy with centro-temporal spikes (SeLECTS). This frequent epileptic syndrome accounts for 6 to 7% of all childhood epilepsies and affects children with normal cerebral MRI and development prior to seizures. Age at onset is usually between 3 to 14 years. EEG usually shows characteristic triphasic high-voltage spike-and-wave complexes that are typically located in the centro-temporal area. These abnormalities usually activate in drowsiness and sleep. Typically, most patients present rare, brief, nocturnal focal seizures involving orofacial and brachial regions and normally resolved by puberty [1]. Because of infrequent seizures, typical and frequent IED and a low need for anti-epileptic

drugs, SeLECTS constitute an ideal model to study IED impact on cognition. At the most severe end of the same continuum, this link between IED and cognition is illustrated by the concept of epileptic encephalopathy with continuous spike and waves during sleep (EE-CSWS), defined as severe global or task-specific cognitive regression associated with almost continuous and diffuse IED during sleep. Because this encephalopathy may arise as a complication of SeLFE, these two entities are part of the same spectrum [2–4]. Interestingly, cases of Landau–Kleffner syndrome (i.e., an EE-CSWS with specific language regression in the form of auditory agnosia) are reported in patients without seizure [5]. At the opposite end of the spectrum, a SeLECTS EEG pattern is more frequently present in patients diagnosed with a developmental language disorder or attention-deficit/hyperactivity disorder than in the general population [6–8]. This suggests a close relationship between IED and cognition rather than an impact of seizures or anti-epileptic drugs.

Children with SeLECTS are at higher risk of developing learning disorders and academic difficulties, which are for the most part reversible after epilepsy remission [9]. Despite the common assertion that SeLECTS patients display a normal-ranged intellectual quotient (IQ), it appears statistically inferior to healthy peers [10,11]. Even if mean IQ may be overestimated by excluding patients with an IQ below 80 from most studies, it seems that, through a lack of sensitivity, exclusive analysis of IQ fails to adequately apprehend cognitive impairment in these patients [9]. Through a more comprehensive neuropsychological assessment, the literature concludes that SeLECTS is associated with an elevated frequency of language deficits, behavioural disturbances, attention-deficit/hyperactivity disorder, as well as verbal and non-verbal memory, attentional processes and executive functions impairment, with no typical cognitive pattern [9].

Associations have already been shown in SeLECTS between IED intensity, defined by quantitative parameters in wake and/or sleep EEG and academic or behavioural problems [12], specific learning disorders [13], verbal memory [14,15], verbal IQ [16], word and sentence reading [16], performance in selective visual attention [17] or central information processing speed [18].

Fewer authors used qualitative parameters to measure IED intensity, such as Massa et al., who isolated five EEG and clinical criteria predictive of complicated evolution (i.e., academic and behavioural problems) in SeLECTS [19]. In 2021, a case–control study driven by our team showed that a qualitative EEG score inspired by the aforementioned Massa study and focusing on IED spreading offered better sensitivity, specificity and agreement between readers with different levels of expertise than the usual quantitative indices (i.e., Spike Wave Index and Spike Wave Frequency) to differentiate EE-CSWS from typical SeLFE [20].

While the association between SeLECTS and possible language impairment is now broadly accepted, data regarding visuo-spatial abilities are still scarce. In this matter, poorer visuo-spatial performance were reported in children with SeLECTS compared to their healthy peers [9,21–23]. The few studies investigating the link between these skills and IED intensity showed an association between visuo-spatial memory and sleep Spike Wave Index (SWI) or awake Spike Wave Frequency (SWF) [24–26].

To date, no studies have explored the link between IED and cognition in a continuous approach within a population of children, including the whole SeLECTS continuum, from learning disorders without any history of seizures to EE-CSWS. Moreover, there are no guidelines in the literature regarding the EEG index that should be used to evaluate the epileptic activity impact on cognitive performance. The objective of this study was to answer the three following questions: first, in a population of children with SeLECTS, is there an association between IED intensity and cognitive performance, evaluated through a comprehensive neuropsychological assessment? Second, can this association not only be found in sleep but also in wakefulness? Finally, could a qualitative EEG analysis, focusing on IED spreading instead of intensity, show associations between cognitive functioning and interictal epileptic activity?

2. Materials and Methods

This retrospective study was approved by the Ethics Committee of the Hôpital Universitaire Des Enfants Reine Fabiola (HUDERF), Brussels, Belgium (CEH 59/22).

2.1. Patient Selection

Patients with SeLECTS were identified from the database of patients who underwent a long-term EEG in an epilepsy investigation unit in HUDERF between 2016 and 2022.

The patients had to fulfil the following inclusion criteria: (a) a SeLECTS EEG pattern (i.e., triphasic high-amplitude spike-and-wave complexes in the centro-temporal regions, activated in drowsiness and sleep [1]); (b) age range 6 to 12 years; (c) standardised neuropsychological assessment less than six months apart from EEG; (d) normal cerebral MRI; (e) normal neurodevelopment, defined as walking independently before 18 months, first words spoken before 18 months and first sentences before 3 years of age; and (f) no history of treatment with corticosteroids before the analysed period. The fulfilment of these criteria was determined by analysing their medical file.

Patients' files were analysed to identify the presence of a neurocognitive regression in at least two domains of development (i.e., language, behaviour, learning, memory, attention, social interactions, motor skills and global intelligence, as assessed through comprehensive neuropsychological evaluation). Language regression was defined as a regression in at least three of the language domains (i.e., phonology, morphology, syntax, semantics and pragmatics) without auditory–verbal agnosia, and frontal syndrome was defined as behavioural disturbances with a combination of attention impairment, impulsiveness, mood swings and perseveration with deficits in reasoning, thought formulation and learning strategy [27].

2.2. EEG Analyses

All EEG studies were carried out using 21 scalp electrodes according to the International 10–20 system, with the BrainRT system (OSG, Belgium). Extracts of 20 min wakefulness and the first 10 min of NREM sleep were created from long-term EEG studies for each patient by author P.D. These two files were randomly and blindly renamed by a medical doctor not participating in this study.

Each segment was then scored using three EEG indices: (1) the Spike Wave Index (SWI), corresponding to the percentage of spike and wave (SW) activity calculated by dividing the number of seconds demonstrating one or more spike-and-wave complexes in the 20 min awake period divided by 1200 s, or in the 10 min period of sleep divided by 600 s, multiplied by 100 to express the results as percentages; (2) the Spike Wave Frequency (SWF), corresponding to the number of spike-and-wave complexes in the first 100 s of the EEG; (3) a qualitative EEG score that was created by Aeby et al. in 2005, based on the Massa study [19], focusing on the background (normal or abnormal, i.e., intermittent slow wave focus in wakefulness and/or abnormal or absent spindles in sleep), the number of epileptic foci and their diffusion to other electrodes. Five grades are defined: grade 0 (normal EEG); grade 1 (normal background, unique focus of SW of low amplitude); grade 2 (normal background, multiple ($\geq$2) asynchronous foci of SW of low amplitude); grade 3 (normal or abnormal background, high-amplitude SW diffusing to one hemisphere or multiple asynchronous foci of SW of high amplitude or unique focus of high amplitude SW with a mirror focus); and grade 4 (abnormal background, high-amplitude SW diffusing to more than 80% of the electrodes) [28].

Quantitative indices (i.e., SWI and SWF) were determined by a previously published automated spike detection algorithm [29,30]. Two physicians (P.D. and A.A.) independently determined the qualitative EEG score. This EEG score was analysed with the same parameters for each patient of this study: bipolar montage, time constant of 10 s, amplitude of 100 μV/cm, high band filter of 0.3 Hz and low band filter of 70 Hz.

2.3. Neuropsychological Testing

All of the participants underwent a comprehensive neuropsychological assessment, developed by our team in 2016 after an extensive literature review, designed to screen SeLECTS patients for cognitive complications. The tests were performed by two experienced neuropsychologists (S.G. and S.B.). The following functions were assessed: (1) verbal short-term memory, with the WISC-V Digit Span subtest, in which the child has to repeat number lists of increasing length, forward and backwards [31]; (2) verbal long-term memory, evaluated by the RLS-15, a task where the subject must memorize a list of 15 words, with 5 free recalls and a delayed recall after 20 min [32]; (3) visuo-spatial short-term memory with the Block Tapping Test, assessing the ability to reproduce visuo-spatial sequences of increasing length by touching specific blocks placed in a two-dimensional grid [33]; (4) attention through the Test of Attentional Performance, a computerized task measuring the reaction time and its variations to a visual stimulus, with (phasic alert) or without (tonic alert) an auditory warning [34]; (5) executive functions, i.e., visuo-spatial planning with the Tower of London test, in which the child has to reorganise three pearls on three sticks in a given configuration, with respect to specific rules [35], and cognitive inhibition via the Counting Stroop Test, a task where the subject has to inhibit automatic responses [35–37].

2.4. Statistics

Statistical analyses were conducted using Jamovi [38]. The relationship between cognitive performance and EEG indices was investigated with Spearman's nonparametric rank correlation, applying Bonferroni's multiple comparisons correction within each cognitive domain [39]. We used raw scores for all neuropsychological measurements, and age was defined as a control variable. A $p_{corr} < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical Data

Among the 125 patients with a SeLECTS EEG pattern who had a long-term EEG between 2016 and 2022, 26 patients underwent a standardised neuropsychological assessment, 19 of them less than six months apart from the EEG. We excluded two patients for an abnormal neurodevelopment and one for an abnormal cerebral MRI. We finally included 16 patients. Their detailed clinical characteristics are reported in Table 1. Age at EEG was 8 ± 1.25 years. Of all the participants, 14 were male.

Among our 16 patients, two had no history of seizures. Their EEG was performed to rule out absence seizures whilst they presented with learning disabilities. Nine patients were treated with anti-seizure medication at the time of the EEG, six with one anti-epileptic drug and three with two different anti-epileptic drugs (Levetiracetam, Valproic Acid, Clobazam or Topiramate). A cognitive regression (i.e., frontal syndrome or language impairment) concordant with an EE-CSWS was reported in four patients. The duration between the EEG and the neuropsychological testing was 2 ± 1.81 months.

3.2. EEG Characteristics

Detailed EEG characteristics are reported in Table 2. Two patients had no IED during wakefulness, but IED were found in their sleep EEG. The following results are expressed in median and interquartile (IQR). Awake SWI was 7.5; 51.25% (median; IQR), sleep SWI was 68; 31%. Awake SWF was 12; 54.75 (median; IQR), sleep SWF was 75; 144. Awake EEG score was 1; 3 (median; IQR) and sleep EEG score was 3; 2.5. Seven patients had a left unilateral focus, three had a right unilateral focus and six had bilateral foci.

Table 1. Clinical characteristics.

Patient Number	Sex	Age at Seizure Onset (Years)	Age at the Time of EEG (Years)	Treatment during EEG	Type of Regression if Present
1	M	6	7	/	/
2	M	4	6	LVT	Frontal syndrome, language
3	M	3	6	LVT	/
4	M	4	6	LVT	Frontal syndrome, language
5	M	6	7	/	/
6	M	8	9	LVT, VPA	/
7	M	/	8	/	/
8	M	6	7	VPA	/
9	M	/	8	/	/
10	M	9	9	VPA	/
11	M	9	9	/	/
12	M	6	7	LVT, CLB	Frontal syndrome
13	F	10	10	/	/
14	F	4	7	/	/
15	M	8	9	VPA	/
16	M	7	8	VPA, TPA	Frontal syndrome

Abbreviations: CLB = Clobazam, LVT = Levetiracetam, TPA = Topiramate, VPA = Valproic Acid.

Table 2. EEG data.

Patient Number	Awake SWI	Awake SWF	Awake EEG Score	Sleep SWI	Sleep SWF	Sleep EEG Score	EEG Lateralisation of Interictal Foci	EEG Lobar Distribution of Interictal Foci
1	51	56	1	73	52	4	L	CT
2	98	217	4	100	352	4	B	CP, F
3	3	30	1	50	72	1	L	C
4	63	57	1	84	140	3	R	CT
5	7	9	4	54	63	4	B	F
6	87	227	4	71	284	4	B	PT
7	34	38	1	66	79	1	L	CT
8	0	0	0	1	2	2	L	C
9	12	15	2	38	49	2	B	C
10	4	2	4	76	99	4	R	F
11	7	5	1	54	49	2	B	CT
12	82	145	4	95	330	4	R	CT
13	0	2	1	1	2	1	L	F
14	0	0	0	1	2	1	L	CT
15	2	0	1	72	75	3	L	CT
16	8	0	4	96	246	4	R	CP, T

Abbreviations: SWI = Spike Wave Index, SWF = Spike Wave Frequency, R = right, L = left, B = both, C = central, CT = centro-temporal, CP = centro-parietal, F = frontal, PT = parieto-temporal, T = temporal.

3.3. Relationship between IED and Neuropsychological Data

The results are summarised in Table 3. Our results showed a statistically significant negative correlation between the awake EEG score and the Block Tapping Test ($r_s = -0.73$; $p_{corr} < 0.05$), highlighting that higher awake EEG score is associated with poorer performance in visuo-spatial short-term memory (Figure 1). Patients with higher sleep SWF also tended to have lower scores on the Block Tapping Test, but this tendency did not reach statistical significance after Bonferroni's correction ($r_s = -0.58$; $p_{corr} = 0.196$). Additionally, we found a significant negative correlation between sleep SWI and the Tower of London ($r_s = -0.87$, $p_{corr} < 0.05$). In other terms, the higher the sleep SWI, the lower the score in this visuo-spatial planning task (Figure 2).

Table 3. Spearman's correlations (without correction) between EEG indices and neuropsychological parameters.

Field	Neuropsychological Test		Awake SWI	Awake SWF	Awake EEG Score	Sleep SWI	Sleep SWF	Sleep EEG Score
Verbal memory	Forward verbal span $n = 12$	Spearman's Rho p value	0.87 0.05	0.14 0.68	−0.03 0.93	−0.43 0.16	−0.13 0.7	−0.36 0.28
	Reverse verbal span $n = 12$	Spearman's Rho p value	−0.13 0.70	0 1	−0.28 0.39	−0.48 0.11	−0.39 0.23	−0.42 0.19
	RLS free recall mean $n = 11$	Spearman's Rho p value	0.10 0.79	0.26 0.5	−0.19 0.62	−0.41 0.23	−0.13 0.73	−0.38 0.31
	RLS delayed recall $n = 11$	Spearman's Rho p value	−0.09 0.82	0.10 0.79	−0.23 0.55	−0.51 0.13	−0.45 0.22	−0.07 0.85
Visuo-spatial memory	Block Tapping Test $n = 13$	Spearman's Rho p value	−0.38 0.22	−0.42 0.18	−0.73 0.007 *	−0.45 0.12	−0.58 0.05	−0.51 0.09
Attention	Reaction time tonic alert $n = 15$	Spearman's Rho p value	−0.07 0.81	−0.31 0.27	0.29 0.31	0.31 0.26	0.25 0.39	0.38 0.19
	Standard deviation tonic alert $n = 15$	Spearman's Rho p value	0.01 0.98	−0.2 0.5	0.34 0.24	0.24 0.39	0.2 0.50	0.29 0.31
	Reaction time phasic alert $n = 15$	Spearman's Rho p value	0.16 0.59	0.05 0.85	0.34 0.24	0.37 0.17	0.29 0.31	0.29 0.32
	Standard deviation phasic alert $n = 15$	Spearman's Rho p value	−0.19 0.50	−0.49 0.08	−0.22 0.45	0.16 0.58	−0.30 0.29	0.20 0.48
Executive function	Commission errors phasic alert $n = 15$	Spearman's Rho p value	−0.12 0.68	−0.11 0.70	−0.33 0.25	0.04 0.89	0.03 0.92	−0.05 0.86
	Reaction time Stroop test $n = 11$	Spearman's Rho p value	0.28 0.37	−0.01 0.97	0.15 0.64	0.23 0.45	−0.12 0.71	0.24 0.46
	Standard deviation Stroop $n = 11$	Spearman's Rho p value	0.10 0.75	0.09 0.79	0.03 0.93	0.11 0.72	0.07 0.83	0.05 0.87
	Tower of London $n = 11$	Spearman's Rho p value	−0.30 0.4	0.07 0.84	−0.21 0.55	−0.87 <0.001 *	−0.58 0.08	−0.51 0.13

Abbreviations: SWI = Spike Wave Index, SWF = Spike Wave Frequency. * Statistically significant correlations after multiple comparisons Bonferroni's correction: $p_{corr} < 0.05$.

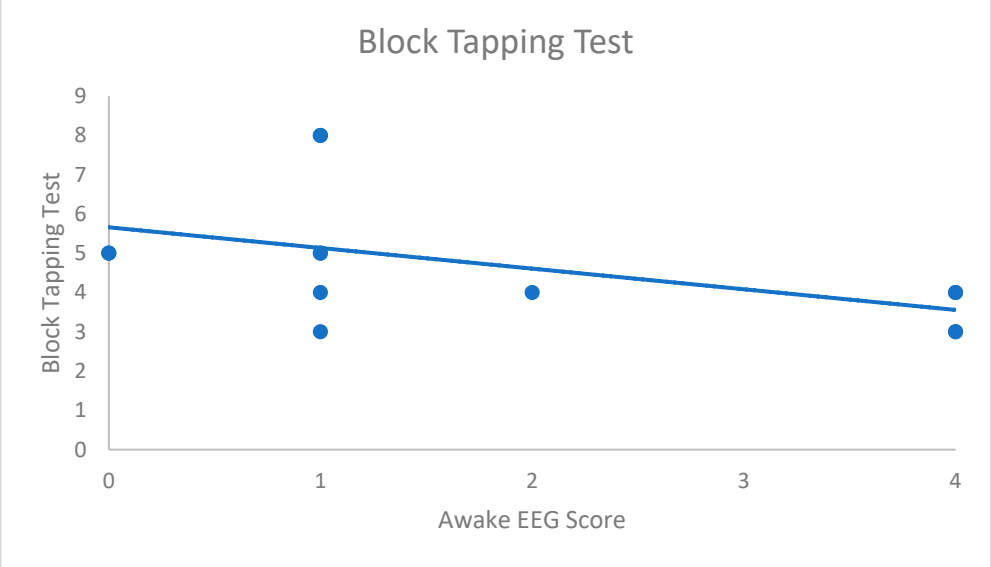

Figure 1. Significant association between the Block Tapping Test and the awake qualitative EEG score. ($r_s = -0.727$; $p_{corr} < 0.05$).

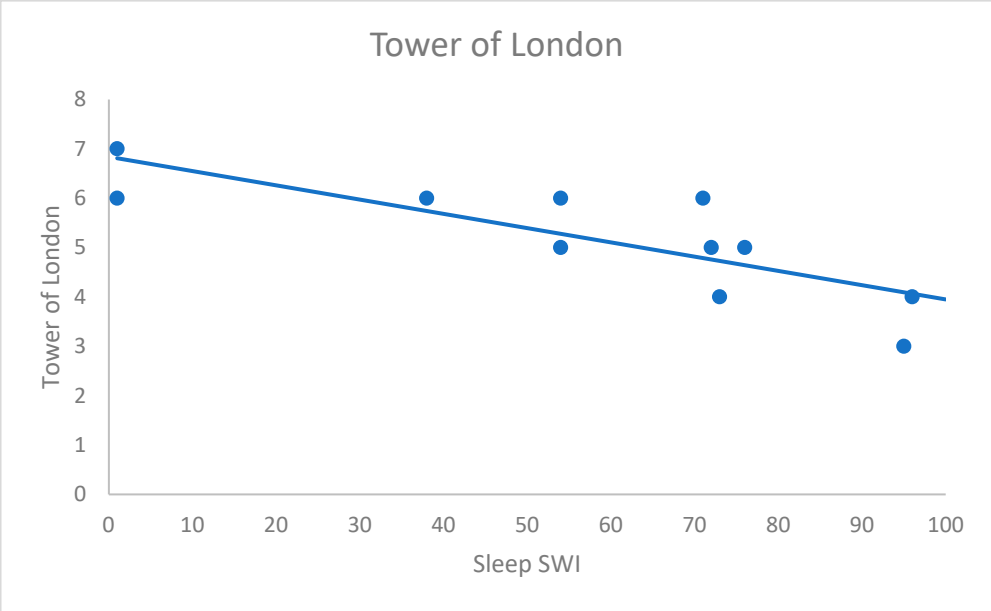

Figure 2. Significant association between the Tower of London and the sleep Spike Wave Index (SWI; $r_s = -0.87$; $p_{corr} < 0.05$).

4. Discussion

This study is the first to investigate the link between cognition and IED in a population of children with an EEG pattern of SeLECTS, including patients without any history of seizures, patients with typical SeLECTS and patients with EE-CSWS, to better illustrate the continuum where these entities fall [4]. Moreover, we examined this link through a linear analysis to better capture the full SeLECTS spectrum, where most existing studies divided their samples by categories of IED intensity or cognitive complications [12,13,17,19,25,40].

Our results show a negative correlation between (1) IED spreading (i.e., spike-and-wave complexes diffusion to the other brain regions) in wakefulness and a short-term visuo-spatial memory task (Block Tapping Test) and (2) IED intensity in sleep and a task involving executive function, and more specifically, visuo-spatial planning (Tower of London). Therefore, our findings confirm the association between IED and cognition in

SeLECTS, suggesting more precisely an association between IED and visuo-spatial skills in these children. Furthermore, they demonstrate that, in addition to epileptic activity intensity in sleep, SW spreading in wakefulness also correlates to cognitive performance in patients with SeLECTS.

Studies investigating the relationship between epileptic activity intensity and visuo-spatial performance in SeLFE are scarce. A negative correlation between long-term visuo-spatial memory performance and awake SWF has been reported by Vintan et al. in a group of 18 patients diagnosed with SeLECTS [26]. Zhang et al. showed in a larger group of 60 children with SeLECTS that patients with a sleep SWI > 55% had lower scores in a visuo-spatial memory test than patients with a sleep SWI < 55%. Moreover, bilateral epileptic foci were associated with worse visuo-spatial performance [25]. Unlike our study, none of these studies reported the application of a multiple comparisons correction during their statistical analyses.

There is evidence of possible visuo-spatial memory impairment in SeLECTS. For instance, Baglietto et al. and Metz-Lutz et al. reported lower scores in the Block Tapping Test in patients with SeLECTS compared to controls [21,41]. By contrast, Lindgren et al. failed to demonstrate a difference in their sample using a similar test [42]. In a study comparing 40 untreated patients with a SeLECTS EEG pattern, with or without related epilepsy, to 40 healthy participants, Weglage et al. showed poorer performance in short-term visuo-spatial memory [43]. In similar-sized samples, Northcott et al. and Volkl-Kernstock et al. reported an impairment of short- and long-term verbal and non-verbal memory in children with SeLECTS [23,44]. In 2021, a meta-analysis regarding working memory in paediatric epilepsy conducted by Poole et al. revealed impairments in the phonological loop and the visuo-spatial sketchpad in epileptic children. Unfortunately, no distinction was made between SeLECTS and other epileptic syndromes and the relationship between these cognitive impairments and IED was not investigated [45].

Galer et al. studied sleep-related consolidation of declarative memories in SeLFE through a verbal (associations of word pairs) and visuo-spatial (location of object pairs presented on a grid) long-term memory task. While their patients displayed similar performance to controls in immediate retrieval, their performance decreased significantly after a night of sleep, becoming inferior to controls. Furthermore, higher SWI during NREM sleep was associated with lower scores in the visuo-spatial task, supporting the hypothesis that IED could interfere with sleep-dependent memory consolidation [24].

Neuroimaging studies suggest that the neural correlates of visuo-spatial memory lie in the frontal and parietal cortices. Functional MRI studies identified a superior frontal–intraparietal network where brain activity, myelination and development of visuo-spatial working memory seem to be related during childhood [46,47]. Interestingly, several functional connectivity studies conducted in SeLECTS precisely showed altered brain functional connectivity in these regions, which seemed partly driven by the IED. Changes in connectivity strength in the default mode network were also reported in SeLECTS. The appropriate regulation of this resting-state network involving the praecuneus, medial prefrontal cortex and lateral parietal cortex seems to correlate with several cognitive functions including working memory and attentional processes. Here again, changes in connectivity seem linked to the IED [48]. In our study, no correlation was found between IED spreading or intensity and performance in attention tasks. This could be caused by our reduced number of patients, or by the great variability of attentional skills depending on cognitive availability.

To our knowledge, this study is the first to correlate visuo-spatial planification to interictal epileptic activity. However, impairments of executive functions such as inhibition, cognitive flexibility, or verbal fluency in SeLECTS were brought to light by previous studies [10,12,49,50]. Croona et al. showed poorer performance in the Tower of London task in 17 SeLECTS patients compared to a control group matched for age, sex and estimated intelligence [51]. Filippini et al. showed lower scores in a structured figural fluency task in SeLECTS patients compared to healthy peers [52]. In our study, only visuo-spatial planification correlated with epileptic activity intensity, and no relationship was found

between IED and cognitive inhibition, which could possibly be related to the small size of our sample.

The finding that in wakefulness, the EEG index that focuses on the spreading of IED correlates with cognitive performance is in line with the results of Aeby et al. in 2021. Their results showed that this EEG score, measured in wakefulness and sleep, offered better sensitivity, specificity and agreement between readers with different levels of expertise than the usual quantitative indices (i.e., SWI and SWF) to differentiate EE-CSWS from typical SeLFE [20]. These observations could be explained by the "remote inhibition" concept, which proposes the existence of epilepsy-induced inhibition of neurons that surround or are remote from the epileptic focus but connected with it via cortico-cortical or polysynaptic pathways [53]. This concept originated from the demonstration, in FDG-PET studies, of focal hypermetabolism at the site of epileptic foci, associated with hypometabolism in remote connected brain areas, such as frontal and parietal cortices and the default mode network [54]. Therefore, we could extrapolate that the more an IED spreads to the other brain regions, the more it could disrupt cerebral connectivity, thus interfering with cognitive networks.

In sleep, the EEG index correlating with neuropsychological measurements is the SWI, in agreement with several studies reporting associations between sleep SWI and word memory retention, sentence reading, verbal IQ or behaviour disorders [14,16,18]. Without guidelines regarding a gold-standard index, a wide variety of indices are used to measure interictal epileptic activity in the literature. Hence, numerous studies used indices based on the number of IED in a specific time period (similar to SWF) instead of the number of seconds containing IED (i.e., SWI) and showed correlations between IED in sleep and language impairments, verbal memory or selective visual attention performance, or with the existence of academic or behavioural problems, correlated themselves with IQ, auditory–verbal, visuo-spatial and attentional capacities [12,13,15,17,19,40]. Although our analyses failed to identify a significant correlation between SWF and cognitive measurements, we found a negative tendency between sleep SWF and the Block Tapping Test, investigating visuo-spatial memory.

Exploring the link between cognition and interictal epileptic activity leads the way to a broad treatment issue. In EE-CSWS, several retrospective studies show that, under corticosteroids, a decrease in epileptic activity intensity correlates with improved cognitive performance and IQ [55,56]. Levetiracetam also appears effective in IED intensity as well as on cognitive capacities [28]. In a pilot study regarding sleep-related declarative memory consolidation in SeLFE led by Urbain et al., normalisation of the sleep EEG in an EE-CSWS patient under hydrocortisone treatment was associated with the normalisation of overnight memory performance, which was not the case in another patient under the same treatment, whose sleep EEG was only partially improved [57]. However, cognitive regression or stagnation can be difficult to identify and, because SeLECTS and EE-CSWS belong to the same spectrum, the threshold for which treatment is appropriate may be debatable. The potential benefit of anti-epileptic drugs has not yet been demonstrated in SeLECTS without cognitive regression. Even though treatment with Levetiracetam or Sulthiame seems to lead to a substantial reduction in IED intensity, it is unclear whether that decrease is associated with a cognitive improvement and this question needs to be addressed by well-designed randomized control studies [58]. A few uncontrolled studies suggest a positive impact of Levetiracetam on cognitive functions [59,60]. Amongst them, McNally et al. reported on seven patients with learning disorders and displaying a SeLECTS EEG pattern without a history of seizures and demonstrated that their parents noticed an improvement in scholarly results and/or language after the empirical introduction of Levetiracetam [61]. Nevertheless, the evidence of a visuo-spatial impairment in SeLECTS brings to light the importance of a comprehensive neuropsychological assessment in the follow-up of these patients, allowing for an early detection of such cognitive deficits, in order to implement rehabilitation strategies such as cognitive rehabilitation and individualised school care.

The main limitation of our study is the limited number of patients. Although this strengthens the significance of our results, we could have possibly brought other significant correlations to light with a larger sample. Furthermore, it could be interesting to compare our patient results with a control group of healthy peers, to better apprehend their cognitive deficits in contrast to healthy children. A certain degree of heterogeneity can also be highlighted in our sample regarding anti-epileptic drugs, a common limitation in the scientific literature [10]. It should be noted that, while the literature suggests a possible detrimental effect of Levetiracetam on behaviour, there is no consistent evidence of cognitive impairment associated with the use of Levetiracetam in children [60]. One controlled study comparing Levetiracetam and Sulthiame in a group of 80 patients with SeLECTS treated for 6 months and evaluated with a longitudinal neuropsychological assessment concluded that anti-epileptic monotherapy did not negatively affect cognitive performance. However, the impact on behaviour was more debatable, with five dropouts due to behavioural problems in the levetiracetam group, even though the study showed a tendency to a decreased CBCL total score after 6 months in both groups, indicating a reduced incidence of behavioural disturbances [62]. By contrast, Valproic Acid, in comparison to other anti-seizure medications, may be associated with an increased risk of impairments in memory, attention and executive function [60]. However, it is worth noticing that almost half of our patients were not under any treatment at the time of their EEG. This heterogeneity is also found in the neuropsychological assessment, which can be explained by the children's cognitive availability to respond to certain tests. Because of the retrospective design, some delay can also be noted between this assessment and the EEG of each child. A shorter interval between both exams would allow us to be more representative of the children's functioning at a given time and, consequently, to discover more associations between the children's cognitive functioning and their epileptic activity. Moreover, in sleep, EEG indices were computed using the first ten minutes of NREM sleep, and we could have possibly brought to light a stronger relationship between IED in sleep and cognition with an entire night analysis. However, other studies have suggested that the first ten minutes of sleep are adequately representative of epileptic activity during a whole night [14]. Another limitation of our study is that, for practical reasons, the majority of our patients could not be tested for language. Therefore, the absence of an association between epileptic activity and verbal memory in our study should be interpretated with caution. Lastly, our study was conducted in a tertiary centre, which might generate a selection bias.

To better apprehend the impact of epileptic activity on cognition, further studies should investigate associations between IED intensity and spreading and performance in a comprehensive neuropsychological and language assessment in a large group of patients displaying a SeLFE EEG pattern without any history of seizures nor anti-epileptic therapy.

5. Conclusions

This retrospective study conducted in a population representative of the SeLECTS spectrum, from learning disorders with IED and typical SeLECTS to EE-CSWS showed an association between IED and cognition, reflecting differently during wakefulness and sleep. During sleep, we showed a negative correlation between cognitive performance and SW intensity, an association already suggested in the literature, while in wakefulness, the parameter having the most significant impact on cognitive function appears to be their spreading to other parts of the brain. Therefore, it would be useful to add an EEG score investigating wake- and sleep-related qualitative parameters in the EEG evaluation of SeLECTS patients in further studies as well as in the day-to-day care of these children. Lastly, as both cognitive functions correlating with IED were visuo-spatial, our results bring to light the relevance of comprehensive neuropsychological assessment, including visuo-spatial skills. Further studies are needed to confirm our results in a larger population.

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brain sciences

MDPI

Review

Epilepsy Networks and Their Surgical Relevance

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Abstract: Surgical epilepsy is a rapidly evolved field. As the understanding and concepts of epilepsy shift towards a network disorder, surgical outcomes may shed light on numerous components of these systems. This review documents the evolution of the understanding of epilepsy networks and examines the data generated by resective, ablative, neuromodulation, and invasive monitoring surgeries in epilepsy patients. As these network tools are better integrated into epilepsy practice, they may eventually inform surgical decisions and improve clinical outcomes.

Keywords: epilepsy; network; resection; ablation; neuromodulation; invasive monitoring; sEEG

1. Introduction

This paper is a narrative review of brain networks in epilepsy and their recent implications in epilepsy surgery. To understand the trend towards understanding epilepsy as a "network disease", clinicians must understand network epilepsy as multiple regions in the brain involved in the development and propagation of seizures [1–3]. These multiple brain regions may be connected both structurally and functionally with varying temporal patterns of spread between regions. In addition, these areas are also connected to a normally functioning brain. It is through the definition of the epileptogenic tissue, as well as the connections that allow seizures to propagate elsewhere, that an epileptogenic network may be defined. As diagnostic and invasive studies have improved, clinicians have developed more insight into these mechanisms. These studies include examples such as tractography (Figure 1), functional magnetic resonsance imaging (MRI) (Figure 2), and invasive interrogations like stereo electroencephalography (Figure 3). Tractography delineates structural connectivity via MRI diffusion sequences. Functional MRI allows for a large-scale sampling of physiological brain coupling via the visualization of multiple brain regions and their associated activity at different times. Stereo electroencephalography provides a better temporal resolution of neurophysiological signal spreading from specific point to point based on the surgical implantation of specific brain regions. All studies contribute to patient specific insights regarding epileptogenic regions of the brain and how they connect to each other and allow the spread of seizures. A better understanding of these networks allows clinicians to better plan how to disrupt them and improve surgical outcomes in epilepsy patients.

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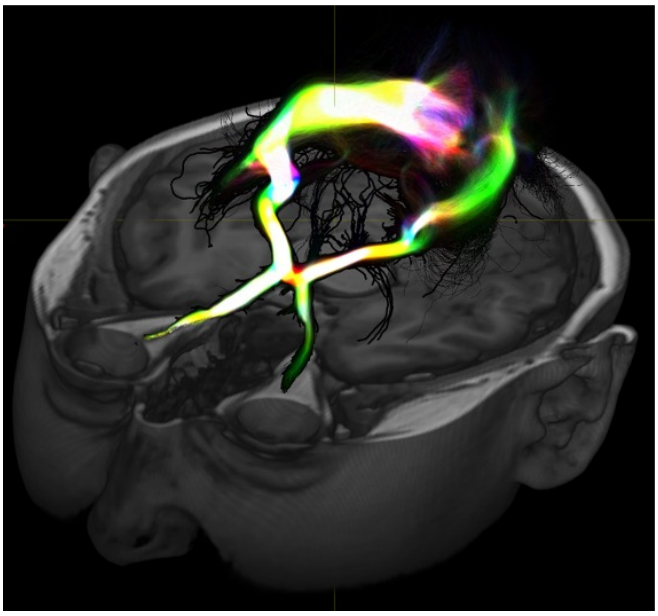

Figure 1. Diffusion-based MRI imaging allows for visualization of white matter structure and here shows the visual pathway. These types of studies may provide information on structural connectivity between areas of interest to help define connections or epileptogenic tissue or even normal tissue to preserve during resection.

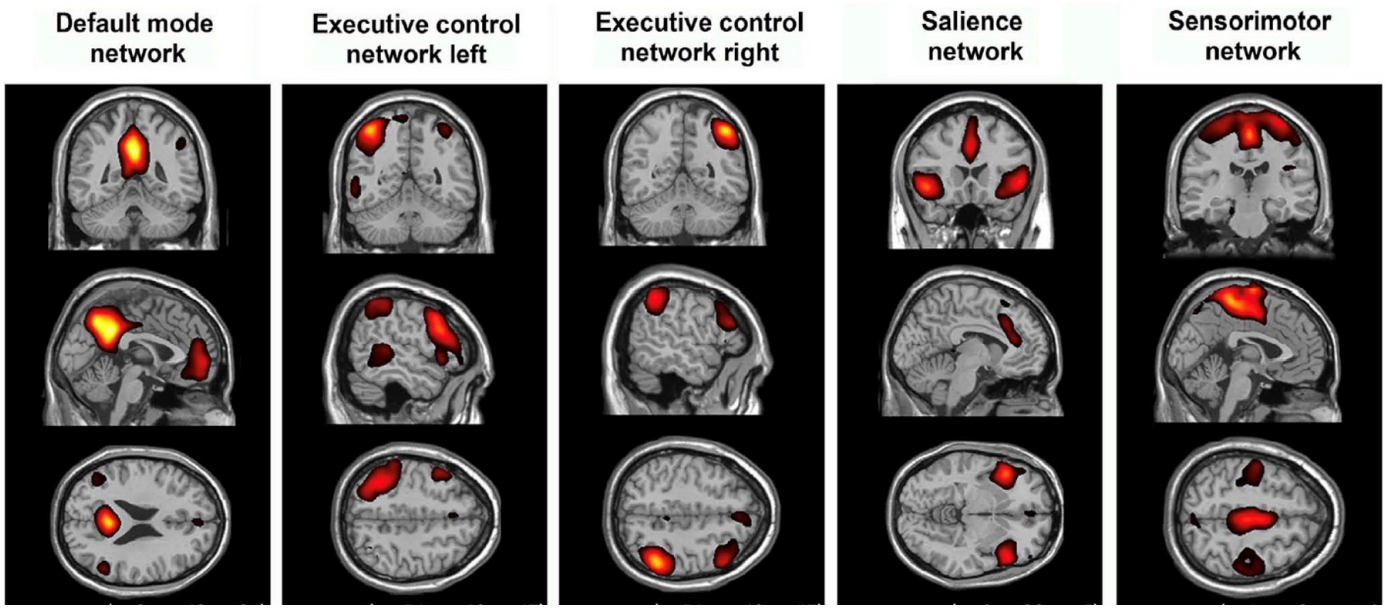

Figure 2. Differences in resting state functional MRI may show how epilepsy modulates established networks and which networks are involved in seizure propagation or generation.

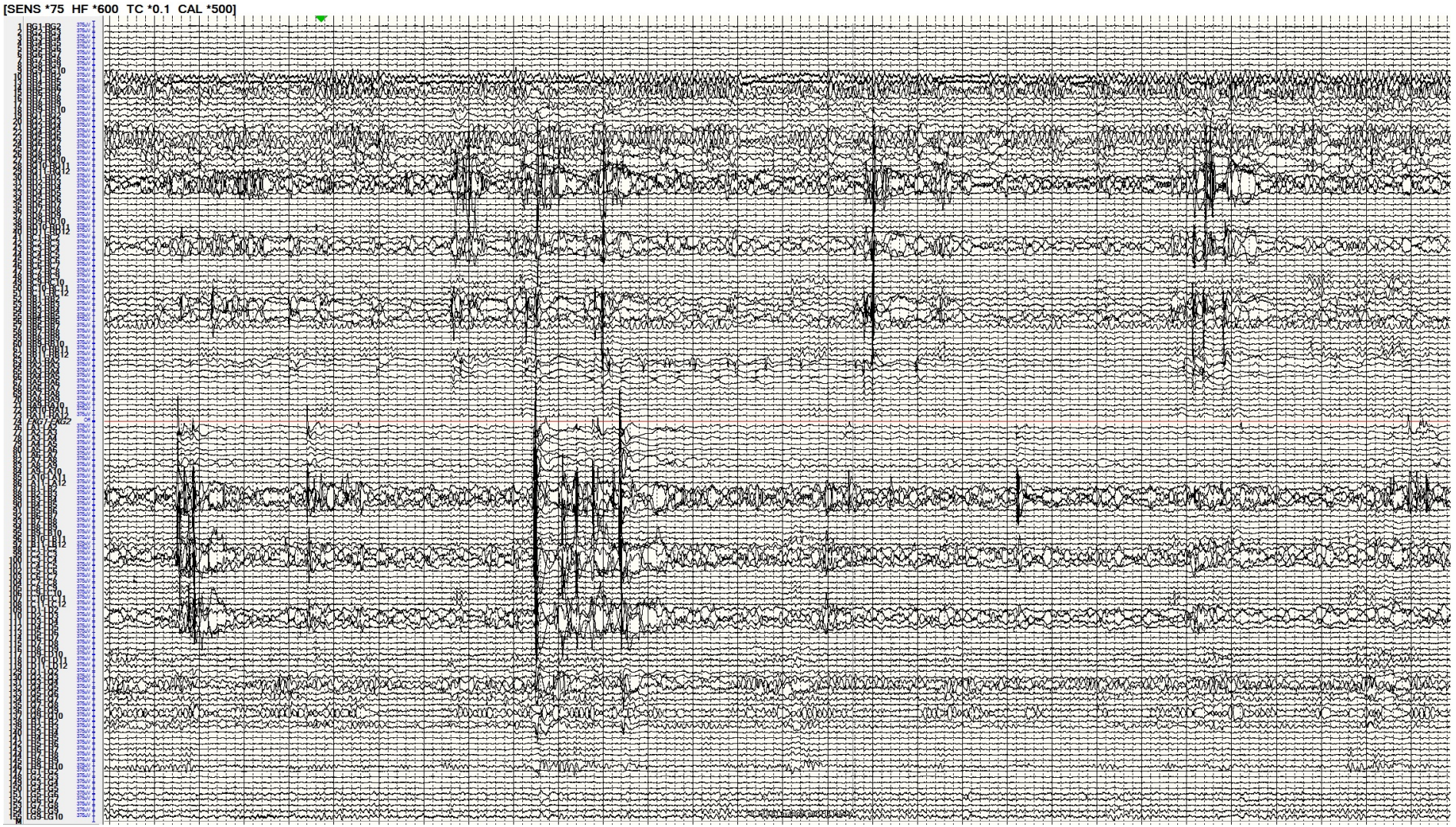

Figure 3. Stereo-electroencephalography interrogation of a patient with bilateral temporal lobe epilepsy. The recording demonstrates bilateral hippocampal spikes implying an epilepsy network involving both these regions.

2. Evolution of Network Epilepsy

Initially thought to be of religious or magical consequence, the understanding of epilepsy has drastically improved over time. With descriptions dating back as far as 1050 BC in Babylonian text, seizures and epilepsy have been described and explored by many [4]. It was not until neurology and epileptology were established in the 19th century that epilepsy was more systematically characterized. Scientists such as John Hughlings Jackson, Robert Todd, Louis Francois Bravais, and Theodore Herpin established seizure types as well as a categorization of the seizures based on which "level" of the brain the seizure originated [4]. Building on this, Paul Broca combined his anthropological background with his interest in infantile seizures to propose trephination as a treatment for epilepsy [5]. As understanding grew, it became clear that focal brain pathology may result in epilepsy. As such, semiology and neurological deficits associated with such pathology began informing epilepsy surgeons when developing laterality and general localization hypotheses [6].

However, the 20th century saw the development of technology that enabled a more accurate localization and characterization of epilepsies. Neuroscientist Herbert H. Jasper pioneered the use of the electroencephalograph (EEG) as a tool to localize epilepsy prior to surgical intervention. It was through his efforts with Wilder Penfield that patients' epilepsies were preoperatively localized so that craniotomies could be performed. In many cases, lesional pathology was resected, alleviating the epilepsy [7]. Through the use of large craniotomies for surface mapping with electrocorticography (invasive EEG), Jasper and Penfield saw that the removal of tissue to attenuate abnormal spikes prognosticated good seizure outcomes in many of their patients. Rasmussen expanded on this with his localization concepts: primary localization was achieved by invasive EEG for the mapping of resection; secondary localization involved understanding cortical recruitment and spread during seizures; and tertiary localization defined the extent of resection necessary to produce satisfactory seizure reduction or cure [8].

While early results were promising, it was noted that EEG methods were not sufficient to localize and resect epileptogenic areas of the cortex while still producing favorable seizure outcomes in a number of cases. As such, the 1960s gave rise to Talaraich and Bancaud's philosophy that clinical, anatomical, and electrophysiologic correlation all together took precedence over surgery solely informed by EEG [9]. This approach facilitated the existence of the epileptogenic zone, which reflects the cortical site of potential initiation and the organization of activity into seizures. This philosophy gave rise to the advent of stereo-electroencephalography, allowing for a three-dimensional interrogation of the spatiotemporal organization of seizures.

In the 1990s, Hans Luders and colleagues modified the term epileptogenic zone to encompass "the area of cortex that is necessary and sufficient for initiating seizures and whose removal (or disconnection) is necessary for complete abolition of seizures" [10]. This definition shifted towards a more conceptual understanding of the goals of surgery to account for the difference between the epileptogenic zone and ictal onset zone. Given the continued failures in epilepsy surgery related to the focal control of the disease process, Susan Spencer advocated for efforts to affect larger networks [11]. The challenge there remains to identify and separate pathologic epileptogenic tissue from normal but affected networks modulated by the epileptogenic zone. As such, some groups have attempted to quantify the recorded anatomy's contribution to the epilepsy network. In one such example, Bartolomei et al. coined the term epileptogenicity index (EI) in their exploration of temporal lobe epilepsies [12]. Using the spectral and temporal characteristics of the structures interrogated on EEG, the contribution to the epilepsy network was quantified. Similarly, in response to the work by Spencer and Bartolomei, John Miller has also advocated for a replacement of the EZ with the "seizure generating network" to explain the dynamic clinical expression of seizures, resection failures, early propagation, and success of newer approaches [13].

As this approach to epilepsy has gained validity, recent investigations have primed the field for a discussion of both epileptogenic tissue and the involved epilepsy network rather than anatomic regions alone. These network relationships inform surgical treatment. One example includes temporal lobe epilepsy. While seizure freedom rates after anterior temporal lobectomy are accepted as high [14,15], surgical failures prompt discussion as to better preoperative diagnosis or surgical planning. This has led to the evaluation of "temporal plus epilepsy". Rather than the temporal lobe anatomically acting as the epileptogenic zone, its network connections to other circuits prevents cure after the resection of the temporal lobe and mesial structures alone [16]. The potential integration of the insular [17], orbitofrontal [18], and occipital networks [19] raises the question of more extensive resections/disconnections to prevent surgical failures in temporal lobe epilepsy patients.

Similar to the well-studied temporal lobe epilepsy, epileptogenic zones arising elsewhere anatomically have the potential for larger network involvement and need for consideration in surgical planning. Occipital epilepsies have the well-documented spread and network involvement of temporal or parieto-frontal networks [20–22]. Insular epilepsies are known to involve the opercular cortex as well as the supplementary motor area cortex [23,24]. The cingulate gyrus network participates in the limbic circuit and even parieto-occipital regions in epilepsy patients [25,26]. These are just several examples of the emerging network relationships that exist in epilepsy patients that may affect surgical prognostication and outcomes. There are many more networks under investigation that need to be accounted for in the current paradigm of epilepsy surgery. These concepts have paved the way for neuromodulation in epilepsy as well as more network-based investigations of networks and surgical treatments. Given the shift towards network epilepsy, we aim to review recent advances in knowledge of the epileptogenic networks derived from epilepsy surgery.

3. Networks in Resective and Ablative Epilepsy Surgery

In the populations of surgical patients who have undergone advanced imaging such as functional MRI or tractography, the response to surgery may be correlated with connectivity to discern the areas participating in an epileptogenic network. In 2019, Alizadeh et al. examined tractography density differences between pathologic and healthy hemispheres in patients undergoing LITT or ATL for temporal lobe epilepsy (TLE) [27]. They found that epilepsy patients with increased white matter density in the ipsilateral lingual, temporal pole, pars opercularis, inferior parietal, and contralateral frontal pole segments were more likely to have residual seizures after surgery. They also noted that patients with left versus right mesial temporal sclerosis (MTS) exhibited differences in connectivity patterns. Bilateral and widespread white matter changes were also more prominent in left MTS compared to right-side MTS. This provides insight that even the laterality of seizure onset may affect network involvement. Network information in the form of resting state fMRI and EEG data was leveraged by Neal et al. in 2020 to map a hypothetical irritative zone in patients undergoing temporal lobectomy [28]. The group compared the preoperative network connectivity to that of the irritative zone in seizure-free and non-seizure-free patients. They also compared temporal lobe connectivity in patients undergoing temporal lobectomy to healthy controls. They found that temporal lobe networks in epilepsy patients demonstrated higher connectivity than healthy controls. When comparing the network connectivity in seizure-free vs. non-seizure-free outcomes, the authors noted that a higher percentage disruption of epilepsy networks was more closely associated with seizure-free outcomes. Finally, patients with a greater disruption of epileptogenic networks demonstrated improvements in quantitative neuropsychological testing. Liang et al. also surgically demonstrated that disconnection, as opposed to resection, affects epilepsy networks [29]. Functional connectivity in 30 Lennox Gastaut Syndrome patients was derived from EEG data before and after callosotomy. Patients with good seizure outcomes had a shifting of the "hubs" of connectivity on the EEG from paramedian regions to a more lateral cortex. Patients with poor seizure outcomes did not see this shift towards a more

homogenously connected state as the hubs remained all paramedian. Together, these studies suggest the presence of increased connectivity in abnormal epileptic networks and the surgical disruption of those networks correlated with surgical outcomes. Further bolstering the need for network-based approaches to resection are the results of Josephson et al.'s systematic review of the outcomes in mesial temporal lobe epilepsy (mTLE). Despite the focus on mTLE, the authors found that standard resection including neocortical removal conferred higher seizure freedom rates than the selective resection methods such as transylvian or transcortical amygdalohippocampectomies [30]. This is presumed to be related to the disruption of a wider set of network pathways that seizures may originate in or spread through.

In addition to connectivity studies, the examination of resected anatomy associated with seizure freedom provides information on structural involvement in network epilepsy. Recent advances in the understanding of the piriform cortex have supported this notion. In 2019, Galovic et al. found that in 107 patients undergoing temporal lobectomy for unilateral TLE, the proportion of the piriform cortex resected was directly associated with seizure freedom [31]. As no other structure shared this association between the extent of resection and the outcome, this finding alludes to the role that the piriform cortex may play in the generation and propagation of seizures in temporal lobe network epilepsy. Until recently, the piriform cortex has not received much attention in human epileptology. It has inputs from the olfactory bulb, other olfactory cortical areas, and the contralateral piriform cortex. In addition, its outputs include strong limbic connections such as the amygdala and entorhinal cortex, orbitofrontal cortex, insula, and even mediodorsal nucleus of the thalamus as well as the hypothalamus [32]. This demonstrates that even small structures may play key network roles in epileptogenesis if highly connected. Similar to temporal lobe epilepsy, such concepts may be applied to other regions. Giampiccolo et al. used tractography in 47 patients undergoing frontal lobectomy to identify the "disconnectome" and predict late seizure recurrence [33]. They found that long-term seizure freedom as durable as five years was associated with the disconnection of anterior thalamic and corticostriatal fibers. In addition to the epileptogenic frontal lobe resection, the disconnection of the epileptogenic network was crucial in the prevention of recurrence.

The utilization of minimally-invasive ablative surgery in epilepsy has also led to a greater understanding of epilepsy networks. As opposed to resection, the ablation of a seizure focus lends itself to more accurate postoperative volumetric analysis because the ablation and remaining tissue are less prone to brain shift or postoperative distortion. As such, volumetric analysis after laser interstitial thermal therapy (LITT) has yielded a depth of information on networks in epilepsy. For example, by normalizing ablation cavities in 175 patients undergoing LITT for mTLE to a common atlas space, Wu et al. demonstrated that the ablation of anterior, inferior, and mesial structures is more associated with seizure freedom while ablation posterior the mesencephalic sulcus provides diminishing returns with respect to seizure freedom [34]. When examining the structures associated with seizure freedom, this analysis also supports the recent development in the epilepsy literature that the piriform cortex is strongly associated with epileptogenic networks and seizure outcomes after epilepsy surgery for mTLE. As we continue to utilize volumetric analysis for ablative or resective lesions in epilepsy, we will improve our understanding of which structures are associated with seizure freedom and thus are implicated in network epilepsy.

4. Networks in Neuromodulation Epilepsy Surgery

While resection or ablation may provide information by exclusion about what structures were previously participating in epilepsy networks, neuromodulation procedures provide information about structures actively included in the network by modulating their function. One of the oldest forms of neuromodulation in the treatment of epilepsy is vagal nerve stimulation (VNS). Investigators found that the cycled stimulation of the vagal nerve decreases frequency by at least 50% in 30–50% of patients with an increasing effect over time [35–37]. While the mechanisms of seizure reduction are still under investigation,

VNS is thought to lower seizure burden by brainstem, limbic, and cortical modulation through the vagal afferent network. Cortically, VNS alters connectivity and this alteration in network connectivity contributes to the increasing and sustained efficacy over time [38]. In patients who have surgically implanted VNS, network alteration has been documented post-operatively. Zhu et al. found that when compared to their preoperative altered regional activity on resting state fMRI, patients implanted with VNS had reorganization and increased regional homogeneity in superior and middle temporal gyri that correlated with an improved seizure outcome after 3 months of stimulation [39]. Similarly, Wang et al. found that after 6 months of stimulation, VNS patients reorganized resting state networks into more regionally homogenous states and demonstrated a suppression of excessive salience network activation [40]. As experience with neuromodulation and VNS grows, a better understanding of network remodeling will lead to better patient selection and prediction of seizure outcomes in response to VNS therapy.

An increasingly common example of the neuromodulation approach is deep brain stimulation (DBS) for treatment of epilepsy. When epileptogenic tissue is too diffuse, in eloquent tissue, or otherwise unable to be resected, the modulation of the network may be the patient's best opportunity for the palliation of epilepsy. The most widely used target for DBS in epilepsy is the anterior nucleus of the thalamus (ANT). The stimulation of ANT has been shown to modulate frontal and temporal circuits on EEG [41]. By targeting the ANT, the limbic circuitry including the mammillothalamic tract, cingulate, hippocampus, and parahippocampus may be modulated. In the SANTE trial in 2010, it was demonstrated that patients have a 41% seizure reduction within 1 year of implantation [42]. After 5 years of treatment and programming optimization, the median seizure reduction increased to 69% [43]. An interrogation of the functional network via EEG after DBS has provided some biomarkers for DBS treatment in epilepsy. Scherer et al. found that responders to DBS were more likely to demonstrate widespread cortical desynchronization of alpha and theta bands whereas non-responders did not [44]. In addition to cortical changes, newer sensing technology allows for the interrogation of thalamic local field potentials in epilepsy patients who have undergone DBS implantation. While the potential for clinical integration remains high as this may allow for closed loop DBS in the future, consistent biomarkers remain elusive as no single frequency bands appear to differentiate ictal from non-ictal activity [45]. While large studies investigating the stimulation effect on epilepsy networks are limited, several centers have begun to investigate functional MRI differences between on and off stimulation in the ANT [46–48]. This work has demonstrated that stimulation increases in the activity of limbic structures such as the bilateral thalamus, anterior and posterior cingulate cortex, amygdala, and hippocampus. In addition, the default mode network areas such as the precuneus and medial prefrontal cortex showed increases in activity during stimulation. Other research has demonstrated network modulation when examining the proximity of an estimated field of electrical stimulation to the mammillothalamic tract (MTT). Specifically, Schaper et al. noted that responders to ANT DBS were more likely to have stimulation closer to the MTT [49]. Given the activity seen in limbic circuitry including Papez's traditional components, it is not surprising that the MTT white matter tracts may play a large role in temporal lobe epilepsies and their treatment. These data lay the foundation for patient-specific epilepsy networks that can be used to inform response to therapy. These methodologies may be adapted to larger studies investigating long-term network effects of deep brain stimulation. At this time, there is unfortunately a dearth of literature in patients undergoing alternative thalamic target stimulation (such as centromedian or pulvinar nuclei). As more patients undergo DBS for centromedian [50,51] or pulvinar stimulation [52], on/off testing in combination with EEG and radiographic data may demonstrate variable network modulation and thus differentiate target utility in a patient-specific manner. While stimulation-related functional data are being generated, structural investigations elucidating the connectivity of these targets is already underway. Using a finite element model approach, Diaz et al. estimated the volume of tissue activation (VTA) in 10 patients who underwent DBS CM placement for treatment of generalized phar-

macoresistant epilepsy [50]. This VTA was used to seed both tractography and functional MRI studies. They demonstrated that responders were correlated with VTA-modulating reconstructed networks that included a sensorimotor, supplementary motor area, cerebellum/brainstem, and reticular activating network. Similarly, as the pulvinar nucleus is being investigated as a target, its network implications must also be investigated. Leh et al. used diffusion tensor imaging (DTI) in six healthy controls to construct pulvinar fiber tracts and found that the pulvinar nucleus is interconnected with the thalamus, caudate, as well as cortical areas including visual, associative visual, posterior parietal, inferior visual temporal, and frontal eye field cortical areas [53]. Large as it is, it is important to note that the pulvinar subregions have different connectivity as more lateral regions are more closely connected with posterior quadrant networks [54]. As potential targets for neuromodulation in epilepsy arise, it is important to interrogate them via functional and structural testing for a more complete understanding of their therapeutic mechanisms.

While the nodes of thalamic connectivity move towards informing DBS targeting, responsive neurostimulation (RNS) employs closed loop stimulation to modulate epilepsy networks [55]. Similar to other forms of neuromodulation, the efficacy increases over time as neuromodulation alters epilepsy networks [56]. To further support the network modulation effects of RNS, Khambati et al. retrospectively analyzed interictal ambulator intracranial EEG readings at each contact [57]. By categorizing which contacts were in the seizure network, the authors used the theta, alpha, beta, and gamma bands to estimate functional connectivity. Beta bands were noted to diverge within network foci in responders, with the earliest evidence of increased beta connectivity appearing around 270 days in responders. In addition, theta, alpha, and gamma bands diverged between foci. This study provides evidence in the changes in functional connectivity occurring after sustained responsive neurostimulator treatment. As experience with RNS has accumulated, investigators note that patient-specific structural and functional connectivity may correlate with seizure reduction and epilepsy outcomes. Charlebois et al. noted that patient-specific tractography and connectivity was correlated with RNS effectiveness. The increased connectivity of the estimated stimulation field to the medial prefrontal cortex, cingulate cortex, and precuneus was associated with larger seizure reductions [58]. The highly connected pulvinar nucleus has already been employed in a closed-loop manner with success. Using it to modulate the network connections as described above, Burdette et al. described the placement of pulvinar depth electrodes paired with occipitoparietal leads in an RNS system to treat three cases of posterior quadrant epilepsy [59]. All patients responded during follow-up and two had 90% or greater reduction in seizure frequency. While these results require rigorous verification, these promising results support using network information in RNS and epilepsy surgery.

5. Networks in Stereoelectroencephalography

In addition to destructive and neuromodulatory surgical insights, invasive monitoring also has yielded significant surgical information on network epilepsy. Stereoelectroencephalography (sEEG) allows for the physiological examination of functional connectivity with excellent temporal resolution compared to other modalities such as rs-fMRI [60]. Azeem et al. combined sEEG with probabilistic white matter tractography. By seeding regions of interest with spike propagation relationships, they were able to compare white matter connections between the two points in epilepsy patients and matched controls from the Human Connectome Project [61]. They demonstrated that spike propagation in epilepsy patients was associated with a higher likelihood of direct white matter connection. As tractography and invasive monitoring methods improve, more indirect inferences may be drawn from combining the two modalities. Mitsuhashi et al. generated a novel dynamic tractography model to localize interictal spike sources and monosynaptic spike propagation through the white matter in sEEG [62]. In their model, they found that spike sources were more likely to be included in the resection in patients who achieved ILAE class 1 outcomes. This manner of combining structural tractography data and physiological sEEG data may

aid in surgical decision making after rigorous validation by surgical results. Koubeissi et al. used invasive monitoring in combination with forniceal-hippocampal-evoked responses to examine functional connectivity as well as the efficacy of forniceal stimulation for the reduction of seizures [63]. They noted that hippocampal-evoked responses as well as posterior cingulate-evoked responses during forniceal low frequency stimulation demonstrated the intimate connectivity of the memory circuit and default mode network. This effect decreased seizure frequency after low frequency stimulation without compromising memory function. Not only did these epilepsy networks correlate closely with memory and default mode networks, but this study also demonstrated differential network effects based on the type of stimulation applied. Such effects of differential stimulation on networks will be paramount in defining these networks during invasive monitoring.

In addition to combining sEEG with advanced imaging modalities or stimulation, sEEG mediated radiofrequency ablation has been used to disrupt epilepsy networks. This technique allows neurologists and neurosurgeons to review intracranial EEG findings after implantation and while the leads are still in place, use radiofrequency ablation to target epileptogenic contacts noted in recordings [40]. This is beneficial because the intervention is based on current epilepsy network data without the need to insert a probe or move the contacts prior to direct intervention. In addition, the ablation is performed with the patient awake so that as the current is applied to each target contact, the surgeons may monitor for adverse effects. While long-term seizure freedom rates are generally reported at low rates [64–66], the risk and additional burden to the patient that RF ablation imposes is minimal. It is particularly effective in some disease processes that may have been considered inoperable in the past. In patients with periventricular heterotopia (PVH), sEEG allows for the interrogation of multiple lesions that could be epileptogenic, many of which are poorly accessible. A review of sEEG-mediated RF ablation in PVH shows reasonable response rates at 81% and even a 38% seizure freedom rate [65]. Lastly, even if patients who have sustained seizure freedom for several months recur, the effect of ablation has a high positive predictive value for the success of subsequent lesional epilepsy surgery. It is a powerful tool to validate a surgical hypothesis and proposed resection [67].

In addition to monitoring the white matter tracts involved in epilepsy networks, there has been increased interest in monitoring deeper nodal structures such as the thalamus. Illyas et al. utilized extensions of sEEG electrode trajectories to include thalamic nuclei for monitoring [68]. Their targets included the anterior (ANT), centromedian (CM), and mediodorsal (MD) nuclei of the thalamus. During seizure activity, they observed changes in high frequency activity (HFA) based on the target nucleus and various ictal time points. While ANT and MD were noted to have greater HFA at seizure onset, CM HFA activity peaked towards the end of seizure activity. These findings suggest that the CM may play a role in seizure termination. In a similar study, Soulier et al. monitored the ANT and pulvinar nucleus (PUL) in TLE patients undergoing sEEG. They noted that the nodal "in" connectivity, as defined by the EEG propagation of the signal towards the target nucleus, of both increased as the seizure progressed after onset inferring that these thalamic nuclei may act as more of a sensor than propagator in focal TLE. Interestingly, they did also note that the PUL outward connectivity increased during synchronous activity at the end of recorded seizures. In this instance, PUL may play a key network role in seizure termination versus propagation [69].

Lastly, sEEG of thalamic monitoring may allow for more rigorous phenotyping of seizures and their associated networks. Wu et al. targeted the thalamic nuclei during sEEG implantation of multiple epilepsy types to simultaneously interrogate different SOZs as well as thalamic PUL, ANT, and MD nuclei [70]. By categorization of seizure by spread types and onset location, 20 of the 22 seizures were noted to involve thalamic nuclei. The only exception was one seizure that remained focal and another that spread to the contralateral cortex without any recorded thalamic involvement. Even more interestingly, thalamic nuclei EEG morphologies were similar among the same seizure types. The similar thalamic EEG signatures among seizure types allows for a future investigation of elucidating seizure

networks by thalamic involvement or even the prediction of an ideal neuromodulation target after sEEG.

6. Conclusions

The surgical treatment of epilepsy continues to evolve as new treatment paradigms are developed. Through the use of a network approach, understanding of the pathophysiology and surgical outcomes continues to improve. The resection or ablation of highly involved nodal networks reduces seizure recurrence. The neuromodulation of various targets reduces seizure frequency while allowing the study of involved cerebral circuitry and white matter pathways. Finally, invasive diagnostics such as sEEG also provide valuable information on epilepsy networks that may inform future surgical decision making. As research is conducted to standardize radiographic technique and larger databases are accumulated, it is likely that surgeons will be able to leverage this information to improve seizure outcomes in their patients. The future of epilepsy surgery will be not only defining surgical targets as an anatomical epileptogenic zone, but also delineating involved networks to ensure an adequate disruption of seizure spread through highly connected neurons.

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Abbreviations

MRI	Magnetic Resonance Imaging
EEG	electroencephalography
EI	epileptogenicity index
EZ	epileptogenic zone
ATL	anterior temporal lobectomy
LITT	laser interstitial thermal therapy
MTS	mesial temporal sclerosis
mTLE	mesial temporal lobe epilepsy
TLE	temporal lobe epilepsy
VNS	vagal nerve stimulation
DBS	deep brain stimulation
ANT	anterior nucleus of the thalamus
MTT	mammillothalamic tract
CM	centromedian nucleus
VTA	volume of tissue activation
DTI	diffusion tensor imaging
RNS	responsive neurostimulation
sEEG	stereoelectroencephalography
PVH	periventricular heterotopia
SOZ	seizure onset zone
PUL	Pulvinar nucleus
MD	Mediodorsal nucleus

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Review

Pathophysiology to Risk Factor and Therapeutics to Treatment Strategies on Epilepsy

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Abstract: Epilepsy represents a condition in which abnormal neuronal discharges or the hyperexcitability of neurons occur with synchronicity, presenting a significant public health challenge. Prognostic factors, such as etiology, electroencephalogram (EEG) abnormalities, the type and number of seizures before treatment, as well as the initial unsatisfactory effects of medications, are important considerations. Although there are several third-generation antiepileptic drugs currently available, their multiple side effects can negatively affect patient quality of life. The inheritance and etiology of epilepsy are complex, involving multiple underlying genetic and epigenetic mechanisms. Different neurotransmitters play crucial roles in maintaining the normal physiology of different neurons. Dysregulations in neurotransmission, due to abnormal transmitter levels or changes in their receptors, can result in seizures. In this review, we address the roles played by various neurotransmitters and their receptors in the pathophysiology of epilepsy. Furthermore, we extensively explore the neurological mechanisms involved in the development and progression of epilepsy, along with its risk factors. Furthermore, we highlight the new therapeutic targets, along with pharmacological and non-pharmacological strategies currently employed in the treatment of epileptic syndromes, including drug interventions employed in clinical trials related to epilepsy.

Keywords: epileptic syndromes; neurotransmission; comorbidities; clinical trials

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1. Introduction

Globally, epileptic disorders are generally classified into two distinct groups: common epilepsies, representing around 95% of cases, and rare epilepsies, characterizing approximately 5%. Within this last group, epileptic syndromes stand out, forming a broad and heterogeneous group of diseases that affect individuals of pediatric age [1]. Epileptic seizures are chronic in nature, characterized by the recurrent manifestation of unprovoked seizures [2]. The classification developed by the International League Against Epilepsy (ILAE) in 2017 established three distinct levels of diagnosis, including the nature of the

seizure, the type of epilepsy, and the epileptic syndrome. In this context, the importance of considering the etiology and comorbidities at each diagnostic level is emphasized [3,4].

This pathology is also divided into three etiological categories: idiopathic, acquired, and cryptogenic. In childhood, the idiopathic form of epilepsy manifests itself without visible neurological signs, while the acquired form is related to identifiable structural lesions in the brain, resulting from trauma, tumors, infections, hippocampal sclerosis, as well as cerebrovascular, immunological, perinatal, and childhood disorders. In turn, cryptogenic epilepsy remains an etiological enigma, as its cause can be difficult to identify, discouraging the use of the term "cryptogenic" in modern studies due to its unclear implications [4,5].

One of the characteristics of epileptic syndromes is their pharmacological resistance, in addition to epileptic polymorphism and severe alterations in the electroencephalogram patterns [6,7]. Affected patients experience a wide range of neuropsychiatric symptoms, ranging from mild to severe, encompassing neurological impairments, mental retardation, sensory and communication deficits, and other significant changes in psychiatric, motor, and behavioral aspects [1].

An extensive combination of genetic polymorphisms, epigenetic modifications, and environmental factors, such as pollutants, diet composition, and brain injuries, emerge as key factors in the reconfiguration of brain circuits, culminating in the emergence of epileptic disorders [1,8]. Brain adaptations resulting from these factors can upset the delicate balance between excitatory and inhibitory processes. As a result, a seizure can manifest itself in different areas of the brain and propagate to other synaptically connected regions, compelling an increase in the severity of the condition [9,10]. This review provides a broad discussion of the neurological mechanisms causing the development and progression of epilepsy, as well as its risk factors, in addition to revealing the new therapeutic targets and pharmacological strategies currently used in the treatment of epilepsy syndromes. Additionally, we will also explain the drug interventions used in epilepsy-related clinical trials.

2. Neurological Mechanisms Underlying the Development and Progression of Epilepsy

Epilepsy is a complex neurological condition, characterized by recurrent spontaneous seizures [11]. It is a prevalent neurological disorder that affects over 50 million people [12]. Patients experience repetitive seizures, which result from abnormal, excessive, and synchronized firing of neuron groups within the brain. Seizures originating from a specific brain region are called focal or location-related, while generalized seizures occur simultaneously in both cerebral hemispheres [13]. Seizures occur when there is abnormal synchronous neuronal firing in a specific section of the brain or throughout the brain. These abnormal networks can be caused by structural, infectious, or metabolic disturbances [14]. Frequent confusion occurs between seizures and epilepsy, although these two terms are not interchangeable. The onset of a seizure is defined as either focal, generalized, unknown, or unclassifiable. The isolated occurrence of a seizure in an individual does not necessarily imply that he has epilepsy, as the seizure could have been triggered, but not be repeated. The term "epileptogenesis" encompasses the developmental trajectory that leads to status epilepticus. This concept encompasses the sequence of events that transforms the brain from a normal state to one predisposed to seizures. This transformation presupposes the hyperexcitability of neuron groups, which become prone to discharge abnormally [15].

Thus, epileptogenesis is the process of developing and expanding tissue capable of generating spontaneous seizures, leading to the development of an epileptic condition and/or the evolution of epilepsy once it is established [16]. Furthermore, epileptogenesis is connected with pervasive neuronal damage, gliosis, and microgliosis, creating an inflammatory state in the microenvironment of the neural tissue [17]. Inflammatory processes can originate in the central nervous system or be acquired from systemic circulation due to a breakdown in the blood–brain barrier (BBB) [18].

Synapses connect with all of the physical parts of an astrocyte, encompassing its terminal projections and soma. A crucial requirement for the realization and effectiveness

of most of these regulatory functions is the extensive electrical and metabolic interconnection between astrocytes, facilitated by low-resistance aqueous channels known as gap junctions (GJs). This leads to the formation of large syncytium-like functional networks that overlap neuronal synaptic networks and allow their coordinated regulation and synchronization [19]. Astrocytic junctions consist primarily of the channel proteins connexin43 (Cx43) and connexin30 (Cx30), whose relative expression levels vary considerably between developmental stages and brain regions [20,21].

The progression to epilepsy is characterized by the presence of neuroinflammation, along with structural and molecular changes in the brain. These subsequent changes lead to increased neuronal hyperexcitability and a long-lasting propensity for recurrent spontaneous seizures [22]. Microglia play a role in regulating neuroinflammation and axonal sprouting and have also been reported to modulate neurogenesis. After seizures, microglia are activated and act as resident macrophages in the brain, responding quickly to injury while trying to keep physiological processes in check [23]. Changes in neuronal homeostasis are also observed, highlighting the various ways in which microglia can contribute to the development of epilepsy [22].

After seizures, cytokines such as IL-1β, IL-6, and TNF-α are released, modulating inflammatory responses in the brain. Studies indicate that these cytokines influence NMDA receptors, synaptic plasticity, GABAergic neurotransmission, and neuronal excitability, contributing to the development and recurrence of seizures. Inhibiting IL-1β activity, for example, has shown a reduction in seizures in rodent models. TNF-α, released by microglia and astrocytes, positively regulates synapses, glutamate release, and GABA levels. Furthermore, IL-6, regulated by TNF-α and IL-1β, negatively influences neurogenesis in the hippocampus and increases microgliosis, possibly contributing to epileptogenesis. (Figure 1A) [17,18,24,25]. Prostaglandins activate the EP3 receptor on astrocytes, leading to increased glutamate release and inducing hyperexcitability and neuronal cell death. Conversely, inhibiting the EP3 receptor may delay the onset of seizures [26].

In addition to epileptogenesis, studies regarding genetic and lesion-induced epilepsies also indicate common pathologic mechanisms. The proposal of an imbalance between excitation and inhibition has been considered as a mechanism of ictogenesis and epileptogenesis. This imbalance is associated with an increase in extracellular glutamate in the brain and/or a decrease in GABA concentrations, resulting in excitotoxicity, convulsions, and cell death [27]. The implication of an imbalance between excitation and inhibition highlights the importance of understanding the relationship between epilepsy and glutamate, a crucial neurotransmitter in the central nervous system and the most abundant amino acid in the mammalian brain. Glutamate plays an essential role in several processes, such as learning, memory, cognition, and emotion, and all activities related to glutamate in the brain occur in the extracellular space [28,29].

Glutamate is released by glutamatergic neurons into the extracellular space, acting on the ionotropic and metabotropic receptors. Neuronal and astrocytic transporters prevent overexcitation by removing glutamate from the synaptic cleft. In the astrocytes, glutamate is converted to glutamine, transported back to the neurons, and reverted to glutamate, allowing for continued release. Glutamate regulation involves both the neurons and astrocytes, and any dysfunction in the system can cause an imbalance between excitation and inhibition [28,30]. Specifically, astrocytes may play a role in the pathogenesis and pathophysiology of epilepsy by modulating synaptic transmission through the release of glycotransmitters such as glutamate, ATP, and D-serine, thus contributing to homeostatic control [19,20]. Astrocytes also contribute to the supply of glutamine to the GABAergic neurons, converting it into glutamate and subsequently, into gamma-aminobutyric acid (GABA) through the action of glutamate decarboxylase. This GABA is then packaged into vesicles for release. GABA is the main inhibitory neurotransmitter, and it is crucial to maintain an adequate balance between it and glutamate. An imbalance between excessive glutamate and/or inadequate GABA can result in overexcitation of the central nervous system, predisposing the occurrence of seizures [29,31].

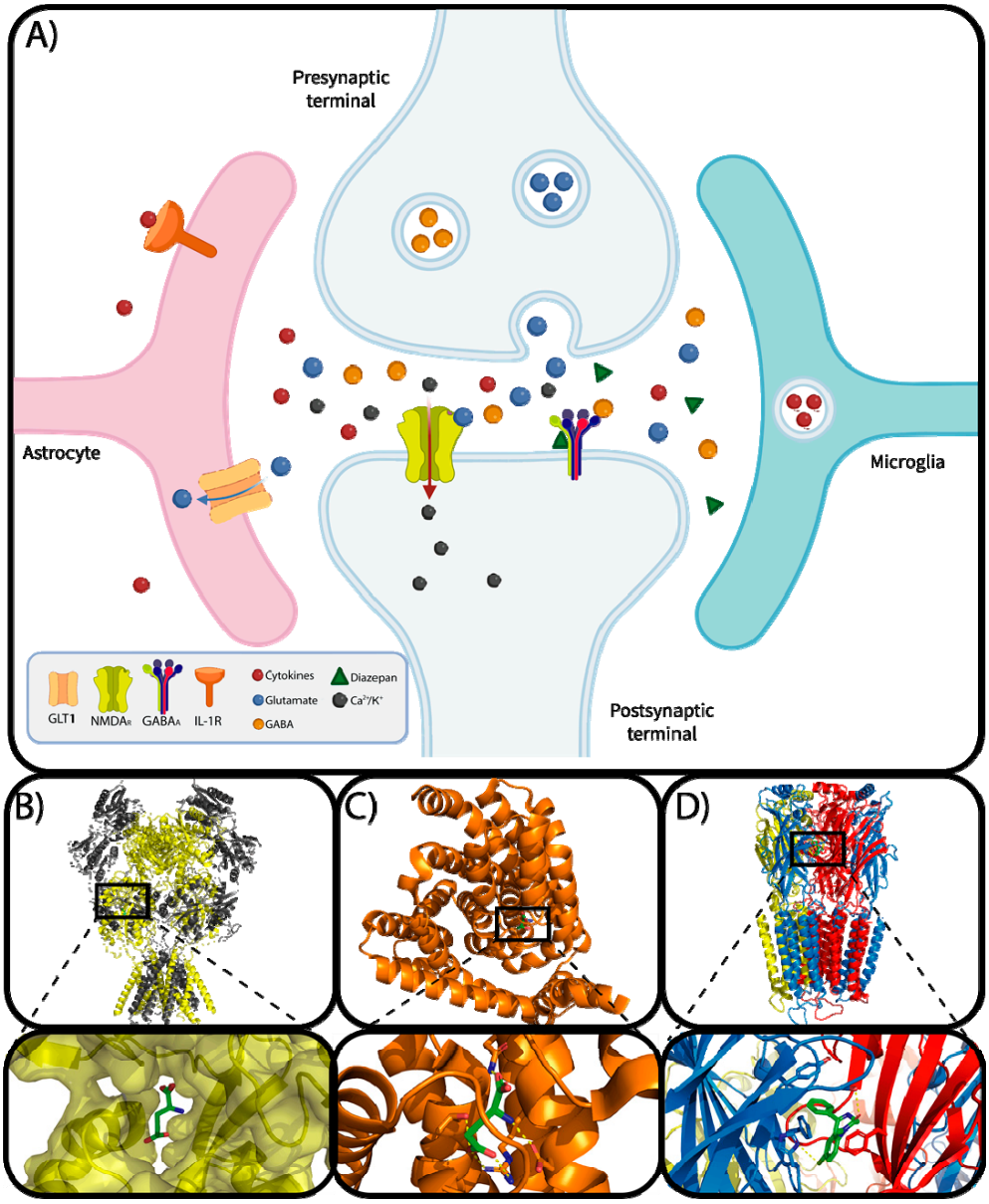

Figure 1. Epileptogenesisis activated by neuroinflammation and the molecular targets for the treatment of epilepsy. (**A**) Synaptic cleft demonstrating a scenario of neuroinflammation influencing epileptogenesis. Due to the initial mechanisms of inflammation (breakdown of the blood–brain barrier and/or genetic factors), there is an expression of cytokines by the microglia, directly influencing glutamate reuptake activity. Furthermore, astrocytes, which naturally contribute to CNS homeostasis, can promote the maintenance of epilepsy by causing an imbalance of glutamate in the synaptic cleft. (**B**) NMDAR transmembrane protein focusing on the glutamate binding site. In a normal situation, this receptor is responsible for transporting ions to the cellular interior of the postsynaptic neurons. In an epilepsy situation, glutamate levels are elevated, promoting greater activation of this receptor, resulting in hyperexcitability. (**C**) In the astrocytes, GLT-1 transports protein in combination with glutamate, focusing on the binding site. Naturally, this protein acts in the reuptake of glutamate, and in the presence of neuroinflammation, the cytokines released by the microglia through the activation of IL-1R promote the reduction of GLT-1 expression, increasing glutamate levels in the synaptic cleft. (**D**) GABAA transmembrane protein in the diazepam complex, focusing on the binding site. In a normal situation, this receptor is responsible for the uptake of GABA, resulting in a suppression of neuronal activity. In the presence of diazepam, this receptor has an increased affinity for GABA, reducing epileptic effects.

Dysregulation in the glutamatergic mechanisms in epilepsy involves dysfunctions in the interactions between the neurons, the astrocytes, or both of these. This may include dysregulation of the ionotropic or metabotropic receptors, abnormal expression of the astrocytic glutamate transporters, and the malfunction of the neuronal or astrocytic enzymes. Genetic mutations in the NMDA receptors, such as GRIN1, GRIN2B, and GRIN2D, and mutations in the AMPA receptors, which increase AMPA expression, are suspected of contributing to physiological imbalances in epilepsy, remodeling and reconfiguring the neural networks. Glutamatergic dysregulation can lead to the accumulation of glutamate in the synapse, as well as the overactivation of glutamate receptors, resulting in excitotoxicity and eventually, cell death [32,33].

3. Genetic Influence on Epilepsy

Currently, cases of severe epilepsy are closely related to genetic factors. About 0.4% of the human population have genetic epilepsy; this percentage corresponds to about 30% of all known epilepsies. More than 50 genes associated with this pathology have recently been identified [34]. It is known that the main genetic influence on severe epileptic conditions is related to mutations in the ion channel neurotransmitters, causing neuronal hyperexcitability or exhaustion of the inhibitory mechanisms, which results in seizures. However, other genes, such as those that cause mutations in transcription factors, intracellular signaling molecules, chromatin remodelers, metabolic enzymes, and even genes in the mitochondrial complex [34], have been identified in individuals with genetic epilepsy.

One of the most important identified mutations is in the existence of R-type calcium channels. Individuals exhibiting the CACNA1E variant present early epilepsy, which is characterized by presenting not only seizures, but also macrocephaly, developmental delay, severe hypotonia, congenital contractures, hyperkinetic movements, and early death. This type of epilepsy is known as early infantile epileptic encephalopathy [34,35].

In addition to GABRB3-type mutations, an apparent connection with early childhood epileptic disorders is also observed, accompanied by significant impairment in the intellectual development of those affected. Additionally, mutations in the GRIN2A and GRIN2B genes, related to the NMDA receptors, can trigger epileptic disorders in neurodevelopment, epilepsy spectrum disorders, and idiopathic focal epilepsy. The GRIN1 gene, in turn, is associated with cases of epilepsy accompanied by developmental delay, along with hyperkinetic movement disorders and infantile hypotonia [36]. The KCNA2 gene was recently identified as one of the genes responsible for epilepsy. KCNA2 is related to the current-dependent potassium channel, which includes four subunits that can assume different conformations, and which is expressed in the central nervous system [37]. Studies have shown that a mutation in KCNA2 can cause dramatic loss or gain of function. Studies with mice show that individuals in whom this gene was deleted were more likely to develop seizures. In addition, some mutations were observed in humans, such as Q213* and G398C, which caused loss of function, as well as mutation L298F, which affects the second arginine of the amino acid sequence and causes a 13-fold increase in channel current, and E157C, which showed an up to 5-fold increase in current [37].

A newly discovered gene related to the onset of focal epilepsy, DEPDC5, was also shown to be associated with other epileptic syndromes, and it is related to the cases of childhood epilepsy accompanied by sudden death. Although it is not included in the group of epilepsies that cause impairment in intellectual development, some individuals with this variant may present with intellectual impairment and the development of ASD [38]. Another study showed that patients with developmental encephalopathy and epilepsy exhibited groups of genes that were previously unrelated to epilepsy, including FGF12, GABBR1, GABBR2, ITPA, KAT6A, PTPN23, RHOBTB2, and SATB2 genes [39].

4. New Therapeutic Targets and Pharmacological Strategies

Studies related to the development of new anti-epilepsy drugs have been increasingly requested due to the recent emergence of resistance to anti-epilepsy drugs currently on

the market, as well as scientific evidence that proves the long-term ineffectiveness of the use of anti-epilepsy drugs [40]. About one-third of people with epilepsy disorders exhibit incomplete or partial control of seizures, even with the administration of anticonvulsant drugs, used in combination or alone [40,41]. Over the past few years, a variety of therapeutic targets have been elucidated to develop anti-epileptic drugs. For a drug to be considered an anticonvulsant, it must promote a balance between the excitation and inhibition of the neurotransmitters, primarily including the GABA and glutamate pathways [42,43]. As the targets of their main mechanisms of action, molecular agents act directly on the physiological process of the designated neuronal element, such as ion channels, enzymes, transport proteins, receptors, or even agents that regulate gene expression, allowing for a partial or total reduction of symptoms observed in an epilepsy condition [42,44].

According to the Epilepsy Foundation (https://www.epilepsy.com/ (accessed on 10 July 2023)) and DailyMed (https://dailymed.nlm.nih.gov/dailymed/ (accessed on 10 July 2023)), in regards to the current commercially available drugs for the treatment of seizures, there are 36 antiepileptic drugs (AEDs) approved by the FDA (Supplementary Table S1). Their mechanisms of action are mostly unknown, but some drugs exhibit more clearly elucidated or proposed mechanisms of action; the most common treatment targets are the GABA system, the voltage-gated channels, the synaptic vesicle protein 2A, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor, and the N-methyl-D-aspartate (NMDA) receptor. Examples of currently marketed AEDs include benzodiazepines, carbamazepine, and levetiracetam [45–47]. While most anticonvulsant medications demonstrate effects related to controlling and reducing symptoms, they do not necessarily act specifically by modulating pathogenic disease mechanisms, as the majority of cases are still not fully elucidated in regards to their pathogenesis [48].

However, symptom reduction is noted for different types of treatment, according to the observed pathology, with monotherapy methods using only one type of medication, and polytherapy techniques targeting different therapeutic targets, altering pharmacological doses, or alternating active ingredients derived from the same class of action [49–51]. Due to the complexity of the disease and its multiple variants, it is necessary to search for new therapeutic targets in order to develop innovative pharmacological strategies, as the same drug may have different effects, depending on the patient [52–54].

Over the last few years, epigenetics has greatly contributed to advancement in the understanding of epileptogenesis and epilepsy syndromes, demonstrating that there is a wide range of alteration in the expression of genes related to cellular physiology, including neural structure assembly, as well as the abundance of neurotransmitters and ion channels that play a critical role in the nervous system [55]. Furthermore, studies reveal the role of neuroinflammation as a process that can not only favor, but also intensify epileptogenesis; when seizures occur, the brain releases a series of chemical mediators, mainly glutamate, causing neuronal hyperexcitability (Figure 1A) [33,54,56]. The key chemical mediators of neuroinflammation resulting from a seizure event include the activation of pathways such as the TLR, IL-1, and IL-6 receptors, the prostaglandin-arachidonic acid cascade, oxidative stress, TNF, and caspase 1 [57–61].

Neuroinflammation, among other epileptogenesis-associated pathways, can lead to neurophysiological changes in the tissues, including neuronal dysfunction, stress, and acute or chronic neurodegeneration [62,63]. In recent years, the neuroinflammation pathway has been isolated as an emerging target for the development of new drugs with anti-neuroinflammatory potential for the treatment of epilepsy [64]. These pathways may be even more promising, as they act directly on the disease mechanism, and not merely by fighting symptoms [65]. Neuroinflammation can be considered one of the most relevant causes for the emergence of this phenomenon, and this occurs mainly due to the influence of cytokines released by microglia that directly interfere with the function of other glial cells [66,67].

When a neural stimulus is released in a normal state, there is a balance between the excitatory and suppressive neurotransmitters, such as glutamate and GABA, which bind to

their respective postsynaptic neuroreceptors, such as $NMDA_R$ (Figure 1B) and $GABA_A$ [68]. Furthermore, the presence of glial cells such as astrocytes plays an important role in glutamate reuptake [69]. In a neuroinflammation scenario, the presence of cytokines in the surroundings of the synaptic cleft can activate astrocyte membrane receptors, triggering a series of intracellular routes that result in the production of factors that influence the expression of GLT-1, a transporter protein that acts in the reuptake of glutamate, keeping the levels of this neurotransmitter stable (Figure 1C) [70,71]. Without the action of this agent, the synaptic cleft contains more glutamate than GABA, resulting in a greater interaction with the excitatory neuroreceptors, causing the phenomenon of frequent seizures [72,73].

Inhibiting microglial activity using anti-inflammatory drugs may directly impact the function of regulating glutamate levels mediated by the astrocytes, as GLT-1 can remain functional [74]. This protein is made up of three identical subunits that join together, forming a complex capable of transporting up to three glutamate units [75]. Just as external factors, such as LPS, can activate microglia, the seizure event itself can prolong the effect of neuroinflammation, due to exacerbated levels of excitatory neurotransmitters and pH changes in the brain, causing injuries that lead to a cycle of chronic neuroinflammation, progressively increasing the severity of the seizures [76].

Among the existing medications, diazepam, a drug belonging to the benzodiazepine class, is well characterized, capable of quickly crossing the blood–brain barrier and interacting with GABAergic receptors, increasing its affinity for γ-aminobutyric acid, promoting a relaxation and suppression of motor neuronal action (Figure 1D) [77,78]. The $GABA_A$ neuroreceptor is an important target for the development of drugs exhibiting neuronal action, as it possesses different allosteric sites which interact with compounds with analgesic, sedative, antispasmodic, anxiolytic, and anesthetic properties; it is a dimer composed of five different subunits—α1-A, α1-D, β3-B, β3-E, and γ2-C—interconnected together, forming a transmembrane ion channel which is present in the postsynaptic region of the neurons [79].

In addition to neurons, other cellular targets responsible for the maintenance and homeostasis of nervous tissue, such as microglia, a resident macrophage of the central nervous system that plays a regulatory and protective role under physiological conditions, may be promising targets for the development of anti-neuroinflammatory drugs [80]. Several studies focusing on the development of biopharmaceuticals with the potential to inhibit microglia are currently being carried out; for example, antimicrobial peptides with anti-inflammatory action are being investigated [81]. Ca-MAP1 is a multifunctional synthetic peptide with inhibitory action on microglia stimulated by LPS, rationally designed for use in an *in vitro* neuroinflammation model using BV-2 cell culture [82].

Peptides are molecules that may have a high potential for the treatment and prevention of epileptogenesis, as they are easily absorbed by the blood–brain barrier due to their small size, and they interact with specific targets, promoting a neuroprotective effect [83]. Arginine and lysine-rich cationic peptides exhibit a range of biological activities, including immunomodulation, the blockade of ion channels, and tropism of membranes with lytic or cell penetration action, contributing to potential permeability and access to the central nervous system [84–87]. Some peptides, such as TAT-NR2B9c, CN-105, and RD2, have a neuroprotective function and can be used for the treatment of ischemic stroke, hemorrhagic stroke, and Alzheimer's disease. Based on clinical data, cationic arginine-rich peptides have been demonstrated to be safe when administered in therapeutic doses by slow venous infusion [88].

In general, all types of epilepsy are related to increased extracellular levels of Ca^{2+} ions and glutamate, contributing to the hyperpolarization and hyperexcitability of motor neurons. Cannabidiol (CBD) is a compound derived from *Cannabis sativa*, and it has an intracellular mechanism of action that acts directly on receptors such as GPR55 and TRPV1, which play a key role in epileptogenesis, allowing for low levels of membrane polarization in the neurons, in addition to blocking the reuptake of adenosine, promoting an increase in extracellular levels of adenosine in the nervous system [89].

Finally, more recent studies have demonstrated that the cytoskeleton and structuring proteins that promote the attachment and formation of synaptic clefts may be crucial factors that effectively contribute to the progression of the epileptic phenomena [90]. This occurs because Arc proteins are responsible for the formation of neuron projections, which interfere in the period of cell–cell interaction, as well as in the neuroplasticity of cells in the sclerotic hippocampus, a neuronal area that demonstrates relationships with cognitive activity [91,92].

Changes in the expression of Arc protein can trigger greater neuronal plasticity during epileptic seizures, leading to an increase in the formation of new connections between the neurons and promoting a chance of electrochemical imbalance in the neuronal tissue [90]. Furthermore, the upregulation of cytoskeletal protein (Arc) mRNA, related to activity in the dentate granule cells (DGCs) of the sclerotic hippocampus, may be a crucial molecular target in the development of new therapeutic strategies [90,92]. These factors, among the others mentioned above, contribute to epileptogenesis and the emergence of specific lesions in the tissue, resulting in an inflamed region, with impact on the microglia [93].

Although our understanding of the seizure phenomenon is still not completely clear, several molecular targets can be used for the development of new drugs, including regulatory agents of gene expression, i.e., GABAergic, purinergic, or opioid neuroreceptors, which promote the suppression or reduction of seizure neuronal activity [36]. Targets involved in neuroinflammation include the inhibition of the microglia and pro-inflammatory receptors, in addition to blocking the ions involved in neuronal membrane polarization [94].

5. Drug Interventions in Epilepsy-Related Clinical Trials

In the search for new forms of treatment or the reuse of existing drugs for the treatment of many conditions and illnesses, including epilepsy, clinical trial research is a method for ensuring proper drug dosage, efficacy, and safety. A clinical trial comprises many phases, with each one designed to investigate one aspect of drug usage; it is divided into preclinical and phases 0 to IV [95,96]. Within the research regarding new drug treatments for epilepsy and epilepsy-related conditions, we identified 92 clinical trials, completed since 2013, in the platform Clinical Trials (https://www.clinicaltrials.gov/ (accessed on 10 July 2023)) found in the National Center for Biotechnology Information database (Table 1).

Table 1. Most relevant completed epilepsy-related clinical trials since 2013 yielding results involving drug intervention, with data collected from ClinicalTrials.gov. NCT refers to National Clinical Trial; M and F refer to male and female.

NCT Number	Conditions	Interventions	Sex	Age	Phase
NCT02451696	Epilepsy | Tuberous Sclerosis Complex | Focal Cortical Dysplasia	Everolimus	M/F	Child, adult	2
NCT02758626	Epilepsy	Ataluren | Placebo	M/F	Child	2
NCT03940326	Epilepsy, Idiopathic Generalized	Levetiracetam | Valproate	M/F	Child, adult	4
NCT02564952	Epilepsy	GWP42003-P | Clobazam	M/F	Adult	2
NCT03650452	Epilepsy | Dravet Syndrome | Lennox–Gastaut Syndrome	Tak-935 | Placebo	M/F	Child	2
NCT01777139	Epilepsy	Retigabine Immediate Release	M/F	Adult	3
NCT03179891	Epilepsy	Diazepam Buccal Film	M/F	Adult	2
NCT03283371	Epilepsy, Focal Seizures, Partial Seizures	Natalizumab | Placebo	M/F	Adult	2
NCT01963208	Drug Resistant Partial Onset Seizure	Ganaxolone | Placebo	M/F	Adult	3

Table 1. *Cont.*

NCT Number	Conditions	Interventions	Sex	Age	Phase
NCT03478982	Epilepsy	Staccato alprazolam ǀ Placebo	M/F	Adult	2
NCT03222349	Epilepsy	Diazepam Buccal Film	M/F	Child	2
NCT02682927	Dravet Syndrome ǀ Seizure Disorder	Zx008 (fenfluramine hydrochloride) ǀ Placebo	M/F	Child, adult	3
NCT02224703	Epilepsy ǀ Dravet Syndrome	GWP42003-P ǀ Placebo	M/F	Child, adult	3
NCT02565108	Epilepsy	GWP42003-P ǀ Clobazam	M/F	Adult	2
NCT02849626	Partial-Onset or Primary Generalized Tonic–Clonic Seizures	Perampanel	M/F	Child	3
NCT02700412	Epilepsy ǀ Seizures	Epidiolex	M/F	Adult	1
NCT02724423	Acute Repetitive Seizures	Nrl-1	M/F	Child, adult	1
NCT02695537	Epilepsy ǀ Seizures	Epidiolex	M/F	Child, adult	1
NCT03405714	Epilepsy	Brivaracetam	M/F	Child	2
NCT03373383	Drug-Resistant Epilepsy ǀ Focal-Onset Seizures	Padsevonil ǀ Placebo	M/F	Adult	2
NCT01747915	Generalized Tonic–Clonic Seizures	Pregabalin ǀ Placebo	M/F	Child, adult	3
NCT02404168	Epilepsy	Lamotrigine (Brand Lamictal) ǀ Lamotrigine (Generic Teva)	M/F	Adult	4
NCT02721069	Acute Repetitive Seizures ǀ Breakthrough Seizures	NRL-1	M/F	Child, adult	3
NCT01954121	Epilepsy ǀ Partial Seizures	Levetiracetam ǀ Carbamazepine	M/F	Child, adult	3
NCT01832038	Epilepsy ǀ Partial-Onset Seizures	Lacosamide	M/F	Child, adult	3
NCT03428360	Epilepsy	Diazepam Buccal Soluble Film	M/F	Child, adult	3
NCT02036853	Glucose Transporter Type-1 Deficiency Syndrome (Glut1 DS)	Triheptanoin	M/F	Child, adult	2
NCT01964560	Epilepsy	Lacosamide	M/F	Child	3
NCT02224560	Epilepsy ǀ Lennox–Gastaut Syndrome	GWP42003-P ǀ Placebo	M/F	Child, adult	3
NCT02224573	Epilepsy ǀ Dravet Syndrome ǀ Lennox–Gastaut Syndrome	GWP42003-P	M/F	Child, adult	3
NCT03021018	Epilepsy	Brivaracetam ǀ Lorazepam	M/F	Adult	2
NCT01999777	Epilepsy	USL261 ǀ Placebo	M/F	Child, adult	3
NCT01713946	Tuberous Sclerosis Complex-Associated Refractory Seizures	RAD001 ǀ Placebo ǀ Antiepileptic drug (1 to 3 only) ǀ Open Label RAD001	M/F	Child, adult	3
NCT02564029	Reflex Epilepsy, Photosensitive	PF-06372865 ǀ Placebo ǀ Lorazepam	M/F	Adult	2
NCT03116828	Epilepsy with Partial Onset Seizures	Eslicarbazepine Acetate (first add-on) ǀ Eslicarbazepine Acetate (late add-on)	M/F	Adult	4
NCT02100644	Epilepsy	Lamotrigine Tablets	F	Child, adult	4
NCT02495844	Highly Drug-Resistant Focal Epilepsy	UCB0942 ǀ Placebo	M/F	Adult	2

Table 1. *Cont.*

NCT Number	Conditions	Interventions	Sex	Age	Phase
NCT01866111	Partial Epilepsy	Ykp3089 | Placebo	M/F	Adult	2
NCT02926898	Dravet Syndrome	Zx008 (fenfluramine hydrochloride) | Matching Placebo	M/F	Child, adult	3
NCT02351115	Epilepsy	Placebo | Inhaled Alprazolam | Inhaled Alprazolam | Inhaled Alprazolam | Placebo	M/F	Adult	2
NCT03953820	Epilepsy	Diazepam Buccal Film | Diastat® Rectal Gel	M/F	Adult	1|2
NCT02072824	Partial Onset Seizures	Pregabalin dose level 1 | Pregabalin dose level 2 | Placebo	M/F	Child	3
NCT04882540	Healthy Participants	Brivaracetam	M/F	Adult	1
NCT02726074	Epilepsy	Perampanel	M/F	Child, adult	4

5.1. Lacosamide

One well-studied drug for epilepsy is lacosamide, a third-generation antiseizure drug for partial-onset seizures [97] that has been used in 2206 participants enrolled in 10 clinical trials for epilepsy, with or without partial-onset seizures. The lacosamide mechanism of action is predominantly exerted by a slow selective sodium channel inactivation, as it may bind to the collapsin response mediator protein-2 [98,99].The experimental use of lacosamide in clinical trials may be employed using two administration pathways, either oral or intravenous, and the drug concentration ranges from 2 mg·(kg·day^{-1})$^{-1}$ to 12 mg·(kg·day^{-1})$^{-1}$ in oral solution intake; 50 mg·day^{-1} to 600 mg·day^{-1} for tablet intake; and 20 mL of 10 mg·mL^{-1} of lacosamide for intravenous application.

The clinical trial study results using lacosamide showed a wide efficacy in the reduction in seizures or the achievement of "seizure-free" days or months; with a large number of participants, the efficacy rate can vary, depending on the number of participants and their ages. The two clinical trials with the greatest number of participants, as identified with the National Clinal Trial (NCT), are represented as NCT01964560 and NCT01832038, with 540 and 473 participants, respectively.

The NCT01964560 study design included male and female participants, with a mean age of 7.4 $\pm$ 5.4 years, mostly of white ethnicity, and reported that 537 participants experienced a mean percentage of 66.96 $\pm$ 36.18 seizure-free days within the 96 weeks of the study. The NCT01832038 study enrollment involved male and female participants with a mean age of 32.7 $\pm$ 12.0 years, mainly Chinese, demonstrating that 57.1% of the 471 participants described a $\geq$50% reduction in partial-onset seizure frequency from baseline per 28 days. The clinical trials NCT01964560 and NCT01832038 have also reported serious and non-serious adverse events in 77.2 and 86.7% of all its participants, respectively. Those adverse events may or may not be lacosamide treatment-related; the most frequent non-epilepsy-related adverse events include vomiting, diarrhea, pyrexia convulsion, pneumonia, nausea, upper respiratory tract infection, nasopharyngitis, blurred vision, abdominal pain, and many others.

5.2. Cannabidiol (CBD)

One emerging drug used in the treatment of epilepsy is GWP42003-P, also known as CBD [89]; it has been tested on 1604 participants enrollment over 13 clinical trials over the last decade. Clinical trials studying the epileptic condition also include other epilepsy-related conditions, such as infantile spasms, Sturge–Weber syndrome, Dravet syndrome, seizures, and Lennox–Gastaut syndrome. The CBD antiepileptic mechanism of action is

unknown; however, it is proposed that it can act on multiple molecular targets due to its high affinity to the transient receptor potential vanilloid-1 (TRPV1), the desensitizing cation channel, and other ion channels. Another possible molecular target for the CDB anticonvulsant mechanism of action is the equilibrative nucleoside transporter-1 (ENT-1) and its interactivity with the purinergic system or the carrier of Ca^{2+} ion, called the 55-receptor, coupled to G-protein (GPR55) [89,100].

In epilepsy-related clinical trials, the CBD administration route is oral, with concentrations ranging from 2 mg·(kg·day^{-1})$^{-1}$ to 40 mg·(kg·day^{-1})$^{-1}$. The three largest CBD clinical trials are NCT02224573, NCT02224560, and NCT02224703, with 681, 225, and 199 patients, respectively. These three trials are focused on the condition of epilepsy related to Dravet syndrome and/or Lennox–Gastaut syndrome. The clinical trial NCT02224573 includes a male and female enrolment, with 315 participants with Dravet syndrome and 366 with Lennox–Gastaut syndrome; the participant's mean age is 9.7 $\pm$ 4.4 and 15.9 $\pm$ 9.5 years, respectively. The efficacy of CDB treatment for epilepsy has shown that 52.6 and 51.4% of the participants with Dravet syndrome and Lennox–Gastaut syndrome, respectively, have reported a $\geq$ 50% reduction in total seizures in the final 12 weeks of the clinical trial.

Participants in the clinical trials NCT02700412 and NCT02695537, with a focus on the CBD treatment of epilepsy and seizure conditions, demonstrated that in children and adults, there is a significant reduction in seizure severity, frequency, or both [101]. The most common adverse effects found in the participants in CDB clinical trials were convulsion, diarrhea, pyrexia, decreased appetite, somnolence, and pneumonia [102,103].

5.3. Perampanel

One broad-spectrum antiepileptic drug is perampanel, an FDA-approved monotherapy orally active, non-competitive, selective α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist. Its antiepileptic mechanism of action includes the reduction of stimuli in the AMPA receptors via the AMPA receptor antagonism, thus exerting an anticonvulsant effect, inhibiting seizure generation and dissemination [104–106]. In perampanel clinical trials 334 participants were enrolled in five clinical trials, with a focus on the conditions of epilepsy, seizures, and partial-onset or primary generalized tonic–clonic seizures. The dosage range for perampanel is 2 mg·day^{-1} to a maximum of 16 mg·day^{-1}, administered either orally or intravenously, with the largest clinical trial using perampanel (NCT02849626) utilizing a starting maximum dosage of 8 mg·day^{-1}, which could be increased, if tolerated. For participants taking any enzyme-inducing antiepileptic drugs (EIAEDs), the adjunctive dose of perampanel was 12 mg·day^{-1} maximum [107].

The clinical trial NCT02849626 enrolled 180 male and female participants, with a mean age of 8.1 $\pm$ 2.0 years, and resulted in a 40% reduction in focal seizures (FS), a 59% reduction in focal to bilateral tonic–clonic seizures (FBTCS), and a 69% reduction in generalized tonic–clonic seizures with the adjunctive treatment with perampanel. The most common treatment-emergent adverse events were somnolence, nasopharyngitis, dizziness, irritability, pyrexia, and vomiting [107,108]. Another larger perampanel clinical trial is the NCT02726074 trial, which focuses on monotherapy for epilepsy, with 106 male and female enrolled participants, with a mean age of 42.2 $\pm$ 14.2 years, receiving a starting dose of 2 mg·day^{-1}, increasing by 2 mg·day^{-1} every 2 weeks, if necessary, to a maximum dose of 12 mg·day^{-1}. The treatment response was a 100%reduction in the responder rate in 47 and 75% of participants with partial onset seizures, with or without secondary generalization and secondary generalized tonic–clonic seizures, respectively.

5.4. TAK-935

A novel compound TAK-935, also known as soticlestat, is an inhibitor of cholesterol 24-hydroxylase (CH24H), a brain-specific cytochrome P450 family enzyme essential for the homeostasis of brain cholesterol. TAK-935 is capable of restoring the excitatory/inhibitory balance in many preclinical hyperexcitability models. TAK-935 was tested in 230 enrolled participants in five clinical trials over the last decade [109,110]. The TAK-935 clinical trial

was focused on the conditions of epilepsy, Dravet syndrome, Lennox–Gastaut syndrome, 15q duplication syndrome, CDKL5 deficiency disease, and developmental and/or epileptic encephalopathies. The administration pathway of TAK-935 is oral or by PEG tube/G-tube; the concentration can range from 50 to 600 mg·day^{-1}.

The largest clinical trial was NCT03650452, which included 141 mostly white or Asian male and female participants, with a mean age of 9.5 ± 4.0 years. The clinical trial NCT03650452 applied TAK-935 orally or via PEG tube/G-tube, with a dosage of 200 mg/day, followed by 400 mg/day, then 600 mg/day, up to week 20. This treatment resulted in a reduction in seizure frequency of 27.76, 36.50, and 18.46% in epilepsy, Dravet syndrome, and Lennox–Gastaut syndrome, respectively. The most common treatment-emergent adverse events involving TAK-935 were upper respiratory tract infection, pyrexia, nasopharyngitis, decreased appetite, vomiting, somnolence, diarrhea, lethargy, fatigue, pneumonia, irritability, and constipation [111].

5.5. Ganaxolone

Another noteworthy drug is ganaxolone, an FDA-approved first-in-class medication used to treat seizures in patients with cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder [112,113]. Its mechanism of action is thought to be the modulation of the synaptic and extrasynaptic $GABA_A$ receptors through binding in the allosteric sites of the receptor. This causes a hyperpolarization of the neuron and an inhibitor effect on neurotransmission, reducing the chance of a successful potential depolarization [114,115]. Ganaxolone has been employed in clinical trials, with the enrollment of 605 participants in six clinical trials, with a focus on drug-resistant partial onset seizures, CDKL5 deficiency disorder, tuberous sclerosis, PCDH19-related epilepsy, status epilepticus, convulsive status epilepticus, non-convulsive status epilepticus, and epilepsy.

The dosage applied in clinical trials of ganaxolone depended on the administration route, with the intravenous application concentration of ganaxolone ranging from 500 to 1800 mg·day^{-1}. The clinical trial NCT01963208 was the largest trial employing ganaxolone, with 405 mostly white male and female participants, with a mean age of 39.7 ± 11.7 years. The clinical trial NCT01963208 yielded a result of a 21.28% reduction in seizure frequency from baseline to week 14 of the study; within the same clinical trial in the same period, 28.1% of the participants treated with ganaxolone reported at least a 50% decrease in 28-day seizure frequency. The most common adverse events observed with ganaxolone treatment are fatigue, nasopharyngitis, somnolence, dizziness, and headache.

5.6. Everolimus

Two clinical trials used everolimus for seizure control, NCT02451696 and NCT01713946, with 14 and 366 participants, respectively. The trial NCT02451696 was conducted employing participants with TSC and refractory epilepsy. Four participants were approved for treatment with everolimus for 7–28 days, at a concentration of 4.5 mg·L^{-1} orally, yielding plasmatic concentrations of 5 to 15 ng·mL^{-1}. The participants who received treatment had a mean age of 18.25 ± 10.1 years, while the control group had a mean age of 13.1 ± 12.3 years. In study NCT02451696, the presence of adverse effects, which were not reported, and levels of vascular endothelial growth factor (VEGF), mTOR brain tissue-s6 phosphate obtained by Western blotting, and HMGB1 expression in brain tissue were investigated. These methods noted a difference in phospho-S6 expression, which is mainly associated with seizure cases. On the other hand, study NCT01713946 was carried out with three different groups, receiving concentrations of 3 to 7 ng·mL^{-1}, 9 to 15 ng·mL^{-1}, and a placebo group, employing dispersible tablets for oral suspension. The age of the participants ranged from 6–65 years old, and the study lasted for 12 weeks. A total of 24.8% of the participants receiving the lowest dose, and 42% of participants in the highest dose group showed a 50% decrease in seizure frequency [116].

5.7. Ataluren

Another drug used as a possible treatment for seizures involving Dravet syndrome and CDKL5 deficiency disorder is ataluren, a drug whose main action is for preterm infants, allowing for the reading of the ribosome of an mRNA with the para codon generated in a full-length protein [117]. In the clinical study NCT02758626, ataluren was used to control seizures arising from the aforementioned diseases. There were seven children with DS and eight with CDKL5 deficiency disorder included in the study, with a mean age of 6.4 and 3.5 years, respectively, ranging from 2 to 11 years old. The study was a double-blind crossover with the use of a placebo as a control; the participants were authorized to receive doses of 10 mg·kg^{-1} in the morning, and 20 mg·kg^{-1} in the middle of the day, in the form of powder for suspension. However, the drug did not demonstrate efficacy in controlling the seizures. The treated groups did not show any significant difference compared to the placebo group. The drug had some adverse effects, but none were of high severity.

5.8. Levetiracetam (LVT) and Valproic Acid (AVP)

Studies were conducted to compare the tolerability and efficacy of anticonvulsant drugs with different mechanisms of action: levetiracetam and valproic acid. Levetiracetam is a drug that binds to the SV2A protein, preventing the release of glutamate in the neurons, demonstrating its antiepileptic action [118,119]. On the other hand, valproic acid is also an antiepileptic drug, which acts by inhibiting succinyl semialdehyde dehydrogenase, increasing the concentration of succinyl semialdehyde, an inhibitor of GABA transaminase, thereby increasing the concentration of the neurotransmitter GABA [120].

The clinical study NCT03940326 used both drugs in the treatment of 103 participants with idiopathic generalized epilepsy. The participants were divided into two groups: one treated with levetiracetam (LVT) and the other with valproic acid (AVP). The groups had a mean age of 26.2 $\pm$ 8.1 and 29 $\pm$ 9.7, respectively. Levetiracetam doses started at 500 mg/week and increased to a dose of 200 mg per day, with further increases to 300 mg per day in cases of seizures. Valproic acid was also started at 500 mg per week, with a maximum dose of 1500 mg·day^{-1}, increased to 2000 mg·day^{-1} in cases of seizures.

As a result, the patients in the LVT group experienced their first seizures, on average, after 169 $\pm$ 6.1 days, while the AVP group experienced their first seizures after an average of 178 $\pm$ 2.2 days. Regarding the absence of seizures, 88.9% of participants in the LVT group and 86.2% in the AVP group did not experience seizure episodes. In the LVT group, 8.9% of the participants discontinued the medication, while in the AVP group, 10.3% did so, with an average time of 220 $\pm$ 8.7 and 172 $\pm$ 4.1 days, respectively. Some adverse events were observed, and valproic acid showed a higher rate of side effects, mainly in terms of weight gain, which was reported by 27.59% of the participants. In addition to this study, others were conducted using different drugs, but involving LVT as one of the tested drugs. The studies NCT02201251, NCT01982812, NCT02707965, and NCT03695094 employed fewer than 70 participants. However, study NCT01954121, comprising 436 participants, compared LVT and carbamazepine (CBZ), a medicine that modulates synaptic transmission and which is also used as an antiepileptic [121].

5.9. Brivaracetam

Another drug from the class of SV2A protein vesicle inhibitors is brivaracetam. Two studies, NCT04882540 and NCT03405714, evaluated the pharmacokinetics of the drug. The latter specifically included participants with epilepsy who were older than 1 month but younger than 16 years of age. The third study, NCT03021018, involved 46 participants with a mean age of 42.12 $\pm$ 13.06 years. The drug provided seizure control within 6, 8, and 12 h, without presenting serious adverse effects to the patients.

In the study group NCT01954121, the participants were divided into two groups: one treated with LVT (218 participants) and the other with CBZ (215 participants). The mean age of the LVT group was 37.8 $\pm$ 16.2 years, while the CBZ group had a mean age of 33.3 $\pm$ 14.3 years. The first group received LVT at a dose of 250 mg twice daily for 2 weeks,

followed by evaluation over the next 27 weeks after stabilization. In the CBZ group, the starting dose was 200 mg once a day, and the participants were also evaluated for 27 weeks after stabilization. Over 6 months, the participants in both groups were analyzed for the absence of seizures. Out of the 186 participants in the LVT group, 47.3% of them remained seizure-free during this period. In the CBZ group, which consisted of 171 participants, 68.4% remained seizure-free. The most common adverse effect observed during the study was nasopharyngitis, a non-serious adverse effect, reported by 42.20% of the LVT group and 43.26% of the CBZ group.

5.10. Benzodiazepines

Benzodiazepines are a class of drugs widely used to treat epilepsy or seizure episodes. These drugs act as agonists of the inhibitory action of GABA, causing hyperpolarization and stabilization of the neuronal membrane, resulting in antiepileptic effects [122]. Benzodiazepines are divided into subclasses based on their duration of action. Alprazolam (APZ) shows fast action and high potency, while diazepam (DZP) exhibits a long duration and medium potency [122].

Clinical studies were conducted using DZP to explore new routes of administration for the medication. Out of four studies, three evaluated new routes of administration. Studies NCT03222349, NCT03179891, and NCT03953820 focused on these new routes, while study NCT03428360 assessed the safety and tolerability of employing DZP buccal film. Other studies involving APZ were NCT02351115, which evaluated the photosensitivity in participants using the drug, and NCT03478982, which involved 156 participants and aimed to determine the pharmacokinetics of seizure episodes using staccato alprazolam administered via oral inhalation. Another benzodiazepine investigated was clobazam (CBZ). Trials were conducted to understand its interactions with CBD. The NCT02564952 trial showed that out of 18 participants, 4 experienced adverse events from this drug interaction. However, in the NCT02565108 trial, which involved 20 participants, no such interaction was noted.

5.11. Lamotrigine (LMT)

Another class of medication that inhibits neural excitability is lamotrigine (LMT). It inhibits the calcium channels, which are linked to the release of neurotransmitters such as glutamate and aspartate [123]. The studies NCT02100644 and NCT02404168 focused on understanding the drug's pharmacokinetics and its interaction with other drugs. In the study NCT02404168, LMT was used in four participants with epilepsy, aged 22–68 years. The study aimed to assess the parameters of the area under the curve and maximum concentration (CMAX) for both reference and generic LMT, which are important constants in drug bioequivalence studies. The comparative CMAX between the drugs showed 8836 ng/mL for the reference and 9024 $ng \cdot mL^{-1}$ for the generic LMT. Among the adverse effects reported by patients, headache was reported by three out of four participants.

On the other hand, study NCT02100644 involved 33 participants with a mean age of 25.6 ± 7.73). They underwent LMT treatments with AVP, receiving different doses which were divided into escalation, reduction, and maintenance phases. The concentration of AVP ranged from 400 to 1200 $mg \cdot day^{-1}$ during the escalation phase, and LMT gradually escalated from 25 $mg \cdot day^{-1}$. In the maintenance phase, AVP was fixed at 300 $mg \cdot day^{-1}$, and the LMT concentration was set at 200 $mg \cdot day^{-1}$. In the reduction phase, the AVP dose was reduced to 200 $mg \cdot day^{-1}$, and the LMT dose to 100 $mg \cdot day^{-1}$, which could be adjusted in case of seizures occurring during the day. The results showed that during the maintenance phase, the participants were evaluated according to the number of days with seizures. They experienced seizures on only two days during a monitored period of 46 weeks. None of the participants reported serious adverse reactions, but 69.7% reported non-serious adverse reactions.

5.12. Pregabalin

Pregabalin is a drug that acts by binding to α2δ voltage-activated calcium channels, which inhibits the release of excitatory neurotransmitters [124]. This drug has been studied for the treatment of seizures and epilepsy. The trial NCT01747915 was conducted with 219 participants, with a mean age of 25.2 ± 13.1 years, and the participants were divided into three different treatment groups: 5 mg·(kg·day^{-1})$^{-1}$, 7 mg·(kg·day^{-1})$^{-1}$, or 300 mg·day^{-1}; 10 mg·(kg·day^{-1})$^{-1}$, 14 mg·(kg·day^{-1})$^{-1}$, or 600 mg·day^{-1}; and placebo.

However, no significant results were observed in the comparison between the treated groups and the placebo group, and there was no reduction in the patients' seizure rate. The same lack of significant results was found in trial NCT02072824, which involved 175 participants with an average age of 28.2 ± 12.6 years, divided into groups receiving 7 mg·(kg·day^{-1})$^{-1}$ or 6 mg·(kg·day^{-1})$^{-1}$; 14 mg·(kg·day^{-1})$^{-1}$ or 12 mg·(kg·day^{-1})$^{-1}$; and the placebo group. Nevertheless, among the analyses, no significant results were presented in when comparing the treated groups with the placebo group, and no reduction in the patients' seizure rate was observed. The same results were obtained in trial NCT02072824, conducted with 175 participants, aged 28.2 ± 12.6 years, with the participants divided into groups receiving 6 mg·(kg·day^{-1})$^{-1}$ or 7 mg·(kg·day^{-1})$^{-1}$; 12 mg·(kg·day^{-1})$^{-1}$ or 14 mg·(kg·day^{-1})$^{-1}$; and placebo, in which no significant results were obtained when the experimental groups were compared to the placebo group.

5.13. Retigabine

Some drugs, such as retigabine, a drug that inhibits neurotransmission through the modulation of potassium channels [125], have shown efficacy in treating partial-onset seizures. The drug was used in trial NCT01777139, which involved 30 participants with a mean age of 36.0 ± 9.25 years. The participants underwent treatment with the drug and were evaluated, resulting in a 50% decrease in seizures over 28 days. Out of the 30 participants, 23 exhibited a positive response. However, some serious adverse events were reported, including headaches and diabetic retinopathy.

5.14. Padsenovil

New drugs, such as padsenovil (PSL), are still under study for the treatment of patients with seizures or epilepsy. Padsenovil's mechanism of action is not fully elucidated, but it is known that it does not bind directly to the GABA receptors. However, it exhibits an antiepileptic action [126]. Trials involving this new drug were conducted, i.e., NCT02495844 and NCT03373383, which involved 55 and 411 participants, respectively. In study NCT03373383, the participants had a mean age of 39.8 ± 12.4 years. The trial revealed the efficacy of PSL compared to placebo, but without dose-dependent characteristics, while also demonstrating safety.

5.15. Other Clinical Trials

Some trials, such as NCT02721069 and NCT02724423, aim to evaluate the safety of certain drugs in new pharmaceutical forms. These trials divided participants into groups receiving different doses of NRL-1, an intranasal formulation of DZP, which is a benzodiazepine [127]. Another trial, NCT01999777, analyzed the efficacy and safety of intranasal midazolam, USL261 [128].

Trials NCT02926898 and NCT02682927, which involved 87 and 362 participants, respectively, aimed to evaluate the action of fenfluramine hydrochloride, a drug that increases the extracellular concentration of serotonin and acts as a 5-HT2 receptor agonist and a σ1 receptor antagonist. This mechanism allows for antiepileptic activity [129–131]. Both trials showed significant reductions in the frequency of monthly seizures compared to that observed in the placebo group, leading to increased quality of life, with seizure-free periods of up to several days [129,131].

Other trials using emerging molecules for the treatment of epilepsy aim to understand the pharmacokinetics, pharmacodynamics, and possible adverse effects of their use. Studies

such as NCT03283371, NCT01866111, NCT03116828, and NCT02036853 continue to seek better seizure control or, in the case of NCT02564029, to focus on increasing the tolerability in cases of seizures caused by photosensitivity.

6. Non-Pharmacological Strategies for Epilepsy Treatment

6.1. Ketogenic Diet

The ketogenic diet is a type of diet based on the control of the proportion of lipid, protein, and carbohydrate intake. The main characteristic of the ketogenic diet (KD) is to mimic the fasting process, contributing to the process of producing ketone bodies [132]. KD intake is rich in fatty acids, low in carbohydrates, and adequate in protein supply; it has been used since 1920 to treat patients with epilepsy who do not respond well to drug treatments [132]. The mechanism of action of the diet has not yet been completely elucidated; however, it is understood that there is a relationship between mitochondrial function, the influence of ketone bodies on neural function, neurotransmitters modulating effects such as the increased synthesis of γ-aminobutyric acid, and the potential for membrane hyperpolarization. The mechanism of action of the ketogenic diet may also be associated with decreasing the release of glutamate, norepinephrine, adenosine, and fatty acids that contribute to the antiepileptic effects and stabilization of glycemia [133].

There are different approaches to applying the ketogenic diet, differing in food bases, such as the KD of long-chain triglycerides (LCT), the KD of medium-chain triglycerides (MCT), the modified Atkins diet (MAD), and treatment with low sugar levels [132]. The distribution of proportions and also the composition of the diets are both factors that individualize the treatment and contribute to its effectiveness and safety [134].

Types of Ketogenic Diets

LCT KD is a diet that is based on the proportion of lipid, protein, and carbohydrate intake, with the majority of the diet being lipids. In LCT KD, the patient's hospitalization helps with adherence to treatment, and the lipid intake ratio is 4:1 (lipids: proteins + carbohydrates), which can range from 3:5:1 to 3:1 [135]. The MCT-type diet proposes the use of medium-chain lipids, such as octanoic and decanoic acid, lipids that are rapidly converted to ketone bodies by the liver [136].

MAD KD, unlike LCT, does not require the patient to be hospitalized, and 65% of the calories in the diet come from lipids. MAD KD allows caregivers, or even the patients themselves, greater flexibility in treatment, as well as better acceptability [135]. The low glycemic level diet allows for the consumption of carbohydrates; however, only those with a low glycemic level, which prevents a rapid increase in blood glucose levels [137].

The mechanism of action of ketogenic diets is based on the conversion of lipids into ketone bodies and their oxidation by the mitochondria, replacing glucose as an energy source for the brain [138]. The presence of ketone bodies and fatty acids is responsible for regulating the excitability of the plasma membrane. These mechanisms occur due to the influence of ketogenic diets in increasing the action of neurotransmitters such as γ-aminobutyric acid (GABA) [132]. Furthermore, under conditions of ketosis, there is a reduction in the use of the glycolytic pathway that produces adenosine triphosphate (ATP), a molecule that sensitizes the potassium channels, leading to cellular hyperpolarization, thus reducing the electrical excitability in seizures [132,138].

6.2. Neuromodulation Therapy

Neuromodulation therapy acts as a non-pharmacological treatment for epilepsy; this type of therapy directly stimulates or prevents the conduction of electrical potential in the brain [139]. Neuromodulation acts directly on the electrical conduction system of the CNS, modulating or modifying brain excitability and impacting the intensity and frequency of seizures in cases of epilepsy [140,141]. The methods used in treatment can be less invasive, through the dermal surface, or highly invasive, accessing the most cortical to the deepest regions of the brain [140].

Among the invasive treatments are: vagus nerve stimulation, deep brain stimulation, and responsive neural stimulation [142]. These are treatments that require implants in specific regions of the CNS, modulating the electrical stimuli that are responsible for triggering high activity in the hypersynchronized neural network during the seizure process [142]. While other treatments have the benefit of not requiring invasive procedures, these methods are used to stimulate the brain through waves, and even through sensorial methods. These treatments include transcranial magnetic stimulation (TMS), transcranial direct current stimulation (TDCS), ultrasound stimulation, transcutaneous VNS (UST-VNS), and trigeminal nerve stimulation (TNS) [142,143].

6.2.1. Invasive

Vagus Nerve Stimulation

Vagus nerve stimulation (VNS) employs stimulus specifically to the vagus nerve, a capacity discovered when it was noted that massage and compression in the carotid region demonstrated the ability to suppress seizures [144], also taking into account the role of the vagus nerve in the control of the brainstem autonomic system [145]. The use of VNS has no age restrictions and can be applied to different types of seizures. The method consists of implanting an impulse generation system (implanted in the subcutaneous region of the left side of the chest), with wires connected to the vagus nerve (subcutaneous) connected to the commercially available programmable pulse generator device (NCP System; Cyberonics, Inc.; Houston, TX, USA) that controls all the parameters used to control seizures [144]. The pulse programming includes the following parameters: current load (the intensity of the electrical stimulus, in milliamps), the pulse width (duration of the electrical pulse, in microseconds), the pulse frequency, and the duty cycle (time to turn the stimulus on and off) [144,146,147].

Deep Brain Stimulation

Deep brain stimulation (DBS) is also an invasive neurointerventional technique, achieved using the implantation of an electrical stimulation compass and electrodes in specific locations in the brain [148]. Due to the characteristics of epilepsy in a hypersynchronized high-intensity discharge, DBS targets the regions of the anterior thalamic nucleus (ANT), the centromedian thalamic nucleus (CM), The subthalamic nucleus (SN), the caudate nucleus (CN), the cerebellum, and the hippocampus [149]. The mechanism of action of the intervention has not yet been completely elucidated; however, it is known that a high-frequency stimulus occurs in the implanted region, which cancels the stimulation of low frequency pathological synchronized neural activity [149], in addition to causing a rhythmic stimulus, helping to synchronize the thalamocortical region and preventing the disorganized stimulation of the cortical area, leading to seizures [150,151].

Responsive Neural Stimulation

This treatment is carried out through the implantation of equipment that maps the electrical activity of the brain, through electrocortigraphic activity and also sends stimuli to interrupt signs of seizure [152]. One to two electrodes are implanted in regions of the brain, according to the seizure focus. The electrodes' function is to monitor brain activity and send electrical stimuli to prevent seizures [153]. Tools are used to detect seizure activity in real-time, as well as to monitor peaks and rhythmic activities within specific frequency bands, where the amplitude and duration of the "half-wave" records are analyzed. Additionally, a comparison is made between short-term averages (128 ms to 4 s) and a long-term average (4 s to 16 min) to identify changes in signal amplitude and frequency, along with the overall signal energy [153].

6.2.2. Non-Invasive

Transcranial Magnetic Stimulation and Transcranial Direct Current Stimulation

As an alternative to surgery, these techniques some of the emerging treatments for epilepsy, as they are non-invasive and focal, with cortical and safe stimulation, emitting small intracranial electrical currents induced by strong and fluctuating extracranial fields [154–156]. There are different approaches to using TMS, which include single-pulse TMS, paired-pulse TMS, and repeated-pulse TMS [156].

TMS is a procedure that uses a coil to produce an electromagnetic field positioned on the head, in which the magnetic fields can modulate nerve cells, improving the symptoms of epilepsy [154]. This therapeutic intervention can reduce the hyperexcitability of nerve cells in the cortical region of the brain, producing a magnetic pulse (100–400µs), and causing depolarization in a few cm^2 of the nearby axons [154].

tCDS, on the other hand, uses a weaker current (around 2 mA), but it also reaches the cortical region of the brain, promoting a change in the polarity of membrane potentials. This type of cathodic stimulation causes a decrease in the discharges linked to epileptic episodes [157]. tDCS can be used in cases of generalized and focal epilepsy, without age restrictions, and it is cheaper when compared to TMS [157].

Ultrasound Stimulation

Ultrasound stimulation occurs in the form of mechanical pressure exerted at a frequency >20 kHz. Neuromodulation using this method has demonstrated results in improving the nature of acute seizures [158]. As a mechanism related to the use of US, it is understood that the applied frequency can affect neural oscillations, which can desynchronize the rhythmic discharges responsible for seizure conditions and even affect the opening of the ion channels present in the neurons, causing cell hyperpolarization [159].

Transcutaneous VNS

As a non-invasive use of VNS, tVNS also stimulates the vagus nerve, with a mechanism similar to that of VNS, but which is applied to the vagus nerve of the auricular branch composed of three nerves: the vagus, glossopharyngeal, and facial nerves [160,161]. Transcutaneous VNS is a modern, non-invasive method which brings comfort and safety to the patient; the use of this approach has already demonstrated beneficial results, reducing seizures by 50% [162,163].

Trigeminal Nerve Stimulation

This method is also a non-invasive technique that helps in epilepsy treatment by anticipating tonic–clonic and generalized seizures [139]. The mechanism of TNS is a similar to that of VNS, where the trigeminal nerve is part of the nucleus of the solitary tract (NTS) and locus coeruleus (LC), brain regions that are involved in seizure reduction [139,140,157,164].

7. Risk Factors and Comorbidities Related to Epilepsy and AED Treatment

The World Health Organization reports that around 50% of epilepsy cases worldwide remain unknown, and of the 50% which are known, it is estimated that 25% are preventable cases, and 70% are diagnoses in which the crisis can be eliminate with the use of medication [165]. Epilepsy can also trigger comorbidities, just as the disease itself can be triggered by other pathologies and their associated comorbidities. According to the World Health Organization, it is also possible to divide the causes of epilepsy into categories including structural, genetic, infectious, metabolic, immune, and unknown [165].

As a neurological disease, epilepsy brings with it the burden of various adjacent comorbidities. Comorbidities can be defined as the combination of two or more diseases that work together. Epilepsy, as a disease of great social impact, can be accompanied by depression, which directly interferes with the form and quality of life of the affected individual, as it can also cause neurological disorders, given its action on the nervous system [166]. Studies show that about 50% of adults with epilepsy exhibit at least one

comorbidity, and that one in three people express psychiatric comorbidities, the most common of which are dementia and intellectual disability [166,167].

Recent studies have linked the higher rate of epilepsy in elderly people with two main factors, possibly related to its high incidence: first, the increased life expectancy of people who developed the disorder at a young age; and second, the fact that elderly people are more likely to develop cerebrovascular trauma, such as strokes, and neurodegenerative diseases, including Alzheimer's disease. Some studies indicate that elderly people who exhibit these pathologies have an increased risk of developing epilepsy later in life [168].

It was also observed that elderly people with epilepsy had more cognitive problems than elderly people without the condition, showing that this disorder can influence the quality of life of these individuals [168]. In addition to the elderly, epilepsy in children has been widely reported. Along with this, affected individuals tend to develop other comorbidities, as observed in other age groups. Migraines, depression, anxiety, attention deficit, sleep disorders, and autism spectrum disorder are among the main problems that affect children with epilepsy [169].

Neuropsychiatric comorbidities are those that stand out most when it comes to epilepsy, mainly because epilepsy is a neurological disease. Therefore, it is important to highlight the epilepsy–dementia and dementia–epilepsy relationship. A meta-analysis study reported that late-onset epilepsy in elderly people is related to a higher risk of developing Alzheimer's disease and vascular dementia [170].

Another risk factor for the onset of epilepsy is the occurrence of a stroke. A study in Taiwan recorded the development of epilepsy in 402 young people after ischemic stroke from a cohort of 6512 patients. The unhealthy behavior of patients was one of the factors noted for the development of ischemic stroke, with a 2.90-fold greater instance of drug abuse in these patients, whereas the use of statins decreases the risk of developing stroke [171].

It was observed that people with Alzheimer's disease were more likely to experience seizures and develop epilepsy when compared to those who did not have this neurodegenerative condition [172]. In addition to the risk factors discussed here and the genetic influences that will be discussed later, there is also a relationship between the appearance of cognitive impairment and the drug treatment prescribed to the patient. Various drugs present several indications of adverse reactions on the neurological system, with some studies even indicating an increased risk of developing neurodegenerative diseases, including different forms of dementia (Table 2).

Table 2. Side effects of FDA-approved antiepileptic drugs (AEDs); data were collected from the Epilepsy Foundation (https://www.epilepsy.com/ (accessed on 1 October 2023)) and DailyMed (https://dailymed.nlm.nih.gov/dailymed/ (accessed on 1 October 2023)).

Side Effects	Drug	
Suicidal behavior and ideation	Brivaracetam	Lamotrigine
	Cannabidiol Oral Solution	Levetiracetam-XR
	Carbamazepine	Midazolam Nasal
	Carbamazepine-XR	Oxcarbazepine
	Cenobamate	Perampanel
	Clobazam	Phenytoin
	Clonazepam	Pregabalin
	Diazepam Nasal	Primidone
	Divalproex Sodium	Rufinamide
	Eslicarbazepine Acetate	Stiripentol
	Ethosuximide	Topiramate
	Felbamate	Topiramate XR
	Fenfluramine	Valproic Acid
	Gabapentin	Fenfuramine
	Lacosamide	Zonisamide

Table 2. *Cont.*

Side Effects	Drug	
Psychiatric adverse reaction	Brivaracetam Fenfluramine Gabapentin Levetiracetam Levetiracetam-XR Lorazepam Midazolam Nasal Oxcarbazepine	Perampanel Phenobarbital Pregabalin Tiagabine Hydrochloride Topiramate Topiramate XR Zonisamide
Neurological Adverse Reactions	Brivaracetam Cannabidiol Oral Solution Cenobamate Clobazam Clonazepam Diazepam Nasal Diazepam Rectal Divalproex Sodium Divalproex Sodium-ER Eslicarbazepine Acetate Felbamate Fenfluramine Gabapentin Lacosamide Levetiracetam Levetiracetam-XR	Lorazepam Midazolam Nasal Oxcarbazepine Perampanel Phenobarbital Pregabalin Primidone Rufinamide Stiripentol Tiagabine Hydrochloride Topiramate Topiramate XR Valproic Acid Fenfuramine Zonisamide
Hypersensitivity	Brivaracetam Cannabidiol oral solution Carbamazepine Carbamazepine-XR Cenobamate Eslicarbazepine Acetate Gabapentin	Lacosamide Lamotrigine Oxcarbazepine Phenobarbital Phenytoin Pregabalin
Withdrawal of AEDs	Brivaracetam Cannabidiol oral solution Cenobamate Clobazam Clonazepam Diazepam Nasal Diazepam Rectal Eslicarbazepine Acetate Fenfluramine Gabapentin Lacosamide Lamotrigine Levetiracetam Levetiracetam-XR	Lorazepam Midazolam Nasal Oxcarbazepine Perampanel Phenobarbital Phenytoin Rufinamide Stiripentol Tiagabine Hydrochloride Topiramate Topiramate XR Fenfuramine Zonisamide
Hepatotoxicity	Cannabidiol Oral Solution Divalproex Sodium Divalproex Sodium-ER	Phenytoin Valproic Acid
Serious Dermatologic Reactions	Carbamazepine Carbamazepine-XR Clobazam Eslicarbazepine Acetate Ethosuximide Lamotrigine Levetiracetam	Levetiracetam-XR Oxcarbazepine Phenobarbital Phenytoin Topiramate Zonisamide

Some studies have shown the relationship between some medications aimed at treating epilepsy and the appearance of symptoms that can predict the development of dementia. Among these, we highlight topiramate and zonisamide, for which, among the common adverse effects, changes in memory have been reported [173].

Valproic acid, for example, is a medication that, despite dementia not being noted as one of the main adverse effects, presents case reports indicating a possible relationship between the chemical and the appearance of different types of dementia in patients [174]. Another study analyzing the relationship of valproic acid in the treatment of bipolar disorder also showed that the risk of developing dementia was increased in around 73–95% of patients who used this medication [175].

Studies have shown that drugs from the benzodiazepine class, including clonazepam, diazepam, clobazam, and lorazepam, are among the main drugs that depress cognitive activity when used in high dosages [176]. Other studies were carried out to observe the effectiveness and tolerability of drugs used to treat Alzheimer's patients suffering from epilepsy; it was observed that among the drugs tested, lamotrigine and phenobarbital seemed to worsen the patients' cognition [172].

One study noted that the incidence of anxiety and depression was increased in children and adolescents with epilepsy by about 18.9 and 13.5%, respectively, when compared to the levels in healthy children. The same was observed concerning attention deficit hyperactivity disorder (ADHD), with an increased incidence between 2.5- and 5.5-fold more in children and adolescents with epilepsy [177]. However, the relationship between ADHD and epilepsy is not well established, but some studies suggest that the medication used in the treatment of epilepsy, such as phenobarbital, for example, can induce the onset of symptoms related to ADHD [178].

Concerning depression, many studies have shown that there is a relationship between the increased development of epilepsy in people with depression, suggesting similar mechanisms between the two diseases, such as altered serotonin levels and/or the common regions of the brain that are affected by both pathologies. These investigations show that the relationships between the two diseases can occur bidirectionally [169,179]. The emergence of depression due to epilepsy or epilepsy due to febrile seizures are examples of coexisting comorbidities. Epilepsy may favor the development of depression through exposure to chronic stress. Increased interleukin-1β (IL-1β) signaling in the hippocampus may be a factor for temporal lobe epilepsy (TLE), as well as for clinical depression; however, glutamate has been suggested as a potential pathogen for depression [180].

Some studies relate the appearance of depression and anxiety in patients with epilepsy to the medications used, such as levetiracetam, which has been shown to have a significant influence on increasing the rate of depression and irritability in epileptic patients. In addition to this, zonisamide has also been shown to have an important influence on the appearance of depressive conditions [181].

Despite the relationship between febrile seizures and epilepsy, neurodevelopmental delay and electroencephalogram abnormalities in children are major risk factors for the development of epilepsy and febrile seizures. Long-term prophylaxis treatments do not decrease recurrence [182]. Febrile seizures and electroencephalogrammay also be risk factors for drug-resistant epilepsy, as well as status epilepticus, symptomatic etiology, and other types of seizures. Neurodevelopmental delay, poor outcome of short-term therapy, and high initial seizure frequency are not risk factors for drug-resistant epilepsy [183].

Depression as a comorbidity related to epilepsy also raises an alert for the increase in suicide cases among those affected, arousing the interest of the scientific community in the diagnosis and treatment of this comorbidity to reduce the incidence of suicide cases [179]. The increased incidence of suicide or suicide attempts may also be related to drug treatments, such as the use of phenobarbital and pregabalin [181].

In addition to these diseases, epilepsy also shows a strong connection with autism spectrum disorders (ASD), and studies demonstrated that the simultaneous occurrence of the two pathologies may be associated with genetic factors or external agents directly

related to pregnancy, such as acquired diseases [184]. A systematic study pointed out that about 12% of autistic individuals had been diagnosed with epilepsy, and that 9% of individuals with epilepsy also had ASD [184]. Another study showed that the incidence of epilepsy in individuals with ASD in adulthood was higher than in childhood, and that the occurrence was more prevalent in female individuals [185].

Some studies have suggested that there are mechanisms in common between epilepsy and ASD, such as the hyperactivation of mTOR signaling through genetic mutations; this signaling pathway is related to the control of cell growth and was discovered based on rapamycin, which works as an inhibitor of this pathway and is used in the control of some pathologies, such as rheumatoid arthritis and atherosclerosis. A study with animal models showed that rapamycin exhibited positive results in individuals with ASD and epilepsy [186–189]. In addition to this, another relationship was observed between ASD and epilepsy, i.e., the absence or low signaling of the GABAergic marker, which causes an imbalance in the cerebral cortex of humans, as well as in animal models [187].

Furthermore, a study sought to analyze the influence of antiepileptic medicines on children who were exposed to these chemicals during pregnancy. It had been reported that some medications, such as oxcarbazepine, valproate, lamotrigine, and lamotrigine concomitantly valproate, increased the risk of developing ASD in those involved in the study [190].

In this way, we verified that the appearance of comorbidities related to epilepsy may be directly related to the implications of the disease itself, as well as be triggered by the use of prescribed medications. Other non-neuropsychiatric side effects of AEDs include hypersensitivity, serious dermatologic reaction, hepatotoxicity, and withdrawal (Table 2). This demonstrates the need for the characterization of the mechanisms of action of current commercially available AEDs and the development of new AEDs with fewer side effects and less impact on comorbidities [191–193].

8. Conclusions

Epilepsy is a complex symptomatic disease with several risk factors, often associated with a strong genetic predisposition, rather than a single expression and cause. Advances in genomic technology have revealed the complex genetic architecture behind epilepsies. In recent years, we have seen the elucidation of several therapeutic targets aimed at the development of AEDs. To be considered an anticonvulsant, a drug must balance the excitation and inhibition of the neurotransmitters, especially in the GABA and glutamate pathways. The main targets of the mechanisms of action are the molecular agents that act directly on the physiological processes of neuronal actions, including ion channels, enzymes, transport proteins, receptors, and regulators of gene expression. These agents allow for the partial or total reduction of the symptoms observed in epilepsy. The mechanisms of action of most commercially available AEDs are proposed to target, individually or simultaneously, the GABA system, the voltage-gated channels, the synaptic vesicle protein 2A or the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor, or the N-methyl-D-aspartate (NMDA) receptor.

Epilepsy represents a great burden in terms of quality of life, morbidity, and risk of premature mortality, especially for those who continue to have seizures despite treatment. In addition, comorbidities have been increasingly recognized as important etiologic and prognostic markers. Although anticonvulsant drugs can suppress seizures in up to two-thirds of patients, they fail to alter the long-term prognosis and can be associated with numerous side effects, as well has have an impact on comorbidities. Despite being the most effective way to achieve long-term seizure freedom, epilepsy surgery is only a viable alternative for patients with drug-resistant epilepsy. Non-pharmacological approaches can also be deployed concomitantly with drug intervention; these include a ketogenic diet and invasive and non-invasive neuromodulation therapies. In summary, the epilepsy etiology and pathophysiology must be better understood in order to elucidate known molecular

targets and unravel novel targets to develop new forms of treatment with fewer side effects and less impact on comorbidities.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/brainsci14010071/s1. Supplementary Table S1. FDA-approved drug used for the treatment of seizures, data collected from the Epilepsy Foundation (https://www.epilepsy.com/) and DailyMed (https://dailymed.nlm.nih.gov/dailymed/).

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Modified Vertical Parasagittal Sub-Insular Hemispherotomy—Case Series and Technical Note

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Abstract: (1) Background: Hemispherotomy is the generally accepted treatment for hemispheric drug-resistant epilepsy (DRE). Lateral or vertical approaches are performed according to the surgeon's preference. Multiple technical variations have been proposed since Delalande first described his vertical technique. We propose a sub-insular variation of the vertical parasagittal hemispherotomy (VPH) and describe our case series of patients operated on using this procedure. (2) Methods: Data from a continuous series of patients with hemispheric DRE who were operated on by the senior author (CR) using the modified sub-insular VPH technique were analyzed retrospectively. Pre-operative demographic and epilepsy characteristics, functional outcome, and surgical complications were extracted from medical charts. (3) Results: Twenty-five patients were operated on between August 2008 and August 2023; 23 have at least 3 months of follow-up. Of this group, 20 (86.9%) patients are seizure-free. Only two patients developed postoperative hydrocephalus (8.7%). All patients who were able to walk autonomously preoperatively and 20 (86.9%) of those with follow-up were able to walk without assistance. A total of 17 (74%) patients were able to perform adapted social activities at the latest follow-up. (4) Conclusions: Modified sub-insular VPH is a successful surgical technique for hemispheric DRE with seizure freedom rates similar to the largest series reported in the literature. Compared to other series, patients who were operated on with our modified technique had a lower rate of postoperative hydrocephalus and excellent long-term motor and cognitive outcomes.

Keywords: drug-resistant epilepsy; hemispherotomy; surgical procedure

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1. Introduction

1.1. Historical Context

Patients with drug-resistant epilepsy (DRE) as a result of unilateral hemispheric pathology can benefit from surgical management, with a reported seizure freedom of 70–90% [1,2].

The classical anatomical hemispherectomy was first described by Dandy for glioma surgery [3] and involved resection of the entire cerebral hemisphere, leaving the basal ganglia in place. This technique was later used to treat epilepsy with 85% complete or near-complete seizure freedom [4]. Unfortunately, long-term follow-up showed neurological worsening in about 35% of patients. Autopsy reports revealed the appearance of delayed superficial cerebral hemosiderosis, characterized by meningeal inflammation with macrophages filled with hemosiderin, subpial necrotic lesions, and ependymitis [5–7].

Rasmussen was the first to propose a combination of resection and disconnection [5] for surgical treatment of hemispheric DRE. The purpose of his functional hemispherectomy was to reduce surgical morbidity [5–7] while maintaining the good seizure outcomes of

anatomical hemispherectomy. His procedure involved a subtotal resection of the hemisphere, keeping the frontal and occipital lobes in place [1,5]. This new approach led to the introduction of hemispherotomy with even less resection and maximal disconnection [8].

Hemispherotomy can be described as a disconnection of the corona radiata and the internal capsule, the corpus callosum, the fornix, the intra-limbic and limbic gyri, the fronto-temporo-limbic connections, and the anterior commissure [1,9–13].

1.2. Technical Evolution

Several modifications of hemispherotomy have been described including anterior temporal lobectomy with peri-sylvian transcortical incision [14], using a supra-insular window [15,16], or a vertical approach through the central cortex [17,18]. The latter, the vertical parasagittal hemispherotomy (VPH), was described by Delalande and is characterized by a vertical disconnection with limited resection [17]. This method rapidly became the preferred method of performing hemispherotomy.

1.3. Goal of this Study

To date, there is no definitive proof showing the superiority of one technique over another, either in terms of epileptic seizure freedom or complication rates (notably postoperative hydrocephalus, which implies additional surgery and shunt dependence) [1,19,20].

The purpose of this study was to report our experience with a modified sub-insular VPH technique, trying to reduce the complication rate (particularly the rate of shunt placement (15%)) and mortality (3.6%)) of classical hemispherotomy [17], while maintaining good epilepsy outcomes and preserving the lenticular nucleus (LN) as much as possible by performing a disconnection under the insular cortex (sub-insular).

2. Materials and Methods

2.1. Population and Data Collection

All the patients had DRE, according to definitions from the international league against epilepsy (ILAE). Patients had a presurgical workup at the Saint-Luc University Hospital, which included video-EEG seizure recording in the epilepsy monitoring unit (EMU), high-resolution magnetic resonance imaging (MRI), fluorodeoxyglucose positron-emission tomography (FDG PET), and, if possible, a cognitive evaluation. Before surgery, all cases were discussed in our multidisciplinary meeting for DRE. Senior adult or pediatric neurology department staff members assessed postoperative follow-up. All relevant pre-, peri-, and postoperative data were collected retrospectively from medical charts.

Epilepsy etiology was classified as congenital (cortical dysplasia, hemimegalencephaly, Sturge–Weber syndrome), acquired (ischemic or hemorrhagic stroke, postoperative, or post-traumatic encephalomalacia), and progressive (Rasmussen encephalitis).

2.2. Sub-Insular VPH Method Description (Figure 1)

The senior author (CR) performed all surgeries. Our first sub-insular VPH was conducted in August 2008 and included a series of modifications from the original Delalande VPH [17] with the aim of improving disconnection and preserving a maximum of lenticular connections. All surgeries were prepared on the BrainLab® neuronavigation software (Brainlab AG, Feldkirchen, Germany) using MRI acquired just before the surgery. All volumes of interest were identified: Trolard vein (superior anastomotic vein), the targeted ventricle, the pericallosal and anterior cerebral arteries, the homolateral optic nerve, the great cerebral vein of Galen, the homolateral LN, and the amygdala.

Surgery is always performed under general anesthesia. Our paramedian skin incision is centered slightly anterior to the coronal suture to avoid the Trolard vein.

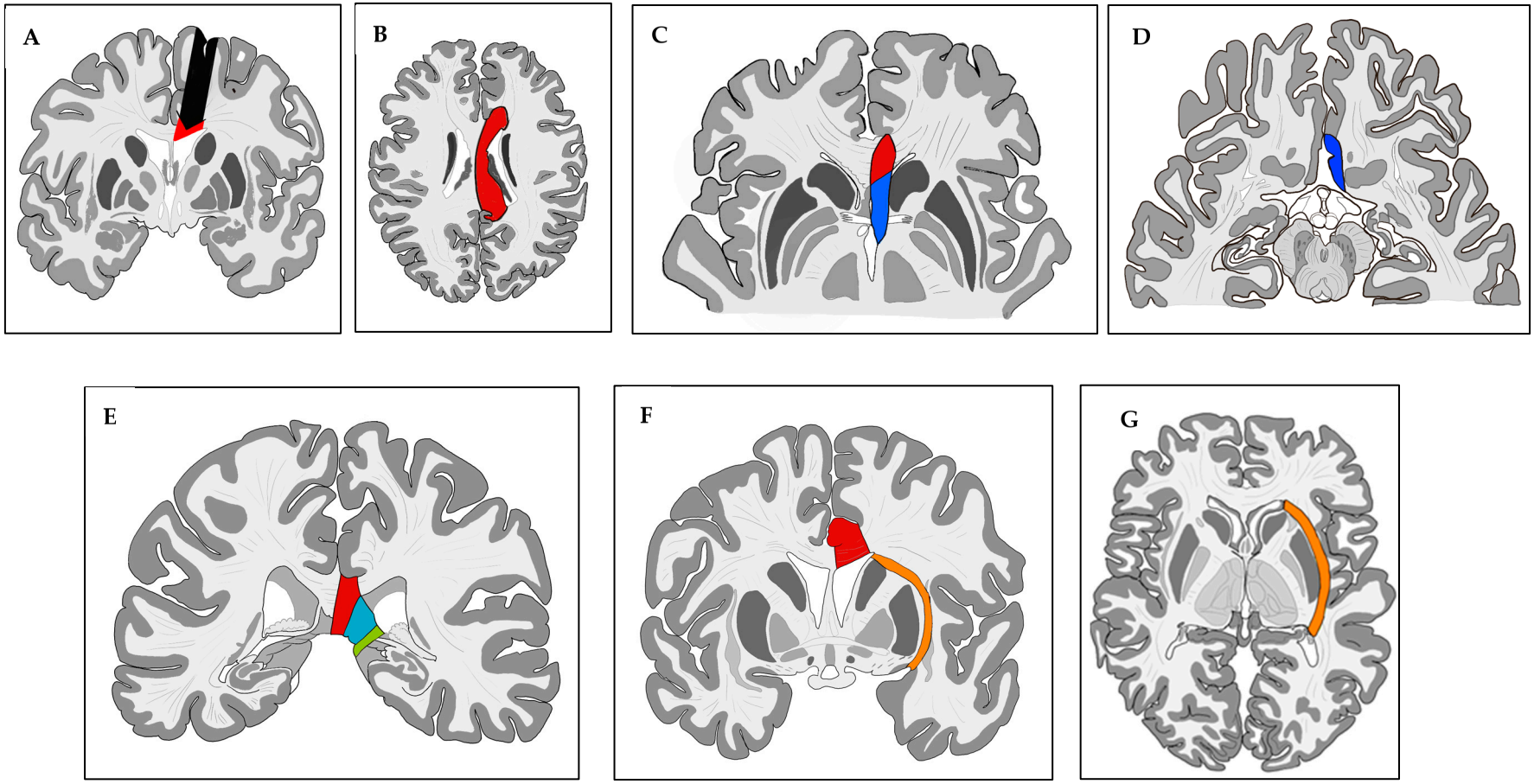
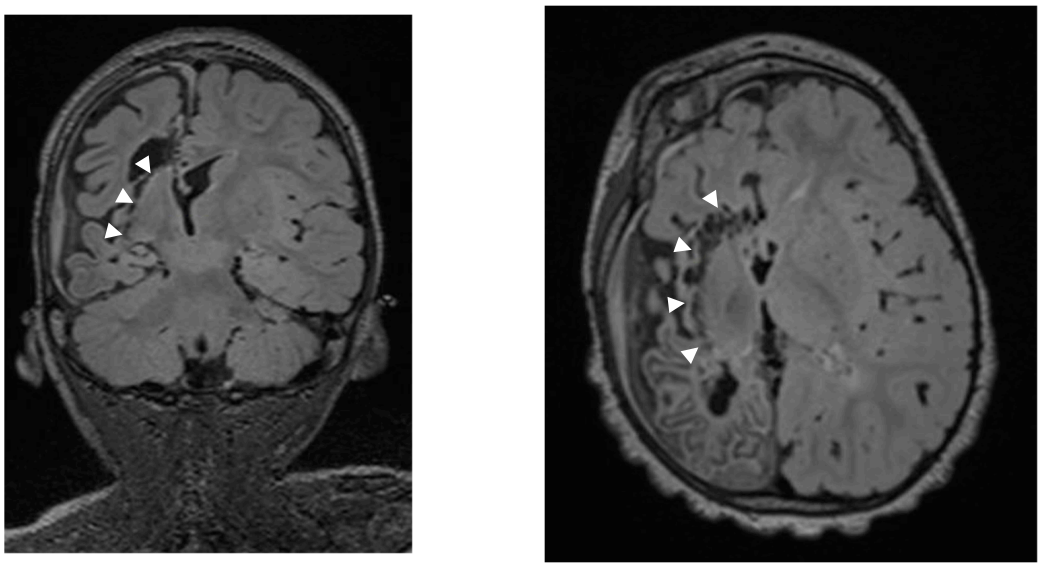


Figure 1. Major surgical steps of the modified sub-insular VPH. (**A**) Coronal section through the interventricular foramen showing the precentral parenchymal resection (black arrow) performed to enter the ventricular body and partial callosotomy (red). (**B**) Axial section through the corpus callosum showing the central callosotomy (red). (**C**,**D**) Axials sections through the anterior commissure and sub-callosal area showing the anterior corpus callosotomy (red) and sub-rostral resection (blue). (**E**) Coronal section through the ventricular trigone showing splenium (red) and ventricular trigone floor disconnection (with disconnection of the posterior column of fornix (light blue) and of the intralimbic and limbic gyri (green). (**F**,**G**) Coronal section through the anterior commissure and axial section through the striate body showing the subinsular trans-claustral disconnection (orange) and corpus callosotomy (red).

After dural opening with maximal preservation of the bridging veins, we perform, using the operative microscope, a minimal precentral parenchymal resection (pallium and body of corpus callosum; ≤20 mm by ≤10 mm). Once the ventricle body is opened and the interventricular foramen of Monro identified, we occlude it with a thin rectangular Gelfoam sponge (Pfizer Inc.®, New York, NY, USA) to avoid blood contamination of the distal ventricular system. Using the ultrasonic aspirator (CUSA Excel® Integra LifeSciences®, Princeton, NJ, USA), we perform an anterior corpus callosotomy (genu and rostrum) followed by a sub-rostral resection of the posterior part of the gyrus rectus, of the cingulum, and of Brodmann area 25. Our next step consists of splenium disconnection down to the great cerebral vein of Galen. The ventricular trigone floor is then disconnected; during that step, we disconnect not only the crus (posterior column) of the fornix but also the intralimbic and limbic gyri to reach the posterior part of the ambient cistern. Once this step is completed, we reach the posterior part of the temporal horn and perform the posterior–anterior sub-insular trans-claustral disconnection, remaining as lateral as possible to the LN (Figure 2).



(**a**) (**b**)

Figure 2. Coronal (**a**) and axial (**b**) MRI of Patient 21 (Sturge–Weber Syndrome) with arrows showing the sub-insular disconnection with preservation of the lenticular nucleus.

We then resect the piriform lobe and end our hemispherotomy by extending our disconnection along the Sylvian fissure until reaching the already resected subgenuorostrum area. At the end of the sub-insular VPH, we take time to achieve complete hemostasis before removing the Gelfoam plug from the foramen of Monro. A Gelfoam plug is placed in the cortical resection cavity and the dura, skull, and skin are closed as usual.

The patient usually remains in the intensive care unit for one day. Immediate postoperative imaging is not routinely performed, and after approximatively one week's surveillance on the neuro-pediatric department, the patient is transferred to an associated institution (Centre Hospitalier Neurologique William Lennox, Ottignies-Louvain-la-Neuve, Belgium) for rehabilitation and follow-up.

The principal differences between the Delalande and Raftopoulos techniques are shown in Table 1.

2.3. Outcome

The Engel classification was used to assess postoperative seizure outcomes [21].

Table 1. Comparison between classical Delalande and modified Raftopoulos sub-insular VPH.

	Delalande	Raftopoulos
Incision	1/3 anterior and 2/3 posterior to the coronal suture	Slightly anterior to the coronal suture
First step	Splenium disconnection	Anterior corpus callosotomy
Subrostral resection	Resection of the posterior part of the gyrus rectus	Resection of the posterior part of the gyrus rectus, of the cingulum, and of Brodmann area 25
Splenium disconnection	Until the roof of the third ventricle	Down to the great cerebral vein of Galen
Posterior disconnection	Posterior column of the fornix disconnection	Ventricular trigone floor (posterior column of fornix but also the intralimbic and limbic gyri)
Lateral disconnection	Lateral to the thalamus, going through the globus pallidus	Sub-insular trans-claustral
Temporal disconnection	Anterior part of the temporal horn resection	Piriform lobe resection

2.4. Statistical Analysis

Qualitative variables and quantitative variables are presented as numbers of patients and percentages with means, respectively. Statistical analysis was performed using GraphPad Prism 9. Results were considered significant when the *p*-value was less than or equal to 5% ($p \leq 0.05$). A non-parametric Mann–Whitney test was conducted to compare the age variable in two populations: transfused and non-transfused patients. Data are presented as mean ± standard error of the mean (SEM). Fisher's exact test was used to assess whether an acute postoperative seizure (APOS) complication influenced the Engel score.

2.5. Ethics

This study was approved by the Clinical Research Ethics Board of the Cliniques Universitaires Saint-Luc (CUSL).

3. Results

3.1. Demographic Data and Clinical Findings

We report on the first 25 consecutive patients with hemispheric DRE who had modified sub-insular VPH by the senior author (CR) at St Luc University Hospital. The mean age at seizure onset was 2.7 years (range: 0.0–9.6). The etiology was ischemic or hemorrhagic stroke in 13 patients (52%), Rasmussen syndrome in 4 (16%), cortical dysplasia in 2 (8%), hemimegalencephaly in 3 (12%), Sturge–Weber syndrome in 1 (4%), and gliosis after trauma or tumor resection in 2 (8%) (Table 2).

Table 2. Preoperative data.

	Overall (25) No. of Patients (%) or Mean
Age at	
Onset	2.7
Surgery	7.3
Sex M/F	14/11
Etiology	
Congenital	6 (24%)
Cortical dysplasia	2 (8%)
Hemimegalencephaly	3 (12%)
Sturge–Weber	1 (4%)
Acquired	16 (64%)
Stroke (ischemic/hemorrhagic)	13 (52%)
Post-traumatic	1 (4%)
Gliosis (post-tumor resection)	1 (4%)
Progressive (Rasmussen)	4 (16%)
Hemispherotomy side (R/L)	17/8

Medical History

Four patients had had previous brain surgery. Patient 5 had had a decompressive craniectomy after trauma in another hospital. Patient 7 had had four epilepsy surgeries, with the most recent procedure being a peri-sylvian hemispherotomy and ventriculoperitoneal shunt (VPS) abroad without seizure improvement. Patient 11 had had drainage of a grade 4 intra-ventricular hemorrhage (HIV) with intraparenchymal hematoma (HIP). Patient 15 had already had a callosotomy by the senior author (CR) without seizure reduction.

All patients had hemiparesis or hemiplegia before surgery without useful hand function. All patients had at least a moderate developmental delay.

3.2. Surgical Procedure and Postoperative Course (Table 3)

The mean age at sub-insular VPH was 7.3 years (range: 0.16–22.1). Seventeen (68%) surgeries were performed on the right hemisphere. There were no intra-operative complications and no deaths. Five (20.8%) patients needed a blood transfusion during surgery or immediately afterward (average: 123 mL). The mean patient age was significantly lower in transfused than in non-transfused patients (Figure 3).

Table 3. Demographic data and outcome.

Case No	Sex	Age at (y)		Side	Etiology			Complications				2nd Surgery	FUp (y)	mRS	Sz Outcome (Engel)
		Onset	Surgery		Cong	Ac	Prog	BTF (mL)	HCP	APOS	AF				
1	F	6.6	6.8	R			+	0	-		+		14.91	1	I
2	M	7.4	8.9	R		+		0	-	+	-		13.59	1	I
3	F	3.2	5.7	R			+	100	-	+	+	+	11.77	5	IV
4	M	0	6.8	L		+		0	-		-		9.72	2	I
5	M	9.6	12.7	R		+		0	+		+		9.96	3	II
6	M	3.2	5.5	R	+			0	-		-		7.32	3	I
7	M	0.1	12.8	R	+			0	-		-		10.04	3	I
8	M	1	22.1	L		+		0	-	+	-		9.71	3	I
9	M	1.8	10	R		+		0	-		+		7.74	3	I
10	F	3	15.8	L		+		0	-		+		4.49	NA	II
11	F	0.3	9.7	R		+		0	-		-		7.6	3	I
12	F	1.5	4.9	L		+		180	-		+		7.02	2	I
13	M	3	5.5	L		+		0	-		+		6.86	3	I
14	F	3.7	4.2	R			+	0	+		-	+	6.2	2	I
15	M	1.3	6.9	R		+		0	-		-		4.31	3	I
16	F	6	7.4	R			+	0	-		-	+	5.15	2	I
17	F	4.6	5.5	R		+		0	-		+		4.6	3	I
18	M	0.3	1.5	L	+			90	-		-		4.95	3	I
19	M	0	1.6	R	+			0	-	+	+		4.93	4	I
20	F	3	4.8	L		+		+	-		+		4.82	4	I
21	F	0.17	2.38	R	+			0	-		-		3.95	2	I
22	M	0.75	7.19	R		+		0	-		-		3.33	3	I
23	F	5.98	10.51	L		+		0	-		+		0.32	3	I
24	M	0	0.16	R	+			100	-	+	-	+	0.16	NA	III
25	M	0.5	3.28	R		+		0	-	+	-		0.08	4	I
M/F	1.27														
Mean		2.68	7.3										6.5	2.8	
Range		9.6–0	22.1–0.16										14.9–0.08		

Cong, congenital; Ac, acquired; Prog, progressive; BTF, blood transfusion; HCP, hydrocephalus; APOS, acute postoperative seizure; AF, aseptic fever; FUp, follow-up; mRS, modified Rankin scale; Sz, seizure; NA, not available/not applicable; -, absent; +, present. Second surgeries (3, completion of VPH and KPS; 14, amygdalohippocampectomy; 16, amygdalohippocampectomy; 24, amygdalohippocampectomy and completion of callosotomy).

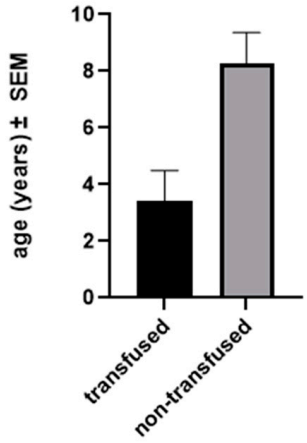


Figure 3. Comparison of age between transfused and non-transfused patients. Patients who were transfused during the operation were significantly younger than patients who were not transfused, $p = 0.0154$; SEM, standard error of the mean.

3.2.1. Acute Postoperative Seizures

Six (24%) patients had postoperative seizures (APOS) within the first week; however, four of them still achieved prolonged seizure freedom (Engel I). The two others (patients 3 and 24) needed a second surgical procedure to complete the hemispherotomy and were not seizure-free at the most recent follow-up appointment. There was no link between the presence of APOS and the risk of not being seizure-free (Figure 4).

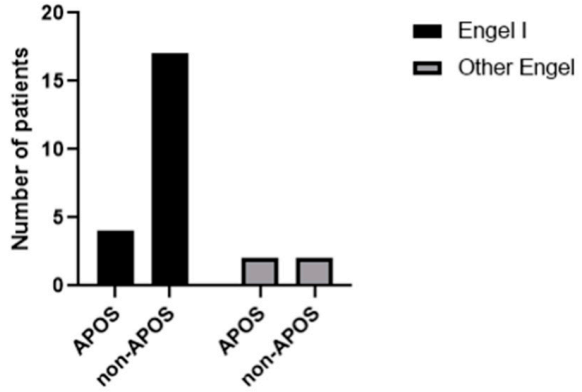


Figure 4. Comparison of epilepsy outcome between APOS and non-APOS patients. There was no statistically significant link between the presence of APOS and the risk of not being seizure-free. $p = 0.234$.

3.2.2. Hydrocephalus and Shunting

No patient required a shunt during the first postoperative week. Two patients (9%) developed symptomatic delayed hydrocephalus requiring a shunt at one (patient 5) and three (patient 20) months postoperatively; the first patient needed a surgical revision three years after shunt placement for disconnection of the distal catheter, no other shunt complication occurred. Patient 3 had a cysto-peritoneal shunt placement during her second hemispherotomy procedure for a persistent subdural hygroma without hydrocephalus. Patient 15 needed a VPS for treatment of a recurrent pseudo-meningocele.

3.3. Seizure Outcome

Our mean follow-up period was 6.5 years (range: 0.08–11.7). Twenty-three patients had at least 3 months of follow-up; of those, 20 (86.9%) were Engel 1 at their most recent follow-up. Our results are comparable with those reported in the literature (Figure 5 and Table 4).

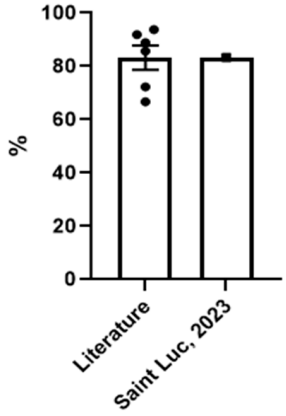


Figure 5. Comparison of proportion of patients with Engel score I (mean ±,SEM) at the final follow-up in the literature and in our Saint Luc University hospital case series. Our results are comparable with those reported in the literature for seizure outcome.

1. Patient 3, with Rasmussen encephalitis, was seizure-free for 10 months after the first VPH before recurrence of catastrophic status epilepticus. Postoperative MRI showed suspected persistence of a callosal connection. We performed a second surgery for completion of the hemispherotomy. Unfortunately, the patient's seizures did not improve despite radiological confirmation of complete disconnection.
2. Patient 5 developed epilepsy after severe head trauma that required decompressive craniectomy. He was seizure-free for two years after VPH and then presented recurrent spasms despite complete disconnection on MRI.
3. Patient 10 still suffered from morpheic seizures after surgery, but her last video-EEG showed a bilateralization of the epileptic foci.

Three other patients needed revision surgery for persistent or recurrent seizures. Patients 14 and 16 had seizure recurrence with predominantly vegetative symptoms and had an amygdala residue resection: they are currently seizure-free [22].

Patient 24 (who was not considered in the analysis of long-term results) needed a second surgical intervention for persistent infantile spasm. Postoperative MRI showed a suspicion of a persistent callosal connection at the genu. He underwent a second surgery for completion of the callosotomy and amygdalohippocampectomy but, despite some seizure improvement, he was not seizure-free one month after this procedure.

3.4. Cognitive Outcome

Six patients (26%) had significant cognitive impairment at their most recent follow-up, with no possibility of social integration. Patient 3 was bedridden since seizure recurrence 10 months after the first VPH with uncontrolled epilepsy, despite two VPH procedures for Rasmussen's encephalitis. Patient 5 was able to walk but had severe pre-operative cognitive impairment due to severe head trauma responsible for the onset of his epilepsy. Patient 7 had severe cognitive impairment as part of extensive cortical dysplasia with refractory epilepsy and encephalopathy for 10 years prior to surgery.

Patient 8 underwent surgery at the age of 22 with severe cognitive impairment present in the context of refractory epilepsy since birth. Patient 19 had global developmental delay, already suspected before surgery, in the context of his hemimegalencephaly, despite excellent control of his epilepsy. Finally, patient 20 had preoperative hemiplegia and severe preoperative congenital delay in the setting of a pre-natal left ischemic stroke. She is currently able to communicate but is unable to walk unassisted due to her persistent motor deficit.

Table 4. Comparison between our UCL results and published series of patients treated using vertical parasagittal hemispherotomy.

Author, yr	N	Etiologies (%)			Mean Age at		Complications										Mean FUp (yr)	Sz Outcome (Engel at Last FUp)							
		Cong	Acq	Prog	Onset (yr)	Surgery (yr)	Mty		HCP		BTF		APOS		Other			I		II		III		IV	
							n	%	n	%	n	%	n	%	n	%		n	%	n	%	n	%	n	%
Delalande, 2007 [1] [17]	83	40 (48)	18 (21)	25 (30)	2.1	8.0	3	3.6	12	14.5	6	7.2	NA	NA	2	2.4	4.4	60	72.3	10	12.0	9	10.8	2	2.4
Honda, 2013 [23]	12	12 (100) [2]	0	0	0.05	0.36	0	0.0	1	8.3	12	100.0	NA	NA	0	0.0	6.5	8	66.7	0	0.0	1	8.3	3	25.0
Dorfer, 2013 [3] [24]	37	13 (32)	26 (65)	1 (2)	1.2	5.5	1	2.7	1 [4]	2.7	2	5.4	NA	NA	0	0.0	37	34	91.9	0	0.0	0	0.0	3	8.1
Kawai, 2014 [25]	7	4 (57)	3 (43)	0	2.1	14.8	0	0.0	0	0.0	NA	NA	0	0.0	0	0.0	3.1	6	85.7	0	0.0	0	0.0	1	14.3
Panigrahi, 2016 [26]	16	1 (6)	10 (62)	5 (31)	2.9	6.5	0	0.0	1	6.3	NA	NA	4	25.0	NA	NA	2.2	15	93.8	NA	NA	NA	NA	NA	NA
Fohlen, 2019 [27]	18	18 (100)	0	0	2	7.2	0	0.0	2	11.1	NA	NA	NA	NA	1	5.6	12.8	16	88.9	2	0.0	0	0.0	0	0.0
Saint-Luc, 2023	23	5	13	4	2.9	7.8	0	0.0	2	8.7	4	17.4	4	17.4	4 [5]	17	6.81	20	86.9	2	8.7	0	0.0	1	4.3

Acq, acquired (infarction, postop/post-traumatic encephalomalacia, brain tumor); APOS, acute postoperative seizure; BTF, blood transfusion; Cong, congenital; Prog, progressive; NA, not available; VPS, ventriculoperitoneal shunt; yr, year; Mty, mortality; HCP, hydrocephalus; BTF, blood transfusion; FUp, follow-up; Sz, seizure. [1]. Engel classification for 81 patients because of 2 postop deaths. [2]. Hemimegalencephaly only. [3]. Analysis included 37 of 40 patients with a follow-up of at least 12 months. [4]. VPS 2.7%: Three patients with hydrocephalus needed a temporary external shunt but only one needed a VP shunt. [5]. One cerebral abscess 6 months postop and 3 s surgeries for recurrence of epilepsy.

All the other patients were able to attend a normal school, or one adapted for their motor deficit. Patients 1 and 2 have a driver's license and have completed university studies. Unfortunately, a quantitative assessment of the developmental quotients was not possible, due to the absence of pre- or postoperative assessment based on quantitative scales.

4. Discussion

4.1. Elegance of the Vertical Parasagittal Hemispherotomy (VPH)

VPH allows complete disconnection of one hemisphere with minimal brain resection and without dissection of the Sylvian fissure, resection of the insula, or vessel sacrifice [15]. VPH provides complete insular cortex disconnection, avoiding potential poor postoperative outcomes due to a residual connected insular cortex as reported by Bulteau et al. [28] in cases of functional hemispherectomy. This complex procedure, involving one whole hemisphere, is carried out through a limited paramedian precentral cortical resection and through one ventricle with an extended view and understanding of the prosencephalic (forebrain) anatomy.

4.2. Gelfoam Plug into the Foramen of Monro

The range of postoperative shunt requirements varies between 2.5% and 23% [1,29]. In their series of 83 cases of VPH, Delalande et al. reported shunt placement in 16% [17], with an incidence of 76.9% in the hemimegalencephaly group with no clear explanation. In our three cases with hemimegalencephaly, no hydrocephalus developed. A similar rate of 13% has been reported with lateral hemispherotomy [30].

Hydrocephalus is considered a major complication after hemispherotomy. Acute hydrocephalus is associated with a risk of intracranial hypertension, cerebral herniation, and an altered level of consciousness, which requires urgent management by an external ventricular shunt. In chronic hydrocephalus with shunt dependence, the patient is exposed to the long-term disadvantages of a shunt (obstruction, disconnection, infection, hardware erosion, ascites). Avoidance of this complication is therefore a key factor in the management of these patients, in order to maximize their quality of life [1,31,32].

In both vertical and lateral approaches, shunt dependency is probably related to blood contamination of the CSF with inflammation of the subarachnoid spaces and arachnoid villi [33] and to the initial volume of the ventricular system (the smaller the ventricular system, the higher the risk of ventricular synechia). We favored the CSF blood contamination hypothesis and thus decided to protect the third ventricle and the rest of the ventricular system by occluding the homolateral foramen of Monro with a thin piece of Gelfoam sponge. This plug is removed at the very end of surgery when hemostasis is complete and the operative field is perfectly clean. This strategy may have played a role in reducing the hydrocephalus rate to 8.7%. Moreover, we had no cases of acute hydrocephalus, which is potentially more dangerous. It would be interesting to know whether this measure was used in other series reporting low rates of postoperative shunt.

4.3. Sub-Insular Disconnection with LN Preservation

The physiology of the LN, i.e., the putamen and the globus pallidum, is important in many aspects: motor adjustment and skill acquisition [34,35], habit memory [36], and cognitive functions [37]. Decreased N-acetyl aspartate/creatine ratios in both LN in patients with bipolar disorder were reported by Lai et al. [38], stressing the role of LN in mood regulation. Abnormalities of the LN microstructure in patients with schizophrenia have also been observed [39]. The LN has multiple connections not only with the cortex, which is disconnected by the VPH, but also with the thalamus, the habenula, and the brainstem. Keeping the LN as intact as possible preserves some of these connections and their roles.

Nevertheless, the clinical impact of keeping the LN intact is currently unknown. Postoperative walking is irregularly reported in the literature, with rates between 33% and 100%, and a great variability depending on the etiology of the epilepsy and the length of follow-up [8,23,24,27]. At their most recent follow-up, 86.9% of our patients

were able to walk independently; this proportion was 100% in those who could walk preoperatively. And 74% of patients were able to follow a conventional educational program with adaptations related to the loss of dexterity of the paretic hand. In patients with a bad functional status at the most recent follow-up, severe preoperative involvement, a longer duration of epilepsy, and underlying pathology (Rasmussen encephalopathy, hemimegalencephaly, etc.) are probably partly responsible for the poorer outcome [9,18]. Because of the small number of patients, it was not possible to demonstrate a statistically significant relationship between epilepsy etiology and functional outcome in our series.

The importance of keeping the LN as intact as possible should be explored and analyzed on a larger series with long-term follow-up.

4.4. Acute Postoperative Seizures (APOSs)

The definition of APOS has evolved over time and the influence of these seizures on prognosis is still unclear. In 1963, Falconer et al. reported 'neighborhood' fits in the first postoperative month without a worse prognosis [40]. From 1991, the time frame of 7 days was used and APOSs during this period were considered not predictive of prolonged seizure outcomes [41,42]. In 2001, the Commission on Neurosurgery of the ILAE recommended using a period of one month to define APOS, and seizures occurring during this period are not considered to have a negative prognostic value [43].

During the first postoperative month after surgery for DRE, 25% of children may have APOS [44]. In their series of patients treated using hemispherotomy, Panigrahi et al. [26] described a 25% rate of APOS, while their rate of seizure freedom (Engel I) at last follow-up was 94%, the highest in the literature.

On the other hand, in their multicenter series, De Palma et al. [29] reported the presence of APOS as the only significant association with poor seizure outcome, and numerous studies have shown a correlation between early postoperative seizures and poor outcomes [45–48].

In our series, 66.6% of patients with APOS were seizure-free (Engel I) at the latest follow-up. There was no statistically significant correlation between the presence of APOS and the risk of not being seizure-free. However, because of the small number of patients, the results should be interpreted with caution.

In this context, we believe that caution be taken in the event of APOS. Surgery should not be immediately considered a failure, but patients/families should be warned that the surgical treatment may prove to be ineffective in one third of cases.

4.5. VPH: Literature Review and Comparison with Our Series (Table 4)

Including the present study, nine original cases series have been reported [17,23–27,29, 49,50] in which the classical Delalande or modified VPH procedures were used, and seven papers presented enough data for comparison.

Our series included predominantly acquired cases of DRE (55%) with an 86.9% rate of seizure freedom. By contrast, in a series by Honda et al. with only congenital cases, the seizure freedom rate was 66.7% [23].

The mean age at surgery in our series (7.3 y) is very similar to that in the population of Delalande et al. (8.0 y) [17]. The lowest mean age at surgery (4.3 y) was reported in the series by Honda et al. [23], probably because all their patients were congenital cases. In their series, all patients received a blood transfusion compared to 20% of our patients. In our population, the need for transfusion was significantly related to patient age ($p = 0.0034$) and our low rate of transfusion (20%) can then be explained by the small number of cases of hemimegalencephaly or Sturge–Weber syndrome.

With 86.9% of patients in Engel class I at one-year follow-up and only one in Engel class IV (4%), our series confirms that sub-insular VPH is a very efficient procedure to treat refractory hemispheric DRE.

5. Limitations

Our study is limited by its observational nature.

The lack of quantitative assessment of developmental quotients and the small number of patients prevents us from demonstrating a formal correlation between LN preservation and better cognitive results and does not enable a quantitative comparison with the literature.

6. Conclusions

The modified sub-insular VPH is a safe and successful surgical technique for hemispheric DRE with a similar seizure freedom rate to those reported in the largest series in the literature. Our modifications permitted a low rate of postoperative hydrocephalus and excellent motor and cognitive long-term outcomes. Longer follow-up periods and quantitative measurement of the pre- and postoperative cognitive status are needed to further assess the impact of the sub-insular approach.

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Informed Consent Statement: All patients' caregivers signed informed consent, thus agreeing to the use of medical data for future clinical research.

Data Availability Statement: The data presented in this study are available in the article.

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Article

Retrospective Clinical Analysis of Epilepsy Treatment for Children with Drug-Resistant Epilepsy (A Single-Center Experience)

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Abstract: Objectives: This retrospective cohort study investigated the clinical characteristics and seizure outcomes of patients aged 1–14 years with drug-resistant epilepsy (DRE) who were treated by different typologies of therapy. Methods: Four hundred and eighteen children with DRE were recruited from Sanbo Brain Hospital of Capital Medical University from April 2008 to February 2015. The patients were divided into three groups: medication (n = 134, 32.06%), resection surgery (n = 185, 44.26%), and palliative surgery (n = 99, 23.68%) groups. Demographic characteristics were attained from medical records. All patients were followed up for at least 5 years, with seizure outcomes classified according to International League Against Epilepsy criteria. The psychological outcome was evaluated with the development quotient and Wechsler Intelligence Quotient Scale for children (Chinese version). Results: The most frequent seizure type was generalized tonic seizure in 53.83% of patients. Age at seizure onset in 54.55% of patients was <3 years. The most frequent etiologies were focal cortical dysplasia (FCD). West syndrome was the most common epilepsy syndrome. Favorable seizure outcomes at the 5-year follow-up in the medication, resection surgery, and palliative surgery groups were 5.22%, 77.30%, and 14.14%, respectively. The patients showed varying degrees of improvement in terms of developmental and intellectual outcomes post-treatment. Conclusions: Pediatric patients with DRE were characterized by frequent seizures, a variety of seizure types, and complex etiology. Recurrent seizures severely affected the cognitive function and development of children. Early surgical intervention would be beneficial for seizure control and prevention of mental retardation. Palliative surgery was also a reasonable option for patients who were not suitable candidates for resection surgery.

Keywords: drug-resistant epilepsy; children; resection surgery; palliative surgery; seizure outcome

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1. Introduction

Epilepsy is among the most common chronic neurological disorders affecting the quality of life in patients. Children constitute the majority of epilepsy patients with an annual incidence rate of 41–187/100,000, which is much higher than that of adults [1]. In China, this ratio reaches 151/100,000 [2]. The causes of seizures are diverse and are generally classified as genetic, structural and metabolic, and unknown [3].

Antiepileptic drugs (AEDs) are the main form of treatment. About 70% of patients respond well to AEDs and achieve seizure-free outcome. In 20%–30% of cases, however, seizures remain uncontrollable [4–6]. The International League Against Epilepsy (ILAE)

defined drug-resistant epilepsy (DRE) as a failure of adequate trials of two tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom [7]. In such cases, surgical intervention is widely used as the primary treatment for pediatric epilepsy [8]. A large histopathological study showed that the main pathology of DRE in children is cortical malformations and tumors. This is clearly different from the pathology in adults, which is hippocampal sclerosis [9]. Notably, not all patients are suitable for resection surgery. For patients with epileptogenic regions involving eloquent cortical areas or diffuse lesions, multiple subpial transections (MST) and corpus callosotomy were the main palliative procedures to reduce seizures in the last century [10]. Due to advances in knowledge and medical technology, neuromodulation for epilepsy is emerging as treatment for epilepsy [11,12]. Regardless of the surgical approach, the goal is to reduce the propagation of epileptiform discharges.

Epilepsy in children with cognitive decompensation was formerly referred to as catastrophic epilepsy although it was not a clinical classification [13]. Furthermore, the term was not recognized by the International League Against Epilepsy (ILAE) [3]. Gradual and severe seizures in children affect their daily life, especially academic performance [14–16]. Thus, DRE is a major public health problem given the significant negative impacts on children's development [17]. Effective treatment of DRE while minimizing the side effects remains a clinical challenge.

However, few studies have systematically compared the efficacy of different typologies of therapy in children with DRE. The aim of our study was to generalize the clinical characteristics of children with DRE. Our hypothesis was that surgical resection would bring children with DRE the most favorable seizure outcomes, and followed by palliative surgery. Good seizure outcomes were associated with improved cognitive function. Moreover, analysis of predictive factors in patients undergoing resection surgery would guide clinical practice. Additionally, this study evaluated long-term cognitive outcomes of children with DRE. To the best of our knowledge, this series reported the largest single-center cohort study on different treatments for pediatric epilepsy.

2. Materials and Methods

2.1. Patient Selection

We retrospectively analyzed the data of 516 patients with DRE who were admitted to Sanbo Brain Hospital of Capital Medical University from April 2008 to February 2015. The inclusion criteria for patients were as follows: (1) aged 1–14 years; (2) uncontrolled seizures after AED and adrenocorticotropic hormone therapy; (3) had undergone examinations by magnetic resonance imaging (MRI), computed tomography (CT), and video electroencephalogram (VEEG); (4) follow-up data were available; (5) informed consent by guardians. The exclusion criteria: (1) Benign epilepsy syndromes in children such as benign occipital epilepsy, benign childhood epilepsy with centro-temporal spikes, and other syndromes which respond better to AEDs; (2) if it was not clear whether the seizures were recurrent or the epileptogenic foci had not been completely removed (Figure 1); (3) patients with a history of surgical resection. The study was approved by the ethics committee of Sanbo Brain Hospital, Capital Medical University.

2.2. Psychological Assessments

The choice of psychological test used was subject to the age and cognitive level of the patients. A Chinese version of the Gesell Developmental Schedules (GDS) or Wechsler Intelligence Quotient Scale (WIQS) was used for psychological assessments of patients who met the inclusion criteria [18,19]. The Chinese version of GDS is a widely used classic psychometrical scale for evaluating neurodevelopmental outcomes of children less than 6 years old. The scale assesses five areas: gross motor skills, fine motor skills, adaptability, language, and social activity. Each area yields a developmental quotient (DQ) [20]. The total DQ is the average of the five DQs mentioned above. DQ was categorized as normal ($\geq$80), borderline delayed ($\geq$70, <80), mildly delayed ($\geq$50, <70), moderately delayed ($\geq$35,

<50), severely delayed (≥20, <35), and extremely delayed (<20). WIQS scores were used to evaluate the children's intellectual quotient. The WISC revised in China (C-WISC) was used for children aged 6–16 years old. The C-WISC covers full scale IQ, verbal IQ, and performance IQ. Our analysis classified them as normal (≥90), mild defect (≥70, <90), moderate defect (≥60, <70), and extreme defect (<60).

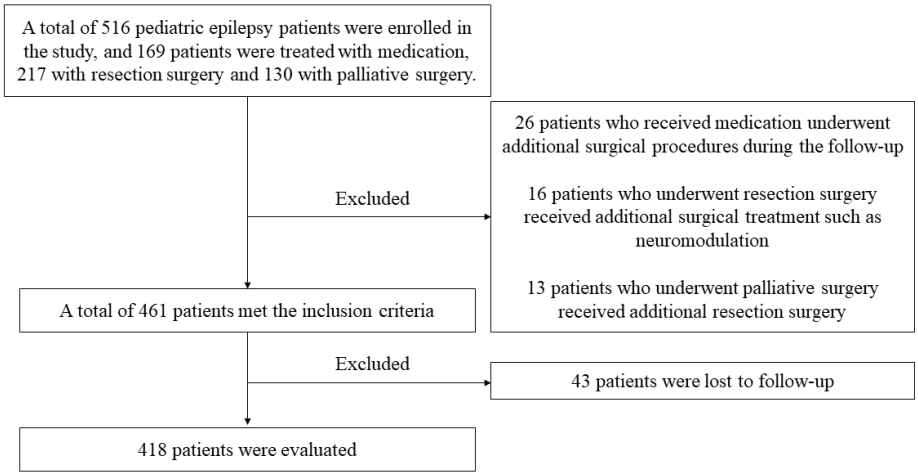

Figure 1. Flowchart describing the procedures and exclusion and inclusion criteria of this study.

2.3. Demographic and Clinical Characteristics

Preoperative evaluations were seizure semiology; a detailed history; neurologic examination; long-term VEEG, MRI, and CT. The seizure type was classified according to the International Classification of Epileptic Seizures; the syndrome type was defined according to the International Classification of Epileptic Syndromes; etiology was determined based on brain MRI and CT and cytogenic findings as well as psychological development at the time of the first admission. If the location of epileptic foci was not clear from the above results, magnetoencephalography (MEG) and positron emission tomography (PET) data were examined. The cases were discussed by a multidisciplinary team of epilepsy neurosurgeons, neuropsychologists, and neuroradiologists at our epilepsy center. The patients' condition and treatment options were explained to the family, and the treatment was decided by the children's guardian(s). All patients were followed up at the outpatient clinic or by telephone interview for at least 5 years. Seizure outcomes were evaluated based on the ILAE classification [21]: ILAE class 1, completely seizure free with no auras; ILAE class 2, only auras and no other seizures; ILAE class 3, one to three seizure days per year with or without auras; ILAE class 4, four seizure days per year to 50% reduction of baseline seizure days with or without auras; ILAE class 5, less than 50% reduction of baseline seizure days to 100% increase of baseline seizure days with or without auras; ILAE class 6, more than 100% increase of baseline seizure days with or without auras. ILAE classes 1 and 2 were defined as favorable and ILAE classes 3–6 as unfavorable outcomes. Resection surgeries included epileptic focal resection, lobotomy, multilobar resection, and hemispherectomy. Palliative surgeries included corpus callosotomy, MST, and vagus nerve stimulator implantation. Patients in whom noninvasive tests could not accurately localize the epileptogenic focus, such as MRI showing multifocal brain abnormalities or negative, some of the patients would undergo stereotactic EEG. If the patient was not suitable for resection surgery after a comprehensive evaluation, we would use intracranial electrodes to perform radiofrequency thermocoagulation (RF-TC). Therefore, RF-TC was considered another palliative procedure.

2.4. Statistical Analysis

Psychological assessments at the 1st, 3rd, and 5th year follow-ups were performed to evaluate the cognitive or behavioral problems. In children with non-resective surgery,

the palliative or medication groups, clinical characteristics, and prognosis were compared. Continuous variables were described as means and standard deviations, and categorical variables as frequencies and percentages. To identify potential predictors of seizure outcomes in patients treated by surgery, a univariate analysis was carried out, where categorical variables were analyzed with the chi-squared or Fisher's exact test. Continuous variables were compared with the Mann–Whitney U test. Variables with a $p < 0.2$ in the univariate analysis were entered into a multivariate logistic regression model by backward elimination. Statistical significance was considered at $p < 0.05$. Analysis was performed using SPSS version 25.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Demographic Characteristics

A total of 516 patients met the inclusion criteria. During follow-up, 55 patients were excluded for changing their treatment regimen at enrollment and a further 43 were lost to follow-up. Hence, 418 patients, 272 males, and 146 females were included in the analysis (Figure 1). The age at seizure onset was <6 months in 82 patients (19.62%), 6 months–1 year in 45 (10.77%), 1–2 years in 52 (12.44%), 2–3 years in 49 (11.72%), and >3 years in 190 (45.45%) patients (Figure 2).

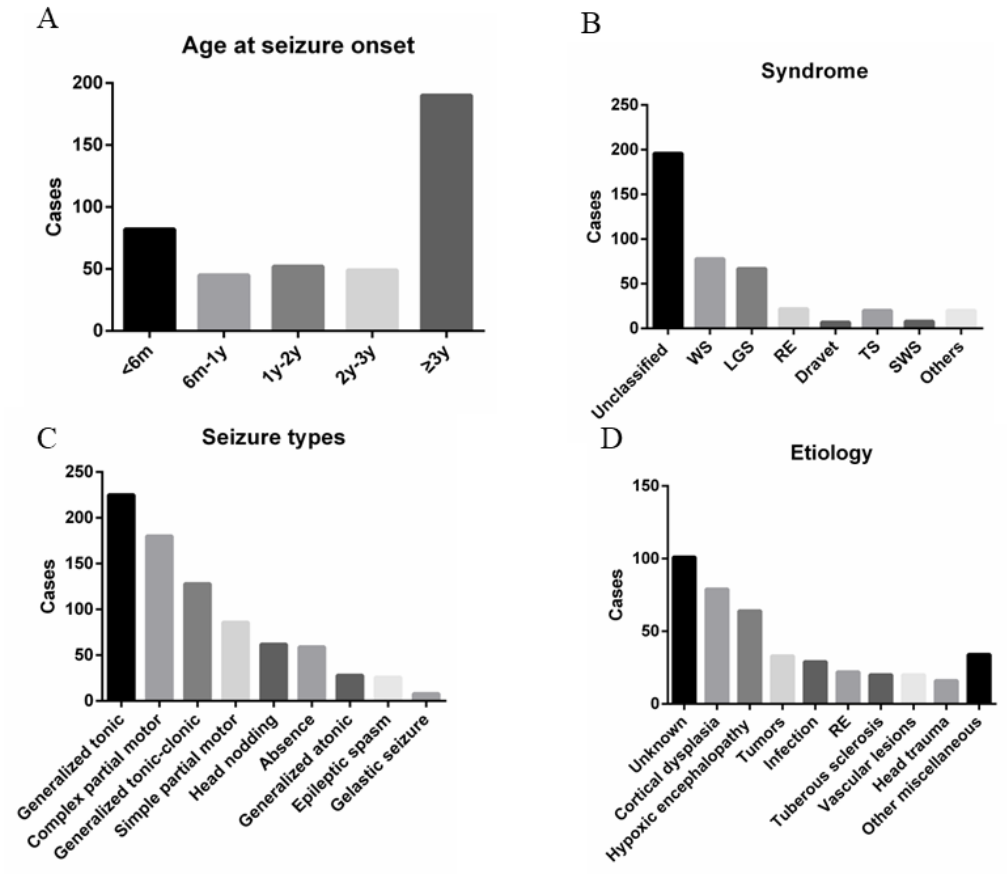

Figure 2. (**A**–**D**) summarized the clinical characteristics of pediatric patients with drug-resistant epilepsy in terms of age at seizure onset, epileptic syndrome, seizure type, and etiology, respectively.

3.2. Seizure Types

A total of 160/418 (38.28%) patients had one type of seizure. Generalized tonic seizure (GTS) was the most common seizure type at 53.83%, complex partial motor at 43.06%, generalized tonic-clonic seizures at 30.62%, and simple partial motor at 20.57%. Partial seizures were recorded in 236 patients, and 68 of them had a secondary generalized tonic-clonic seizure.

3.3. Etiology

The etiology of epilepsy was determined based on the medical records, CT, brain MRI, and cytogenic findings. The five common etiologies were focal cortical dysplasia (n = 79, 18.90%); hypoxic-ischemic encephalopathy (n = 64, 15.31%); tumors (n = 33, 7.90%); central nervous system infection (n = 29, 6.94%); and Rasmussen encephalitis (RE; n = 22, 5.26%). The etiology in 101 patients (24.16%) was unknown.

3.4. Epileptic Syndrome

Unclassified epilepsy was the frequently observed type of epilepsy (n = 196, 46.89%), followed by West syndrome (WS; n = 78, 18.66%), Lennox-Gastaut syndrome (LGS; n = 67, 16.03%), neurocutaneous syndromes (NS; n = 28, 6.70%), and RE (n = 22, 5.26%) (Figure 2).

3.5. Psychological Assessment

DQ was evaluated in 135 patients. Normal patients were 25/135 (18.52%), borderline delayed 9/135 (6.67%), mildly delayed 22/135 (16.30%), moderately delayed 26/135 (19.26%), severely delayed 34/135 (25.19%), and extremely delayed 19/135 (14.07%).

The remaining 283 patients were evaluated with the C-WIQS; normal 30/283 (10.60%), mild retardation 97.283 (34.28%), moderate retardation 51/283 (18.02%), and severe retardation 105/283 (37.10%). Thus, cognitive ability was normal or borderline delayed in 25.19% and 10.60% of patients, respectively (Figure 3).

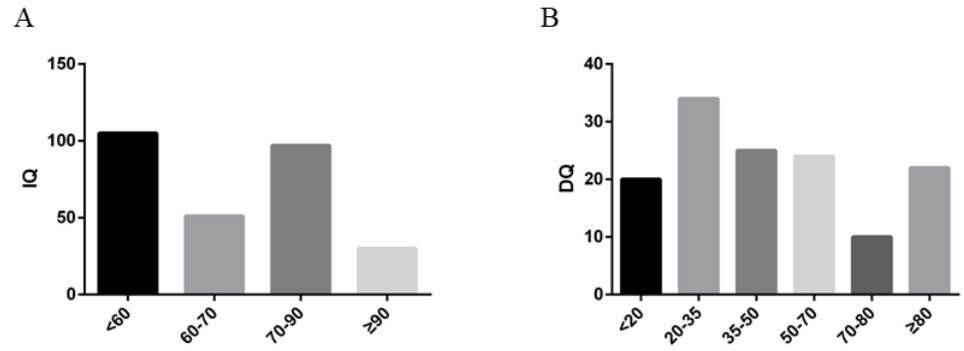

Figure 3. (**A**). IQ of children over 6 years old at enrollment. (**B**). DQ of children under 6 years of age at enrollment.

3.6. Seizure and Psychological Outcomes

Seizure outcomes were evaluated based on the ILAE classification. The three treatment groups were 134 patients (32.06%) on medication, 185 (44.26%) with resection surgery, and 99 (23.68%) with palliative surgery. Although the prognosis of medication should not be assessed with this type of classification, the seizure outcomes were also evaluated with the ILAE classification of patients in the medication group for comparison with the outcome of surgical treatment. Favorable seizure outcomes were observed in 14.93%, 81.62%, and 19.19% of patients on medication, resection surgery, and palliative groups, after 1-year follow-up; 10.45%, 78.92%, and 16.16% after 3-year follow-up; 5.22%, 77.30%, and 14.14% after 5-year follow-up. Cognitive function was evaluated based on DQ and C-WIQS scores. The use of DQ was limited by age, hence, only 63 patients less than 3 years old were followed up at the time of the first admission for three years. Favorable psychological outcomes were observed in 61.11%, 70.97%, and 71.43% of patients in the medication, resection surgery, and palliative surgery groups, respectively, in the children younger than 6 years old. Patients older than 6 years showed favorable psychological outcomes in 71.13%, 81.88%, and 82.50%, respectively, at the last follow-up. The mean number of antiepileptic drugs taken by patients in the medication, resection surgery, and palliative surgery groups at the time of enrollment in this study was 2.32, 2.18, and 2.58, respectively. At the last follow-up, this figure was 2.13, 1.45, and 1.85, respectively. All patients in the medication

group were still taking AEDs, while 43 and 7 in the resection and palliative surgery groups, respectively, were off AEDs (Figures 4 and 5).

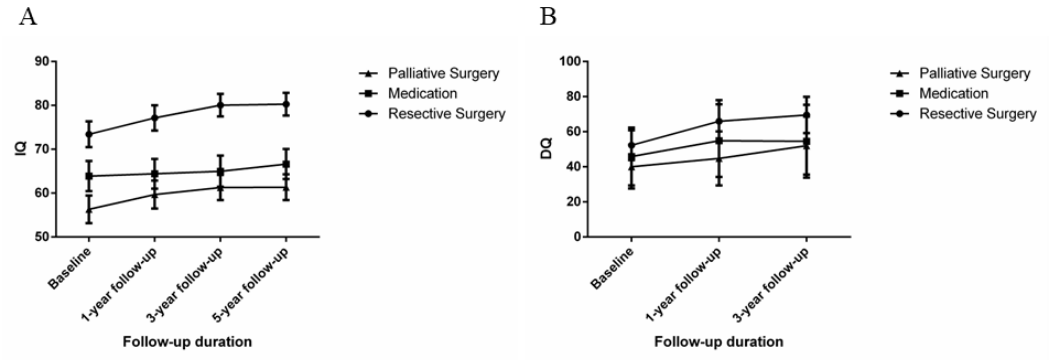

Figure 4. (**A**,**B**) The changes in IQ and DQ of children with drug-resistant epilepsy after different treatment regimens, respectively. It was worth noting that the Chinese version of the Gesell Developmental Schedules was only suitable for children under 6 years of age. Here we only listed the developmental quotients of 63 children who were suitable for observing the changes at follow-up.

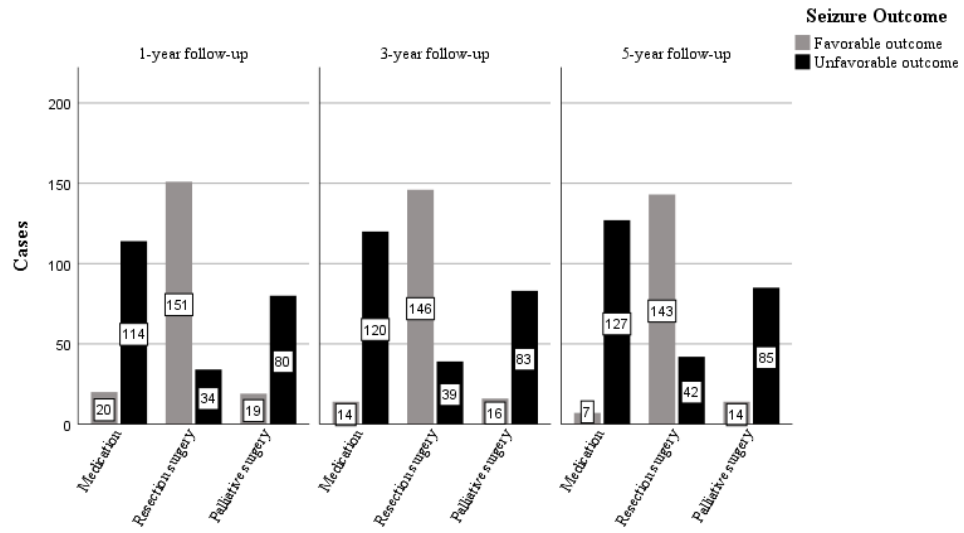

Figure 5. Number of patients with seizure outcomes according to different treatment regimens at different follow-up times.

4. Discussion

The lifetime prevalence of epilepsy has been estimated to be 7.60 per 1000 persons [22], and the global prevalence of active epilepsy is in the range of 4.9–12.7 per 1000 [23]. Children constitute the majority of epilepsy patients, with an annual incidence of 41–187 per 100,000, which is higher than that of in adults [1]. Most intractable epilepsy syndromes such as WS, LGS, NS, and RE are the main causes of DRE [24]. The onset of seizures typically occurs at a young age. In the present study, the earliest onset occurred at 3 days after birth. DRE is often accompanied by developmental delay, systemic comorbidities and has a high risk of mortality. The clinical characteristics of children below 6 years old who developed DRE were investigated [25]. However, the association between different types of therapy and seizure outcomes has not been reported. Few large cohort studies have compared long-term seizure outcomes and cognitive function outcomes following different treatment methods in DRE patients. Based on cognitive function and seizure control in epileptic pediatric patients, surgical treatment should be carried out early in DRE patients, but surgery was not fully utilized [26]. Here, we outline the clinical features of

childhood refractory epilepsy, and compare the effects of different treatment modalities on the prognosis and cognitive function of the children.

Drug-resistant epilepsy, as the name and definition suggest, has poor effect on AEDs. Previous studies showed that the probability of achieving seizure-free by tweaking drug regimens after failure of two AEDs was less than 10% [27]. Figure 5 shows treatment with newer AEDs yielded satisfactory seizure outcomes in only a minority of patients. Given that the lobes of the brain are interconnected via fibers, repeated epileptic seizures can cause secondary pathologic changes [28]. For instance, lesions located in the temporal lobe may lead to secondary hippocampal sclerosis [29]. Pediatric epilepsy surgery aims to remove the epileptogenic area or limit the propagation of epileptiform discharges in the brain. Clinically, the application of surgical treatment for DRE in children is increasingly reported. In our epilepsy center, resection surgery was recommended for suitable patients. In a randomized controlled trial, Dwivedi et al. reported that with the rate of seizure freedom was 7% and 77% in the medication group and surgical treatment group respectively [30].

Epileptic syndromes begin in childhood that have different ages at seizure onset [31]. However, not all children with epilepsy can be classified to have a specific epilepsy syndrome. In the present study, unclassified epileptic syndromes were observed in 46.89% of cases (Figure 2). Excluding those that could not be classified, WS was the most common epilepsy syndrome in this study, accounting for 18.67% of all patients. It was followed by LGS (some patients had a history of WS), which accounted for 16.03%. The peak age at seizure onset in WS has been reported to be 5 months [29]. In our cohort, 67.95% of patients with WS had seizure onset in the first year of life, and 20 were born with hypoxic-ischemic encephalopathy. Children with WS usually have unfavorable seizure outcomes [32,33] and most have mental retardation that evolves into LGS. In general, only 25.64% of patients with WS attained favorable seizure outcomes. LGS is another common childhood epilepsy syndrome, which is characterized by a triad of multiple seizure types, intellectual impairment, and a specific EEG pattern of slow-spike waves, with or without paroxysmal fast activity in sleep. However, not all patients had the three features at the onset of the seizure [34]. Over time, cognitive impairment would become more pronounced. Additionally, uncontrolled seizures would contribute to further worsening of cognitive impairment. Therefore, it had been suggested that the diagnostic criteria should not be so strict to allow patients to receive novel treatment early [35]. Pediatric patients might have multiple seizure types. In the present study, the most common seizure types were generalized tonic seizures. The capture of multiple seizure types was helpful in the diagnosis of epilepsy syndrome.

The major difference between children and adults was that children's brains were not yet fully developed. In this study, the age of seizure onset was seen in more than 50% of children younger than three years old, which is a critical period of brain development. Recurrent seizures would cause the occurrence of ischemia and hypoxia in neuronal tissue. It might affect the normal development of brain tissue. Darra et al. reported most severe cognitive dysfunction and motor impairment in patients were associated with persisting seizures [36]. Effective control of seizures might benefit cognitive function and motor function. Moreover, children with DRE with severe functional impairment from the onset of early age seizures or long-duration seizures show abnormal neurologic and psychological development, even as they become seizure-free [37].

In this cohort study, we compared the demographic and clinical characteristics of patients in the medication and palliative surgery groups. The results showed no statistically significant differences between the two groups for these basic characteristics (Table 1). However, the seizure outcome of patients in the palliative surgery group was significantly better than the medication group. It had been reported that difficult localization of epileptogenic zones, such as bilateral epileptic discharges or no positive MRI findings, may be related to unfavorable seizure outcomes [38]. Moreover, the lesions that overlap with the functional cortex may be linked with the unfavorable seizure outcomes. Patients who show the above preoperative examination results might likely not undergo resection surgery. Palliative surgical procedures such as corpus callosotomy, bipolar electrocoagulation, and neuromod-

ulation are effective for patients without indications for resection surgery [11,12,39–41]. Baba et al. reported a group of 56 patients with refractory WS who underwent corpus callosotomy and were seizure-free after surgery in 32.1% of patients [42]. A previous review showed that vagus nerve stimulation resulted in 6%–27% of patients with refractory epilepsy being seizure-free [43].

Table 1. The comparison of clinical characteristics between AEDs group and palliative surgery group (n = 233).

Clinical Characteristics	AEDs	Palliative Surgery	p
Gender, n (%)			0.629
Male	92 (39.5)	65 (27.9)	
Female	42 (18.0)	34 (14.6)	
Age	8.02 ± 3.69	8.20 ± 3.86	0.718
Age at seizure onset	3.17 ± 3.14	3.31 ± 3.12	0.723
Duration of seizures	4.86 ± 3.20	4.86 ± 2.77	0.992
Duration of AEDs	38.48 ± 37.55	41.71 ± 37.93	0.519
Seizure types, n (%)			0.275
Partial seizure only	8 (3.4)	7 (3.0)	
Generalized seizure only	59 (25.3)	53 (22.7)	
Generalized and partial seizure	67 (28.8)	39 (16.7)	
Brain MRI, n (%)			0.054
Normal or basal ganglia	33 (14.2)	15 (6.4)	
Unilateral	26 (11.2)	31 (13.3)	
Bilateral	75 (32.2)	53 (22.7)	
Interictal EEG, n (%)			0.095
Unilateral	24 (10.3)	10 (4.3)	
Bilateral	110 (47.2)	89 (38.2)	
Ictal onset rhythms, n (%)			0.492
Not captured	18 (7.7)	10 (4.3)	
Unilateral	12 (5.2)	6 (2.6)	
Bilateral	104 (44.6)	83 (35.6)	
PET, n (%)			0.715
No	117 (50.2)	88 (37.8)	
Yes	17 (7.3)	11 (4.7)	
MEG, n (%)			0.745
No	99 (42.5)	75 (32.2)	
Yes	35 (15.0)	24 (10.3)	
Invasive EEG, n (%)			0.166
No	133 (57.1)	95 (40.8)	
Yes	1 (0.4)	4 (1.7)	
Seizure outcome, n (%)			0.019*
Favorable outcome	7 (3.0)	14 (6.0)	
Unfavorable outcome	127 (54.5)	85 (36.5)	
Etiology (%)			0.560
Unknown	59 (25.3)	42 (18.0)	
Other miscellaneous	39 (16.7)	27 (11.6)	
Infection	9 (3.9)	12 (5.2)	
Hypoxic encephalopathy	27 (11.6)	18 (7.7)	

AED: antiepileptic drugs; MRI, magnetic resonance imaging; EEG: electroencephalogram; PET, positron emission tomography; MEG, Magnetoencephalography. (%), percentage of the total values. * $p < 0.05$.

A total of 185 patients with DRE underwent resection surgery. The rate of seizure freedom at the last follow-up was 77.30% (Table 2), which was similar to studies by studies [25,44,45]. Longer duration of seizures was significantly associated with unfavorable seizure outcomes [46,47]. Similarly, the present study also found longer duration of seizures was an independent predictor on unfavorable seizure outcome. In this group, there was no significant association between seizure outcomes and age at surgery (Table 3). In this study, multivariate analysis revealed that unilateral ictal onset rhythm was a predictor of seizure-free. Although 23 patients in this group had infrequent seizures that were not

captured, 22 patients had definite lesions on the MRI. Pathologically, FCD, tumors, and hypoxic-ischemic encephalopathy were the most common etiologies and were observed in 38.38%, 17.84%, and 10.27% of cases, respectively. In a European study of 9523 patients who underwent epilepsy surgery, the most common cause of childhood epilepsy was FCD, followed by tumors [9]. In another study of 543 children who underwent epilepsy surgery, 60% were <2 years old and the most frequent etiologies were cortical dysplasia (42.4%), tumors (19.1%), and atrophy/stroke (9.9%) [48]. FCD and tumors are regional and structural abnormalities that are difficult to visualize by MRI at an early stage [49,50]. As a result, patients with such lesions often show a poor response to AEDs, and are likely to be classified as DRE [51,52].

Table 2. Clinic characteristics of patients in resection surgery group and their relationship with seizure outcomes (n = 185).

Variable	Univariate Analysis		
	Favorable Outcomes	Unfavorable Outcomes	p
Gender, n (%)			
Male	92 (49.7)	23 (12.4)	0.261
Female	51 (27.6)	19 (10.3)	
Age at surgery	8.19 ± 3.87	8.93 ± 4.00	0.281
Age at seizure onset	4.42 ± 3.40	3.18 ± 2.80	0.032 *
Duration of seizures	3.78 ± 2.91	5.76 ± 3.60	0.009 *
Duration of AEDs	29.80 ± 25.97	43.00 ± 36.30	0.021 *
Seizure types, n (%)			0.200
Partial seizure only	49 (26.5)	10 (5.4)	
Generalized seizure only	30 (16.2)	14 (7.6)	
Generalized and partial seizure	64 (34.6)	18 (9.7)	
Brain MRI, n (%)			0.254
Normal or basal ganglia	5 (2.7)	4 (2.2)	
Unilateral	103 (55.7)	27 (14.6)	
Bilateral	35 (18.9)	11 (5.9)	
Interictal EEG, n (%)			0.943
Unilateral	57 (30.8)	17 (9.2)	
Bilateral	86 (46.5)	25(13.5)	
Ictal onset rhythms, n (%)			0.021 *
Not captured	20 (10.8)	3 (1.6)	
Unilateral	78 (42.2)	16 (8.6)	
Bilateral	45 (24.3)	23 (12.4)	
Invasive EEG, n (%)			0.088
No	127 (68.6)	33 (17.8)	
Yes	16 (8.6)	9 (4.9)	
PET, n (%)			0.857
No	107 (57.8)	32 (17.3)	
Yes	36 (19.5)	10 (5.4)	
MEG, n (%)			0.158
No	79 (42.7)	18 (9.7)	
Yes	64 (34.6)	24 (13.0)	
Etiology, n (%)			0.292
FCD	52 (31.9)	19 (10.3)	
Tumors	29 (15.7)	4 (2.2)	
Hypoxic-ischemic encephalopathy	13 (7.0)	6 (3.2)	
Others	49 (26.5)	13 (7.0)	

MRI, magnetic resonance imaging; EEG: electroencephalogram; PET, positron emission tomog-raphy; MEG, Magnetoencephalography; FCD: focal cortical dysplasia. (%), percentage of the total values. * $p < 0.05$.

Table 3. Predictors of seizure outcomes in the resection surgery group for DRE on multivariate analysis (n = 185).

Variables	OR	95%CI	p
Age at seizure onset	0.948	0.840–1.069	0.382
Duration of seizures	1.299	1.115–1.513	0.001 *
Duration of AEDs	0.996	0.982–1.009	0.544
Ictal onset rhythms			
Not captured	Ref.		
Unilateral	4.099	1.075–15.636	0.039 *
Bilateral	2.543	0.695–9.300	0.158
Invasive EEG	0.882	0.389–2.002	0.764
MEG	1.494	0.742–3.006	0.261

OR: odds ratio; CI: confidence interval; AED: antiepileptic drugs; EEG: electroencephalogram; Ref. reference; MEG, magnetoencephalography. * $p < 0.05$.

During the follow-up, the intellectual and psychological development of most patients showed varying degrees of improvement (Figure 4). The most favorable outcomes for both seizures and cognitive function were in the resection group, the palliative surgery, and the medication groups respectively (Figure 5), similar to previous reports [53,54]. A study conducted by Veersema et al. [55] showed that 2 years post epilepsy surgery 24 of 36 patients had a $\geq$10 points increase in IQ. These were similar to our study, where patients who had resective epilepsy surgery had an IQ increase of $\geq$10 points (25.95%) in the resection group. Similar results were recorded in 16.16% and 6.72% of the palliative surgery group and medication group, respectively. Cumulatively, the side effects of long-term AED use diminish efficacy and lead to mental retardation [56,57]. In the present study, 43 and 7 patients in the resection and palliative surgery groups, respectively, were successfully weaned off AEDs. In a systematic review of epilepsy in children, a strong relationship was found between intelligence quotient decline and continued seizures [58]. Moreover, a cohort study of children <3 years old who underwent epilepsy surgery found that early surgery was associated with increased postoperative DQ [59]. As a consequence, evidence shows that improvement in cognitive function was closely linked to better seizure outcomes.

The limitations of our study were: first, it was a single-center retrospective analysis in which not all variables could be controlled, for example, patient selection and time of surgery. Second, seizure outcomes were determined from the medical records of patients or by telephone interview without clinical assessment, which may have introduced recall bias. Future studies should consider factors such as genetic data, and family history of epilepsy. Third, detailed information was obtained from the medical records. Therefore, we did not adopt the 2017 classification of seizure types since the earliest cases enrolled in this study was from 2008.

5. Conclusions

The results of this study demonstrated that compared with adult patients, children with DRE were a special group with their own characteristics. Based on seizure outcomes and cognitive function, surgical resection seemed to be the most effective treatment. Short duration of seizure and unilateral ictal rhythm were associated with favorable seizure outcomes. Palliative surgery could still benefit patients for who resection surgery was not an option. Regardless of the surgical treatment regimen, children with DRE should be treated as early as possible to prevent progressive brain damage and mental retardation caused by epilepsy.

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tion, Y.H.; writing—original draft, C.L., Y.H.; writing—review and editing, H.Z., X.W., and C.L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The studies involving human participants were reviewed. This study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Sanbo Brain Hospital (protocol code SBNK-YJ-2022-024-01).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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brain sciences

MDPI

Systematic Review

Neuromodulation Techniques in Children with Super-Refractory Status Epilepticus

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Abstract: Status epilepticus (SE) is a life-threatening condition and medical emergency which can have lifelong consequences, including neuronal death and alteration of neuronal networks, resulting in long-term neurologic and cognitive deficits in children. When standard pharmacological treatment for SE is not successful in controlling seizures, the condition evolves to refractory SE (rSE) and finally to super-refractory SE (srSE) if it exceeds 24 h despite using anaesthetics. In this systematic review, we present literature data on the potential uses of clinical neuromodulation techniques for the management of srSE in children, including electroconvulsive therapy, vagus nerve stimulation, and deep brain stimulation. The evaluation of these techniques is limited by the small number of published paediatric cases (n = 25, one with two techniques) in peer-reviewed articles (n = 18). Although neuromodulation strategies have not been tested through randomised, prospective controlled clinical trials, this review presents the existing data and the potential benefits of neuromodulation therapy, suggesting that these techniques, when available, could be considered at earlier stages within the course of srSE intending to prevent long-term neurologic complications. Clinical trials aiming to establish whether early intervention can prevent long-term sequelae are necessary in order to establish the potential clinical value of neuromodulation techniques for the treatment of srSE in children.

Keywords: super-refractory status epilepticus; neuromodulation; electroconvulsive therapy; deep brain stimulation; vagus nerve stimulation; children epilepsy

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1. Introduction

Status epilepticus (SE) has been defined as "a condition characterized by an epileptic seizure that is sufficiently prolonged or repeated at sufficiently brief intervals to produce an unvarying and enduring epileptic condition" [1]. SE is a life-threatening condition and medical emergency, which has an incidence of 14.3–28.4 per 100,000 people per year, affecting all ages, particularly children and the elderly [2]. It is also a condition that, depending on the type and duration of seizures, can have lifelong consequences, including neuronal death and alteration of neuronal networks resulting in long-term neurological and cognitive deficits [1]. A duration longer than 5 min, beyond which, a long seizure or cluster of seizures (without returning to baseline) occurs, is considered as SE. It has been reported in animal models that durations longer than 30 min induce neuronal damage [3], and these timeframes may be variable depending on the type of SE.

Two different types of status epilepticus (SE) have been described: convulsive and nonconvulsive. Convulsive SE shows tonic–clonic, tonic, clonic, or myoclonic manifestations. [4–7]. Nonconvulsive SE (NCSE) is further classified as generalised, focal, and

unknown, depending on the EEG findings. In cases of NCSE with coma and persisting behavioural or awareness changes, the EEG is the most accurate method for appropriate diagnosis [1,8,9].

SE is an emergency condition and guidelines for its management are well elaborated for the choice of first, second-, and third-line drugs of treatment. Benzodiazepines are suggested as the first line of treatment for SE [10]. NICE guidelines suggest intravenous lorazepam, but rectal diazepam or buccal midazolam can also be considered [10,11]. If the SE is not resolved, phenytoin, fosphenytoin, levetiracetam, lacosamide, or phenobarbital can be considered for sustained control. General anaesthesia with propofol, midazolam, or thiopental sodium can be tried in refractory cases of SE (rSE), and super-refractory SE (srSE) is established when it outlasts 24 h of anaesthesia [12,13]. srSE has high mortality and morbidity rates [12,14]. To date, there are no class I data to support recommendations for most antiepileptic drugs for established, refractory, and super-refractory SE.

Timings of SE treatment have been suggested by Shorvon and Ferlisi [12] and Trinka et al. [13], but without clear time recommendations for other types of treatments for rSE. Alternative non-pharmacological techniques have been suggested for rSE in children, including plasmapheresis, ketogenic diet, hypothermia, immunomodulation, and neuromodulation [15,16]. Plasmapheresis is a technique that has been found useful in generalised rSE, largely in adult populations [17]. In paediatric cohorts, only 7 out of 37 children appeared to achieve seizure control [18]. A ketogenic diet has been reported in small case series and larger reports (n = 8–17), showing electrographic seizure resolution within 7 days in 20–90% of patients [15]. A recent clinical trial with hypothermia in an adult population did not find efficacy in srSE compared with placebo [19].

Invasive and non-invasive neuromodulation techniques have been suggested as potential treatments capable of complementing standard pharmacological treatment [20] for srSE. A small number of case reports using neuromodulation techniques have shown some promising results controlling srSE when conventional treatment has failed in children and adults [21].

1.1. Non-Invasive Neuromodulation Techniques

Electroconvulsive therapy (ECT) with transcutaneous electrical stimulation of the brain cortex under EEG monitoring is considered a potential treatment for severe major depression and other mental disorders [22] and has also been suggested as a potential treatment for rSE [23]. The technique consists of several sessions of ECT with stimulation intensity and duration parameters, either based on the patient's seizure threshold or a standard protocol [22,24]. Electrodes can be placed in several positions of the head, either bitemporal, right unilateral (left in left-handed), or bifrontal, depending on the clinical aims [25] (Figure 1A). A serious side effect of ECT is amnesia, retrograde, anterograde, or both, usually improving within 2 weeks [24,26], but a close monitoring of cognitive function is needed to prevent adverse cognitive effects [22]. Details on the technique, the parameters, and the possible side effects have been described in several previous publications [19–23].

Repetitive TMS (rTMS) has recently been considered a diagnostic and potential treatment tool for neurological and psychiatric disorders, including depression, epilepsy, and pain [27–29]. rTMS relies on the application of trains of magnetic pulses over the patient's head, depolarising neurons in the target area [30], and can initially reduce seizures in patients with drug-resistant epilepsy. Transcranial direct current stimulation (tDCS) is a painless, non-invasive stimulation technique that uses polarity-specific electric current to modulate brain excitability and has been used in several conditions [31], including patients with mesial temporal lobe epilepsy [32]. rTMS and tDCS have been reported only for the treatment of srSE in adults and thus, details are not included in the present review.

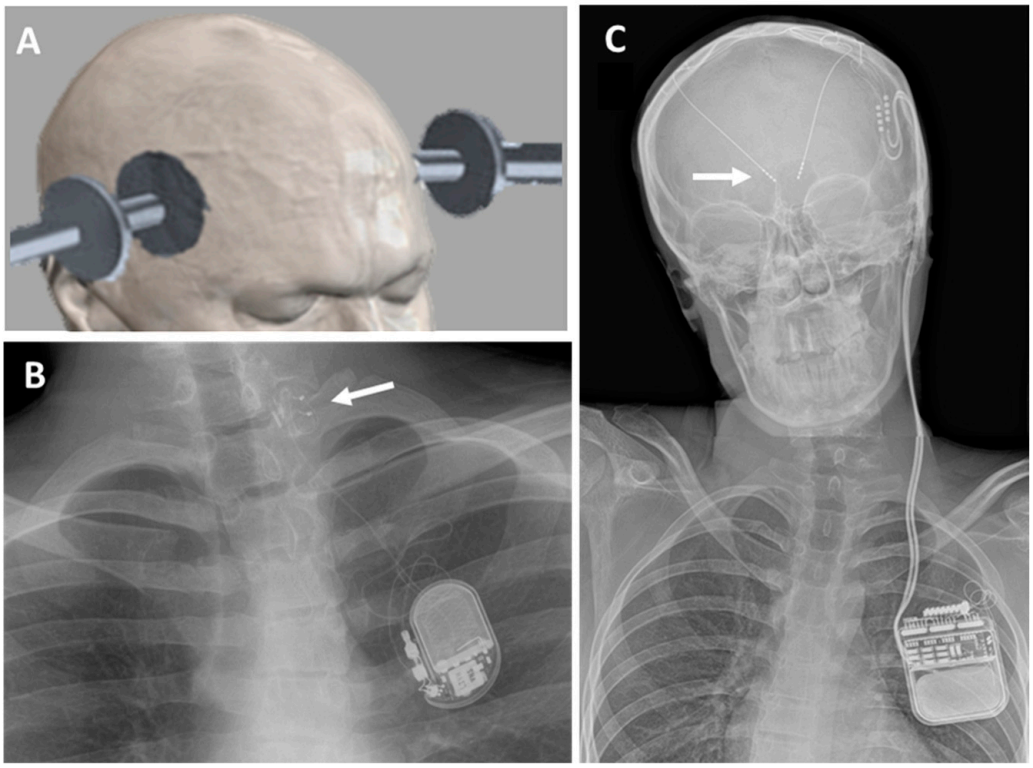

Figure 1. Invasive and non-invasive neuromodulation techniques tried in children with srSE. (**A**) Electrodes and typical positions for non-invasive electroconvulsive therapy (ECT); (**B**) X-ray showing vagus nerve stimulation (VNS) in a 14 y/o child. The white arrow indicates the position of the stimulating contacts in the vagus nerve; (**C**) X-ray showing deep brain stimulation (DBS) in a 12 y/o child. The white arrow indicates the DBS position in the centromedian thalamic nucleus in the brain.

1.2. Invasive Neuromodulation Techniques

Vagal nerve stimulation (VNS) is a NICE-approved procedure for children and adults suffering from drug-resistant epilepsy as an add-on to antiepileptic medication [11]. VNS equipment consists of a VNS pulse generator surgically implanted on the left subclavicular area, including a battery and a 43 cm lead wire with two platinum/iridium helical electrodes (Figure 1B). An external programming system is used to modify stimulation parameters [33,34]. A recent meta-analysis indicates that VNS interrupts srSE in 74% of patients, though the article raises concerns about reporting bias [35]. Reported VNS side effects include dyspnoea, dysphagia, and hoarseness due to vagus nerve damage; bradycardia/asystole during the implantation procedure; postsurgical infections; obstructive sleep apnoea; and tonsillar pain [34,36].

Deep brain stimulation (DBS) includes the implantation of multi-electrode bundles in the brain which are connected to a pulse generator to deliver electrical pulses to modulate the implanted region and functionally connected areas (Figure 1C). Deep brain stimulation is now a technique used worldwide, particularly for the treatment of movement disorders', but also used in obsessive–compulsive disorder, depression, Tourette syndrome, headache, chronic pain, eating disorders, and epilepsy [37–39]. In patients with refractory epilepsy, DBS has been tried for different brain regions, particularly the anterior and the centromedian nucleus of the thalamus [40,41]. The SANTE trial studied the effects of anterior nucleus DBS in patients with focal seizures [41] while centromedian DBS has been mainly tried in patients with generalised epilepsy [42]. Potential DBS side effects include infection, skin erosion, lead migration or fracture, and malfunction of the DBS pulse generator [43]. DBS stimulation-related side effects depend on the area stimulated, the most common being paraesthesia related to DBS intensity [41,44,45].

During the last 4 years, another invasive neurostimulation/neuromodulation has also been described in srSE. This is the responsive neurostimulation (RNS) and consists of depth or subdural electrodes placed in or over one or two predetermined seizure foci. These are connected to a programmable device which is cranially implanted and can provide electrical stimulation in response to detected ictal electrocorticographic activity [46]. About 10 cases have been described and the results are positive [47–50], and likely more cases will be described soon, but the published peer-reviewed cases regard adults and thus further details were considered out of scope of this review for the paediatric population.

Although neuromodulation has potential in the management of epilepsy and srSE, there have been only a few studies in children showing the potential benefits of these techniques after conventional medical treatment for srSE has failed. This systematic review aims at evaluating the potential benefits of neuromodulation for srSE in the paediatric population.

2. Materials and Methods

This systematic review was performed in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guidelines [51]. A comprehensive literature review was performed using PubMed and MEDLINE to find relevant articles published from 1946 to August 2022. The selection criteria included all relevant subject headings and freeform texts relating to neuromodulation techniques and paediatric status epilepticus. The following search strategy for PubMed was performed on 15 September 2021: (VNS OR vagal nerve stimulation OR vagus nerve stimulation) OR (transcranial magnetic stimulation OR TMS) OR (electroconvulsive therapy OR ECT) OR (deep brain stimulation OR DBS)) AND ((status epilepticus OR epilepsia partialis continua OR refractory status epilepticus OR rSE OR super-refractory status epilepticus OR srSE) OR ("Febrile infection-related epilepsy syndrome" OR "FIRES") OR ("New-onset refractory status epilepticus" OR "NORSE")). A similar search strategy was used for MEDLINE using subject headings and freeform text on the same day. All articles were imported for screening to Covidence (© Cochrane). Duplicates were automatically removed before initial screening by the software. Title and abstract screening and full-text assessment were performed independently by two reviewers (H.L.P. and A.V.). Articles in English referring to neuromodulation (VNS, TMS, ECT, or DBS) and status epilepticus lasting more than 24 h with anaesthesia in patients <18 years were included. Original articles, case studies, case reports, and letters to the editor were included while conference articles, literature reviews, and systematic reviews were excluded. Conflicts in screening and full-text assessment were resolved by three reviewers (H.L.P., A.V., and I.S.).

Studies should give an estimation of the number of days between SE onset and initiation of neuromodulation therapy, an estimation of the number of days from neuromodulation to any changes in patient condition, and the final results of the neuromodulation therapy. Neuromodulation therapy was regarded as successful when it led to cessation of both clinical and electrographic SE, and when the patient was stable enough to be transferred out of ICU. This data was then converted into graphs to illustrate the timeline for all relevant patients. Apart from the timeline details, data included demographics (patient's age and gender), type of neuromodulation, and epilepsy before the onset of SE.

3. Results

The Prisma flowchart is presented in Figure 2. After excluding duplications and papers not fulfilling the inclusion criteria, 18 references were included for further analysis in the present review. The neuromodulation techniques reviewed were VNS (n = 15), DBS (n = 6), and ECT (n = 5). The mean age of the patients was 7.4 years, ranging from 0.5 to 17 years. Twelve out of twenty-five patients were female.

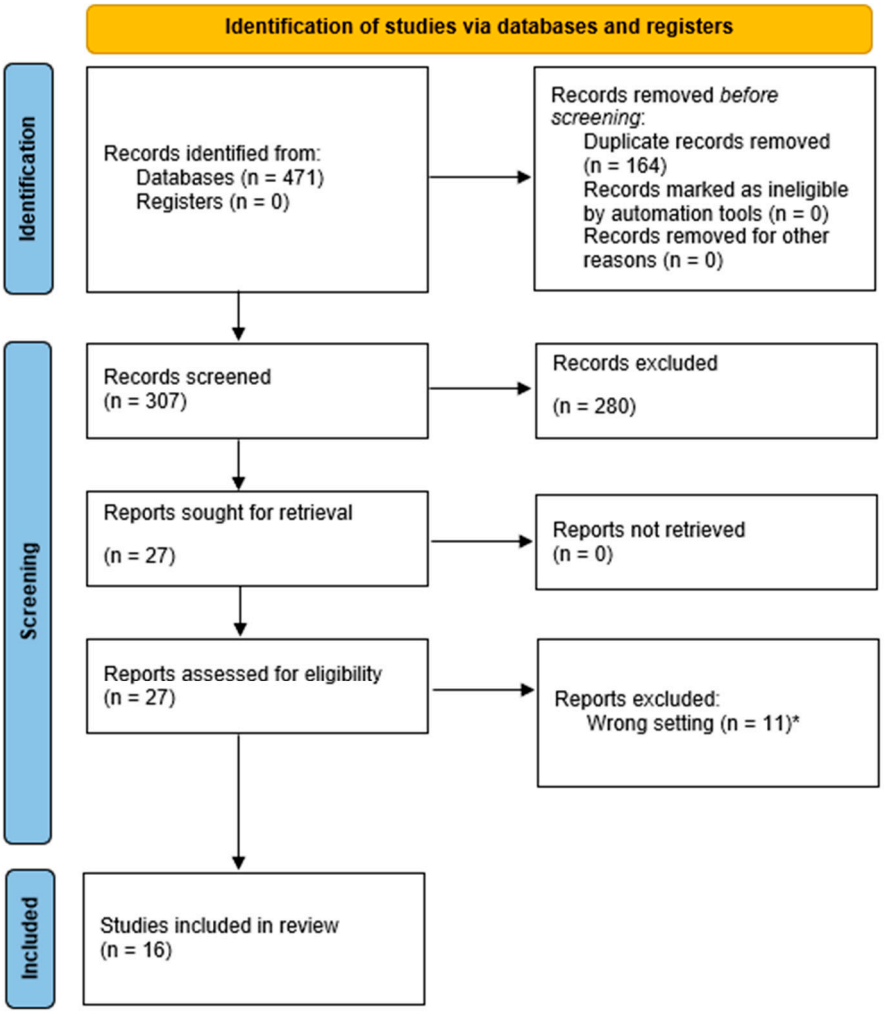

Figure 2. PRISMA 2020 flow diagram for new systematic reviews. * 6 studies reported epilepsia partialis continua, 1 study reported asleep electrographic status epilepticus, 1 study reported Rasmussen's encephalitis, and 3 studies where neuromodulation did not directly treat the status epilepticus.

Basic demographics of patients are illustrated in Tables 1 and 2. Different neuromodulation protocols for srSE in children have been described, with heterogeneous approaches for results description.

Table 1. Basic demographic including age, gender, and type of neuromodulation. * One patient [52] was reported in both VNS and DBS as the patient underwent both neuromodulation techniques. VNS did not offer benefits, and DBS caused resolution of super-refractory status epilepticus.

	Number of Patients	Male	Female	Stimulation Age (Years)	De Novo	Post Febrile
ECT	5	2	3	6.8 (3⁻––16)	3	2
VNS	15 *	8	7	5.73 (0.5–16)	3	3
DBS	6 *	3	3	12.5 (5–17)	4	3
All	25	13	12	7.4 (0.5–17)	9	7

Table 2. Outcomes following neuromodulation. Stim = stimulation; SE = status epilepticus. * One patient [53] was removed from calculation of "Duration of stimulation before SE resolution" as it was not adequately reported. ** Four patients ([54]; [pt 4] [55]; [pt 3 and pt 4] [56]) were removed from calculation of "Duration of SE before stimulation" as it was not adequately reported. *** 3 patients died of unrelated causes after SE resolved ([57]; [pt 1 and pt 2] [58]).

	SE Duration before Stim (Days)	Stim Duration before SE Resolution (Days)	Recovered from SE	Severe Sequelae Post-SE	Died during SE
ECT	53.63 (14–120) N = 5	4.75 (0–12) * N = 4	5	3	0
VNS	23.9 (5–66) N = 11 **	6.2 (0–20) N = 12	12	0	2 ***
DBS	49 (27–86) N = 6	7.7 (0–29) N = 6	6	1	0
All	37.5 (5–120) N = 21	6.3 (0–29) * N = 22	23	4	2

3.1. Electroconvulsive Technique (ECT)

ECT was used in five patients reported in five publications (Figure 3) [53,59–62]. srSE was focal in all patients, showing secondary generalisation in two. In three patients, srSE occurred de novo and in two was due to FIRES. MRI was not reported in one patient, showed bilateral polymicrogyria in one, and was initially normal in three (and later in the course of the srSE showed atrophy in one patient). ECT was applied between bitemporal electrodes in two patients and between frontotemporal electrodes in two patients, and electrode positions were not specified in one patient. The number and frequency of ECT sessions varied as seizure control was attempted. The number of days that each patient had been on srSE before ECT was started was 14, 24, 50, 60, and 120 days. Three patients had ECT initially on a number of consecutive days (7 or 12), one had five ECT sessions within 9 days whereas the fifth patient had ECT in two pairs of two consecutive days each, separated by 5 days. The number of days of ECT before srSE remission was 2, 5 (in two cases), 7, and 12 days. srSE stopped on the last day of ECT in three patients, while in two patients srSE stopped 4 days before the end of ECT was completed (after 29 and 125 days of SE). Therefore, ECT was considered to have contributed to stopping srSE in all five reported cases. Two patients remained out of the srSE without sequelae (one had learning difficulties beforehand), one had right temporal lobe surgery for focal cortical dysplasia, one developed severe epileptic encephalopathy, and one remained seizure-free but with severe motor dysfunction and cognitive decline.

3.2. Vagus Nerve Stimulation (VNS)

Fifteen patients had VNS implantations for srSE in nine articles (Figure 4) [52,54–58,63–65]. In three patients, srSE occurred de novo due to FIRES. srSE was focal or secondarily generalised in 10 patients, myoclonic in one, spasms in two, primarily generalised (tonic, T-C, myoclonic, absence seizures) in one, and GTCS without further explanation in one [65]. Identified aetiologies among the focal srSE included malignant partial epilepsy of infancy in four patients (in one patient due to mother's heroin abuse in pregnancy), neonatal venous thrombosis in one patient, FIRES in three, hemimegalencephaly in one, and one patient had bilateral frontal simplification of cortical gyri together with progressive diffuse cerebral atrophy. Spasms were due to non-ketotic hyperglycinemia in one patient and to microdeletion of 1q43q44 in another patient. Head MRI was normal in seven patients and showed cerebral atrophy/microcephaly in four patients, hemimegalencephaly in one, thalamic lesion/stroke in one, and there was no information about imaging in two patients. There was significant heterogeneity among the VNS parameters. The amplitude varied from 0.25 mA to 3 mA, usually with progressive increments during the course of the treatment until srSE improvement was achieved. The "on" time was 7 s in one case, 14 s

in another case, and 30 s in the remaining patients. The "off" period was between 1.8 and 5 min.

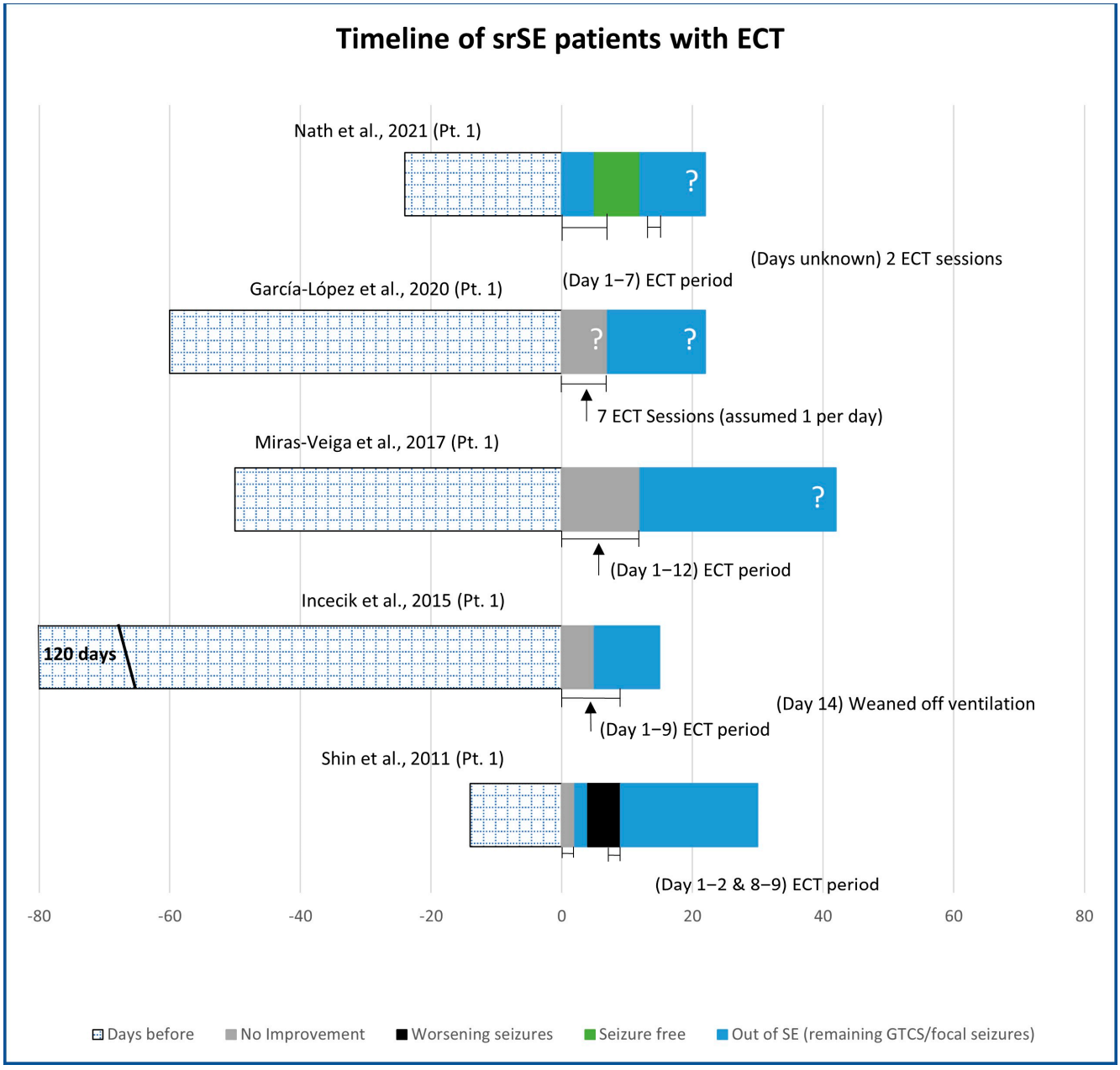

Figure 3. Timeline of srSE patients treated with ECT. (? = the time period is estimated but not clearly stated) [53,59–62].

Among the fifteen patients with VNS, the srSE was resolved in twelve. Two patients recovered without seizures, seven recovered from srSE remaining with seizures and learning difficulties due to underlying conditions, and three patients recovered but died of unrelated causes (dilated cardiomyopathy 5 months late, paediatric acute respiratory distress syndrome 2.5 years later, and tracheostomy-related late bleeding). Three patients continued with srSE after VNS implantation (two died and one was implanted with CMN DBS).

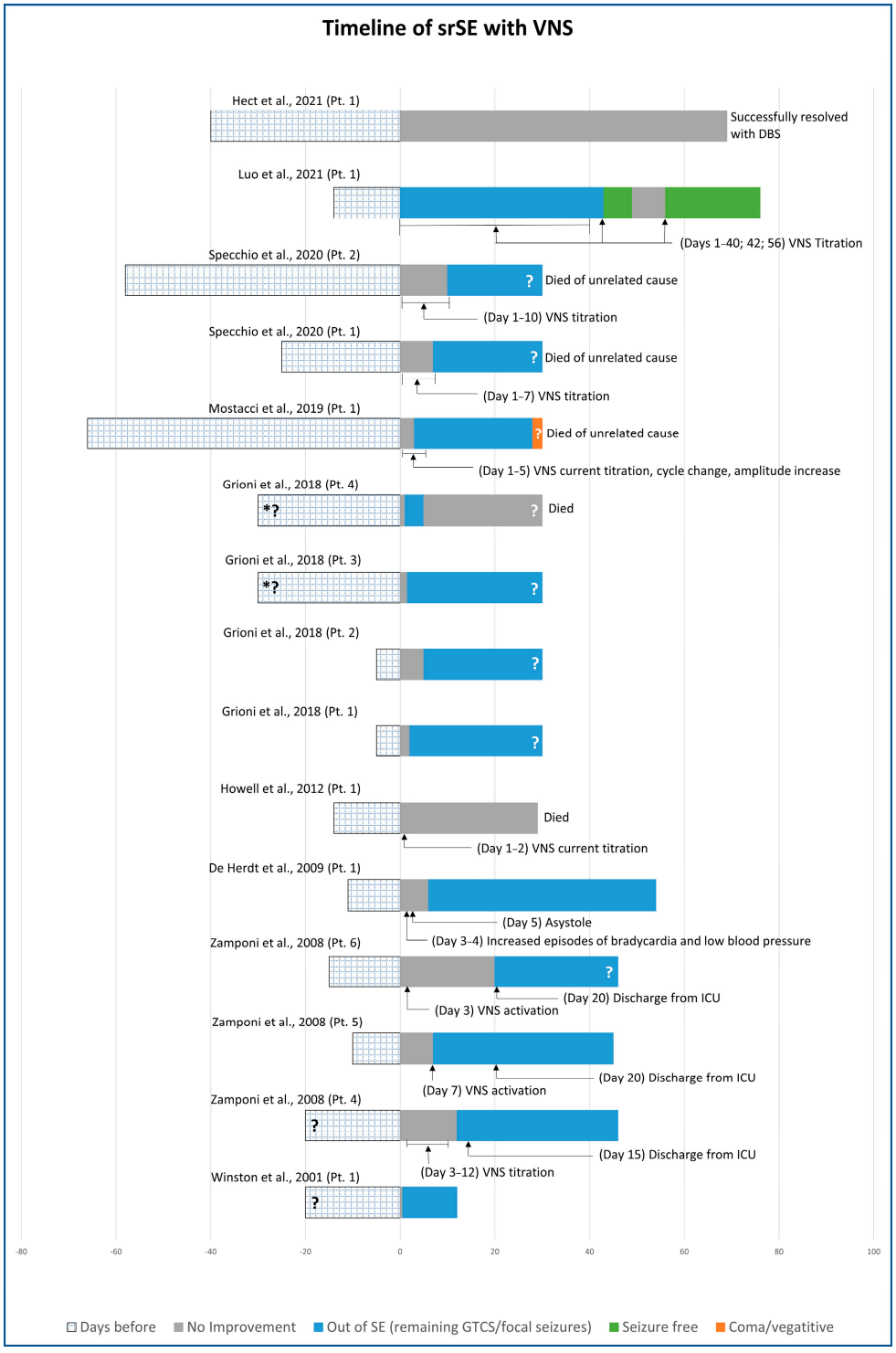

Figure 4. Timeline of srSE patients treated with VNS (* about 30 days but not clearly stated; ? = the time period is estimated but not clearly stated) [52,54–58,63–65].

3.3. Deep Brain Stimulation (DBS)

DBS was performed on six patients reported in five articles (Figure 5) [52,66–69]. In four patients, srSE occurred de novo, due to FIRES in three cases. The centromedian nucleus was stimulated in five patients and the anterior nucleus in one. srSE was focal in five patients, requiring intubation and induced coma in one, and generalised tonic–clonic in one. Head MRI was reported in five patients and was normal in three, showed severe cytotoxic oedema in one, and another patient showed signal abnormalities in basal ganglia,

external capsule, and cortex. Four patients had new onset SE without a previous history of seizures or epilepsy (de novo), including three patients where srSE followed febrile illness, suggesting FIRES. Four patients underwent trials of cessation of the stimulation after an initial improvement (stimulation period A) in order to demonstrate the stimulation effect. As SE returned, stimulation was then re-started (stimulation period B). The duration of stimulation period A was 15, 18, 22, and 67 days. After stimulation period B, patients were discharged with the stimulator on. The stimulation frequencies were 6 Hz (in three patients), 145 Hz, and 180 Hz. DBS had immediate effects on srSE in two patients. In the remaining four children, improvement occurred after 2, 4, 6, and 30 days of stimulation. SrSE resolved in all patients; one patient came back to her previous number of seizures, four children remained with seizures, and one child remained in a vegetative state.

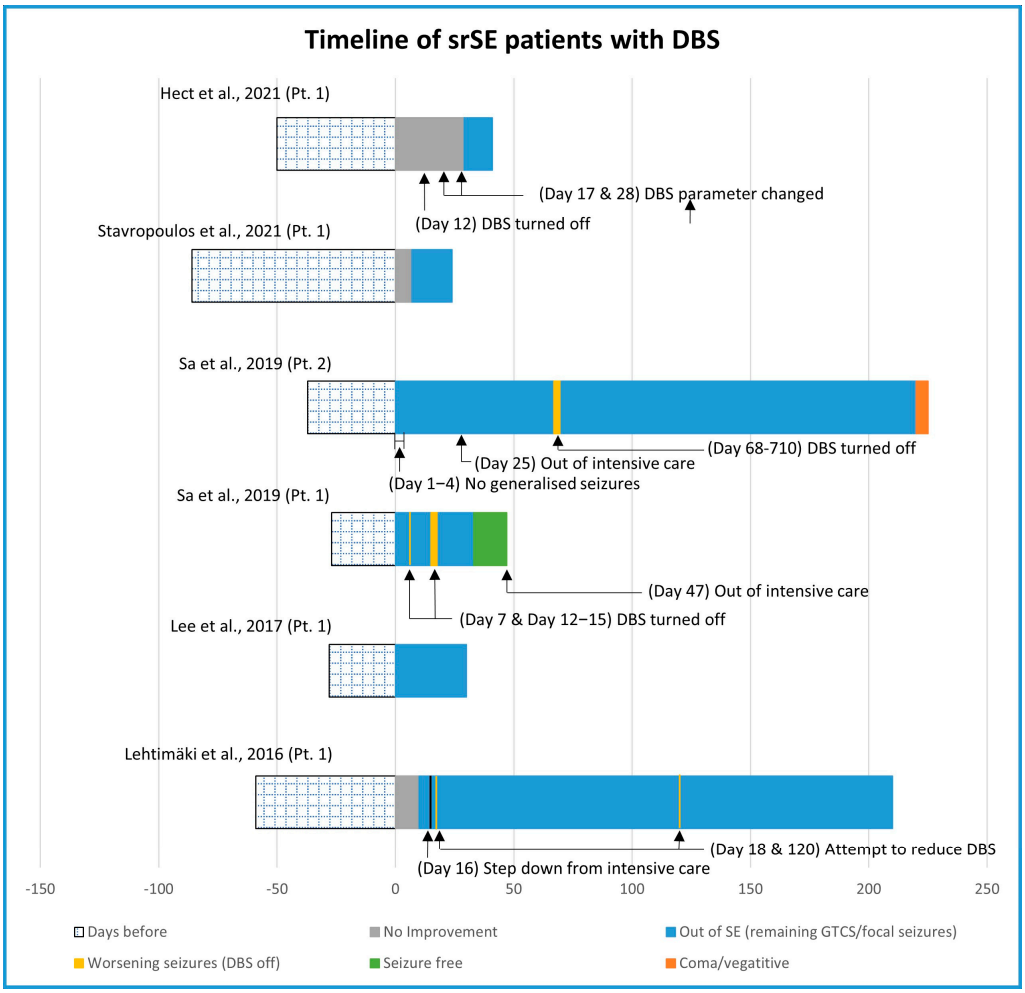

Figure 5. Timeline of srSE patients treated with DBS [52,66–69].

In summary, 23 out of the 25 children treated with neuromodulation techniques recovered from srSE (Table 2). Among these children, twelve recovered without new medical conditions, four recovered with severe sequelae (epileptic encephalopathy or cognitive/motor decline), four developed new seizures after the srSE, and three died for unrelated reasons.

4. Discussion

Status epilepticus (SE) is a medical emergency with high mortality and morbidity rates [70–72]. The treatment protocols for the early management of SE are well standardised [12]. However, refractory and super-refractory SE (rSE and srSE) are often associated with significant and irreversible brain damage whose severity is related to SE duration and

aetiology. A well-defined, effective, and fast-acting therapeutic protocol would be highly desirable to prevent potential longstanding neurologic complications. In this review, we present data showing that neuromodulation could be a potentially efficacious treatment option for shortening rSE and srSE duration in children when the routinely used 1st, 2nd, and 3rd line treatments have failed.

The features of status epilepticus in children can be slightly different from those in adults. As shown in the presented data, srSE in children is often caused by genetic/metabolic conditions, brain malformations, birth injuries, and febrile infection-related epilepsy syndrome (FIRES). The latter is a rare, life-threatening condition that presents with a non-specific febrile illness followed by refractory status epilepticus within 24 h to 2 weeks of the onset of the febrile illness in previously healthy children, with a mortality of up to 30% [73]. Its pathogenesis is unclear, but autoimmune mechanisms have been proposed [74], and a recent international consensus recommendation suggests that first-line immunological treatment should be started during the first 72 h [75].

There are no clinical trials performed to assess the efficacy of any of the neuromodulation techniques on srSE. The literature shows that three different neuromodulation techniques, one non-invasive (ECT) and two implantable devices (VNS and DBS), have been sporadically tried and could be beneficial in cases of rSE and srSE in children. However, the number of reported cases remains small, there is significant diversity regarding the cause of the srSE, and the mechanisms by which neuromodulation affects SE are not elucidated. DBS and ECT appear to have provided benefit in cases with the FIRES condition, while only one out of the three cases published with FIRES and VNS showed resolution of the srSE. All three techniques share unclear mechanisms of action. Animal studies suggest that ECT alters biological processes such as neuroplasticity and neurotransmitter function and might cause internalisation of NMDA receptors or other epigenetic effects [76,77].

Several studies suggest that VNS can modify norepinephrine and serotonin levels at the locus coeruleus and dorsal raphe nuclei [78]. It has also been suggested that VNS can cause changes in limbic structures' functions modifying GABA and glutamate concentrations at nucleus tractus solitaries [79]. Similar mechanisms may be effective against srSE.

The main advantage of DBS is that the electrical stimulation can be applied locally to specific brain areas using implanted intracranial electrodes and that different stimulation parameters can be applied at the implanted region. In some published cases, electrode implantation probably induced a microlesion effect which was associated with major seizure improvement [39,80]. It has also been proposed that upon high-frequency stimulation (>60 Hz), inhibition of the stimulated area might be mediated by activation of GABAergic afferents or inactivation of voltage-gated currents [39,81]. Moreover, low-frequency stimulation (6 Hz) of the centromedian thalamic nucleus has recently been reported to be useful in reducing the severity and frequency of focal seizures in children and adults with srSE [66,67,82], probably via neuromodulation of cortical structures through the thalamocortical pathway [83].

At present, there is no consensus protocol for the use of ECT, VNS, and DBS in the management of rSE/srSE, and existing evidence is based on a limited number of reported patients. Not all neuromodulation techniques are clinically available in most centres, and they are only considered at late stages of rSE/srSE when standard treatment has failed. As brain damage caused by SE can start as early as 30 min from SE onset [3], the appropriate time for the application of neuromodulation for the treatment of srSE in children is a question of major importance.

Even though published paediatric cases show that neuromodulation was applied as a last-resort treatment, the results appear encouraging. Neuromodulation techniques were applied between 5 [56] and 120 days [59] after SE onset, and substantial brain damage may have already been present in most cases by that time. Regarding the use of non-invasive neuromodulation techniques, the use of ECT in children has given limited but promising results, suggesting that the non-invasive safer techniques could be considered earlier in the course of srSE. Despite the side effects and the invasive nature of VNS and DBS, such

damage is unlikely to be induced by neuromodulation, as similar techniques are safe when used for the chronic treatment of epilepsy or other brain conditions [22,39,84,85]. Unfortunately, differences in the timing to apply neuromodulation techniques in different centres/cases do not allow reliable conclusions on the optimal timing for starting this treatment modality.

Regarding invasive procedures, the present series suggests that the effects of DBS and VNS can occur within the first week of treatment. Nonetheless, DBS was used in fewer children, and VNS appears to have been used in a higher number of patients with severe epilepsy (migrating epilepsy, severe cortical malformations, birth injury). DBS implantation led to improvement of srSE, with worsening in seizures when the DBS was turned off [66,67,69,82,86].

Even though the presented data look encouraging, clinical guidance cannot be based on published case reports due to the risk of significant bias [87]. For instance, it is common to find successful neuromodulation cases for srSE published as single case reports, but unsuccessful cases are usually published as part of a case series [56,88] or are not submitted for publication.

As suggested by Rossetti and Lowenstein [20], neuromodulation could be complementary to pharmacological treatment for the management of rSE. Non-invasive techniques such as ECT, tDCS, or rTMS could be considered as add-on treatments after the failure of standard treatment. If no improvement is noted, invasive techniques (DBS and VNS), when available, could be discussed and planned in a timely manner.

5. Conclusions

The evaluation of neuromodulation techniques for the treatment of srSE in children is limited by the small number of published cases and the variability of neuromodulation protocols used for the treatment of srSE. Although neuromodulation strategies have not been tested through randomised, prospective controlled clinical trials, this review presents the existing data and the potential benefits of neuromodulation therapy, suggesting that these techniques could be considered at earlier stages within the course of srSE intending to prevent long-term neurologic complications. Clinical trials aiming to establish whether early intervention can prevent long-term sequelae are necessary to establish the potential clinical value of neuromodulation techniques for the treatment of srSE in children.

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Daytime-Restricted Feeding Ameliorates Oxidative Stress by Increasing NRF2 Transcriptional Factor in the Rat Hippocampus in the Pilocarpine-Induced Acute Seizure Model

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Abstract: Seizure-mediated oxidative stress is a crucial mechanism in the pathophysiology of epilepsy. This study evaluated the antioxidant effects of daytime-restricted feeding (DRF) and the role of the Nrf2 signaling pathway in a lithium-pilocarpine model seizure model that induces status epilepticus (SE). We performed a lipoperoxidation assay and dihydroethidium fluorescence to measure oxidative stress markers in the hippocampus (malondialdehyde and reactive oxygen species). The protein content of Nrf2 and its downstream protein SOD2 was evaluated using Western blotting. The cellular distribution of the Nrf2 and SOD2 proteins in the pyramidal cell layer of both the CA1 and CA3 hippocampal subfields and astrocytes (GFAP marker) were quantified using immunofluorescence and immunohistochemistry, respectively. Our results indicate that DRF reduced the malondialdehyde levels and the production of reactive oxygen species. Furthermore, a significant increase in Nrf2 and SOD2 protein content was observed in animals subjected to restrictive diet. In addition, DRF increased the relative intensity of the Nrf2 fluorescence in the perinuclear and nuclear compartments of pyramidal neurons in the CA1 subfield. Nrf2 immunoreactivity and the astrocyte marker GFAP also increased their colocalization under DRF conditions. Additionally, SOD2 immunoreactivity was increased in CA1 pyramidal neurons but not in the CA3 region. Our findings suggest that DRF partially prevents oxidative stress by increasing the Nrf2 transcriptional factor and the SOD2 enzyme during the development of SE.

Keywords: daytime-restricted feeding; oxidative stress; status epilepticus; Nrf2; SOD2; hippocampus

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1. Introduction

Epilepsy is the third most common chronic brain disorder, affecting nearly 70 million people. It is characterized by recurrent spontaneous seizures due to the hyperexcitability of brain neurons [1]. *Status epilepticus* (SE) results from the failure of the mechanisms responsible for seizure termination or initiation, leading to prolonged seizures within a period of 5 min that can occur or not in epileptic patients [2]. SE must be treated urgently; otherwise, it could trigger serious consequences such as neuronal death, neuronal injury, and alteration of neuronal networks [2,3].

Oxidative stress has recently been recognized as playing a crucial role in the pathophysiology of SE and epilepsy [4–6]. Oxidative stress results in functional cellular damage and may cause subsequent cell death via the oxidation of biomolecules such as proteins, nucleotides, and lipids [4]. Prolonged seizures generate reactive oxygen species (ROS); this

process is carried out by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and NMDA receptor activation [7]. Furthermore, the seizure-induced inflammatory response can activate inducible nitric oxide synthase to produce nitric oxide, which may react with the superoxide radical to form reactive nitrogen species (RNS) like the peroxynitrite radical. These RNS contribute to the severity of oxidative stress in the pharmacological model of SE [7,8].

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcriptional factor related to the natural cellular defense system. Nrf2 induces the gene expression of numerous ROS-eliminating enzymes [9]. Under physiological conditions, Nrf2 is targeted for proteasomal degradation in the cytosolic compartment; however, upon oxidative or electrophilic stress, it translocates to the nucleus, binds to the antioxidant-responsive element (ARE) sequence, and promotes the transcription of antioxidant enzymes, including NAD(P)H quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO1), and superoxide dismutase 2 (SOD2) [10]. Several recent reports have demonstrated that the Nrf2-ARE signaling pathway could represent an important target for protecting neurons after ischemic damage [11]. However, the possible protecting role of Nrf2 has not been fully studied in epilepsy.

Many metabolic-based therapies have been tested in patients with epilepsy in clinical trials and in pharmacological models of temporal lobe epilepsy. These therapies include ketogenic diets, calorie restriction, and intermittent fasting [12]. In this regard, our group has shown that daytime-restricted feeding (DRF), an intermittent fasting schedule, has an anticonvulsant effect attributed to metabolic activation (increased AMP activating-protein kinase), epigenetic mechanisms (increased histone 3 acetylation) [13], and anti-inflammatory and neuroprotective effects (preventing the activation of microglia and astrocytes) [14]. Hence, this study aimed to determine whether the DRF schedule could have an antioxidant effect after a pilocarpine SE-induced seizure and whether such an effect is due to the increase in antioxidant-related enzymes.

2. Materials and Methods

2.1. Experimental Animals

Eighty adult male Wistar rats (n = 5 per group) weighing approximately 250–280 g were used. The rats were maintained at a 12 h light/12 h dark cycle under constant temperature and humidity conditions (25 °C and, 50–70%, respectively). The animals were fed with the standard diet of Lab Diet Rodent Laboratory Diet 5001 pellets (PMI Nutrition International, Inc., Brentwood, MO, USA) and had free access to water.

2.2. Daytime-Restricted Feeding and the Pilocarpine-Induced Seizure Model

The experimental animals were randomly assigned to one of the following four groups: (1) a control group with ad libitum access to food (AL), (2) a pilocarpine-induced SE group with ad libitum access to food (ALSE), (3) a group under daytime-restricted feeding (DRF), and (4) a pilocarpine-injected DRF group (DRFSE). As previously described [13,14], DRF consisted of giving the rats access to food for only two hours daily for 20 days (from 12 to 2 pm) with free access to water and, after this period, we proceeded to perform the acute seizure model at day 21 (Figure 1).

We chose the lithium-pilocarpine model because it is one of the most widely used models to induce SE [13,14]. The animals were first injected with lithium-chloride (127 mg/kg, i.p.) on day 20; 18 h later, they received a scopolamine methyl nitrate injection (1 mg/kg, s.c.) to minimize the peripheral cholinergic effects of pilocarpine. Thirty minutes later, pilocarpine chloride was administered (30 mg/kg, s.c.) to induce SE. Ninety minutes later, the seizures were stopped with a diazepam injection (5 mg/kg i.m.).

The behavioral grading of the seizures was performed via video monitoring for 2 h. The scoring was based on Racine's scale [15] with the following stages: (0) no abnormality; (1) akinesia and facial movements; (2) head nodding; (3) forelimbs clonus; (4) rearing; and (5) rearing and falling. Only the animals that reached stages four to five were used; the rest were discarded. The AL control and DRF animals only received a saline injection instead of

a pilocarpine injection. Importantly, the food was removed 12 h before the usual start of food restriction to ensure that all animals were in the same metabolic condition. Therefore, SE was induced after 6 h of fasting in the AL-pilocarpine rats and after approximately 22 h of fasting in the DRF-pilocarpine rats. Moreover, both AL- and DRF-pilocarpine animals received a saline solution injection to avoid dehydration. Twenty-four hours after the pilocarpine injection, the animals were euthanized with an overdose of sodium pentobarbital (26 mg/kg) to perform biochemical analyses (Figure 1). All the experiments from the present study were approved by the Ethical Committee of the Faculty of Medicine at UNAM following all the statements to minimize animal suffering.

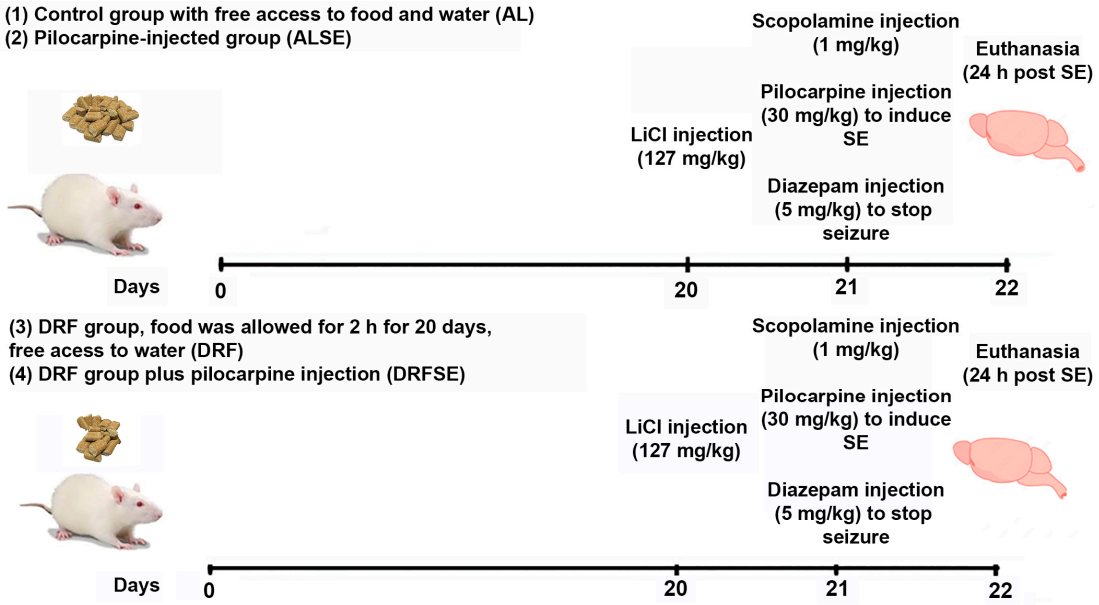

Figure 1. Schematic representation of the experimental procedure of the day-time restrictive feeding schedule and status epilepticus induction via pilocarpine injection.

2.3. Determination of Lipid Peroxidation

The rat hippocampi were dissected as quickly as possible and were immediately washed with phosphate-buffered saline solution (PBS, pH 7.4) and transferred into clean plastic tubes. To measure lipid peroxidation products such as malondialdehyde (MDA) from hippocampal tissue, we used the ALDetect lipid peroxidation assay kit (Enzo Life Science, Farmingdale, NY, USA, BML-AK170) following the manufacturer's instructions. The results were expressed as nmol of MDA per mg of protein.

2.4. Determination of Reactive Oxygen Species in Hippocampi

The rats' brains were quickly dissected after euthanasia and frozen with butanol at −70 °C. Coronal slices (20 µm) were obtained in a cryostat and placed in slides with oly-L-lysine. The slides were incubated with a solution of dihydroethidium (DHE, 10 µM) at room temperature for 30 min and protected from light. Once the incubation time had elapsed, the sections were mounted and observed under an epifluorescence microscope. Although DHE has been described to measure superoxide anion, it also can detect other reactive oxygen species such as hydrogen peroxide and hydroxyl radicals.

2.5. Western Blotting

Hippocampi homogenates were transformed into cytoplasmic extracts using a commercial buffer lysis (Thermo-Fisher Scientific, Waltham, MA, USA). Homogenate samples were collected and stored at −70 °C for later analysis. Protein extracts were quantified using a BCA assay kit (Pierce, Appleton, WI, USA). For this, 60 µg of protein was loaded on 10 or 15% SDS-PAGE gels. The proteins were transferred to a nitrocellulose membrane in a

wet tank transfer system (Mini Trans-Blot Central Core, Bio-Rad Laboratories, Hercules, CA, USA). Then, the membranes were rinsed with Tris-buffered saline (TBS) and blocked with a solution containing 5% non-fat dry milk in TBS-Tween 20 0.1% (TBST) overnight at 4 °C. The blots were probed with anti-rabbit polyclonal Nrf2 (1:1000, Santa Cruz Biotechnology, Dallas, TX, USA, SC-722), and anti-rabbit polyclonal manganese superoxide dismutase (SOD2) (1:1000, Boster Biological Technology, Pleasanton, CA, USA, A00349) in TBST at 4 °C for 48 h. After three rinses with TBST for five minutes each, the membranes were incubated with anti-rabbit (1:10,000 Cell Signaling Technology, Beverly, MA, USA) or anti-mouse IgG secondary antibodies (1:5000, Santa Cruz Biotechnology, SC-516102) for 90 min at room temperature, followed by three rinses with TBST for five minutes each. As loading control, we used mouse monoclonal antibody against beta-actin (1:1000, Genetex, Irvine, CA, USA, GTX629630). The membranes were revealed using the chemiluminescence reagent Luminata Crescendo Western HRP, Millipore. Images were obtained with the Fusion FX photo documentation device (Vilber, Lemont, IL, USA). Image analysis was performed with the Fiji image processing software version 1.54 developed at the National Institutes of Health and available online (https://imagej.net/software/fiji/, accessed on 20 November 2022). The data obtained from the density analysis of each protein under study were normalized with the data of its corresponding protein.

2.6. Immunofluorescence and Immunohistochemistry

The animals were euthanized as previously mentioned and perfused transcardially with 300 mL of ice-cold PBS followed by 250 mL of 4% paraformaldehyde in phosphate buffer (pH 7.4) as a fixative solution. Their brains were removed and immersed in the fixative solution overnight. Then, the brains were dehydrated using alcohol solutions and xylene and embedded in paraffin wax. Coronal sections of 5 µm of thickness were cut in a microtome and placed in poly-L-lysine coated slides. The paraffin was removed, and the sections were pretreated with a heat retrieval Diva Decloacker solution and placed in an electric pressure cooker (decloaking chamber, Biocare Medical, Pacheco, CA, USA) for 15 min. The coronal sections were rinsed with distilled water and PBS. Moreover, to avoid autofluorescence of the sections, we submerged the slides into a Coplin glass filled with a saturated solution of Sudan black B (0.25%) in 70% isopropyl alcohol. The hippocampal sections were rinsed with PBS for 5 min, permeabilized with PBS Triton-100× (0.3%) for 20 min, and blocked with 1% normal goat serum (Vector Laboratories, Inc., Newark, CA, USA) in the PBS solution for 30 min. Then, rabbit polyclonal Nrf2 (1:100, Santa Cruz Biotechnology, SC-722) and anti-mouse antibody against the glial fibrillary acidic protein (GFAP, 1:200, Biocare Medical, CM065) were incubated in blocking solution at 4 °C for 48 h. After three rinses in PBS, the sections were incubated with anti-mouse Alexa Fluor 488 and Alexa Fluor 594 conjugated donkey anti-rabbit IgG antibody (1:300, Thermo Fisher Scientific) in the blocking solution for 2 h at room temperature. Then, the slides were rinsed three times with PBS (5 min each). After washing, the nuclei were labeled with Hoescht (1:5000, Roche Lab, Indianapolis, IN, USA) in PBS (for 1 min. The tissue was washed again with PBS for 3 min and covered. For immunohistochemistry, the coronal sections were permeabilized with PBS containing 0.3% Triton X-100 and 0.3% H_2O_2 solution for 30 min and left with the blocking solution (Background Sniper, Biocare Medical) for 1 h to reduce the background staining. Then, the brain sections were incubated with rabbit polyclonal anti-SOD2 (1:300, Boster Biological Technology, A00349) overnight at 4 °C and rinsed three times for 5 min with PBS. Immediately after, the sections were incubated with Trekkie Universal Link for 1 h at room temperature rinsed with PBS again and then with TrekAvidin-HRP (Starr Trek Universal HRP Detection, Biocare Medical) for 1 h, and rinsed one more time with PBS. Afterwards, coronal sections were revealed by using 3, 3-diaminobenzidine (Betazoid DAB Chromagen Kit, Biocare Medical) dehydrated, mounted, and observed under a Brightfield microscope (Leica Microsystem, Wetzlar, Germany). The negative controls underwent the same procedure but without the primary antibodies in both techniques.

2.7. Confocal Microscopy Analysis

The brain sections were evaluated with a Nikon Ti Eclipse inverted confocal microscope equipped with an A1 imaging system, both controlled from the proprietary NIS Elements v.4.50 software. Imaging was performed using a 20X objective (dry, NA 0.8). The dye was excited in a sequential mode using the integrated laser lines: 403 nm (Hoechst), 488 nm (Alexa 488), and 620 nm (Alexa 594). The corresponding fluorescence was read in the following ranges: 425–475 nm (Hoechst 33342), 500–550 nm (Alexa fluor 488), and 570–620 nm (Alexa fluor 594) using the manufacturer-provided filter sets. Images were acquired and analyzed using NIS Elements v.4.50. The intensity of the red channel pixels (Alexa 594) per area was quantified with FIJI software. The intensity per area was calibrated using a spatial scale derived from the maximum and minimum intensity values contained in the bitmap of each image (image provided by the software). This calibration allowed us to establish the basal intensity values of each image. The density was calculated in a similar manner to the intensity per area; it was determined relative to the control groups and expressed in pixels/mm^2. The count area was adjusted to 1 mm^2.

2.8. Statistical Analysis

All data are presented as the mean ± standard deviation of the mean (S.D.) and examined with the appropriate normality test. A one-way ANOVA with Tukey's multiple comparison test or the Kruskal–Wallis test followed by Dunn's post hoc test were used to measure the optical band's density from the Western blot assay and the relative intensity from DHE fluorescence and Nfr2 nuclear factor immunofluorescence. All statistical tests were performed using GraphPad Prism statistics software version 7 (GraphPad Software, San Diego, CA, USA), and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Dietary Restriction Reduces the Levels of Malondialdehyde in Seizure-Induced Animals

Since SE has been associated with an increase in lipid peroxidation, we evaluated the levels of malondialdehyde (MDA) in the hippocampal tissue of the experimental groups. There was a significant increase in the MDA levels in the AL-injected pilocarpine group compared with the AL control group ($p < 0.001$, Figure 2). Remarkably, we found a significant decrease in MDA levels between the AL-injected pilocarpine and DRF-injected pilocarpine groups ($p < 0.01$, Figure 2). Moreover, the MDA levels were slightly higher in the DRF-schedule group than in the AL control group, but this was not significant.

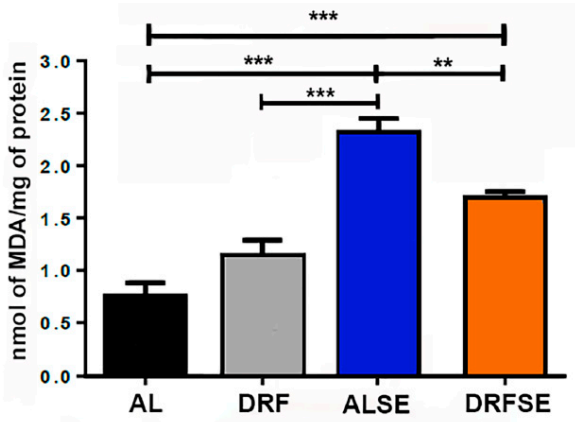

Figure 2. Effect of daytime-restricted feeding on lipid peroxidation after SE induction. Malondialdehyde was significantly increased in the ALSE group compared to the AL control group. The DRF group had slightly more MDA than the AL control. The DRFSE group showed lower MDA levels than the ALSE group. These results suggest an antioxidant effect of DRF. All data are presented as mean ± S.D. (n = 5 rats per group). Statistical analysis: One-way ANOVA followed by Tukey's multiple comparison test, ** $p < 0.01$, *** $p < 0.001$.

3.2. Daytime-Restricted Feeding Reduces the Levels of Reactive Oxygen Species in Seizure-Induced Animals in CA1 and CA3 Hippocampal Regions

Using DHE fluorescence, we found that the reactive oxygen species (superoxide anion, hydrogen peroxide, and hydroxyl radical) levels were significantly increased in the CA1 subfield of the hippocampus in the ALSE group compared to the AL control group ($p < 0.001$, Figure 3A,B). Moreover, the DRFSE group significantly reduced the relative intensity of DHE fluorescence to the levels of the ALSE group ($p < 0.01$, Figure 3A,B). Regarding the CA3 subfield, we also observed a significant increase in the relative intensity of DHE in the ALSE group compared to their respective control ($p < 0.05$, Figure 3C,D). Furthermore, the restrictive diet did not statistically reduce DHE relative intensity in the DRFSE group compared to the ALSE group (Figure 3C,D).

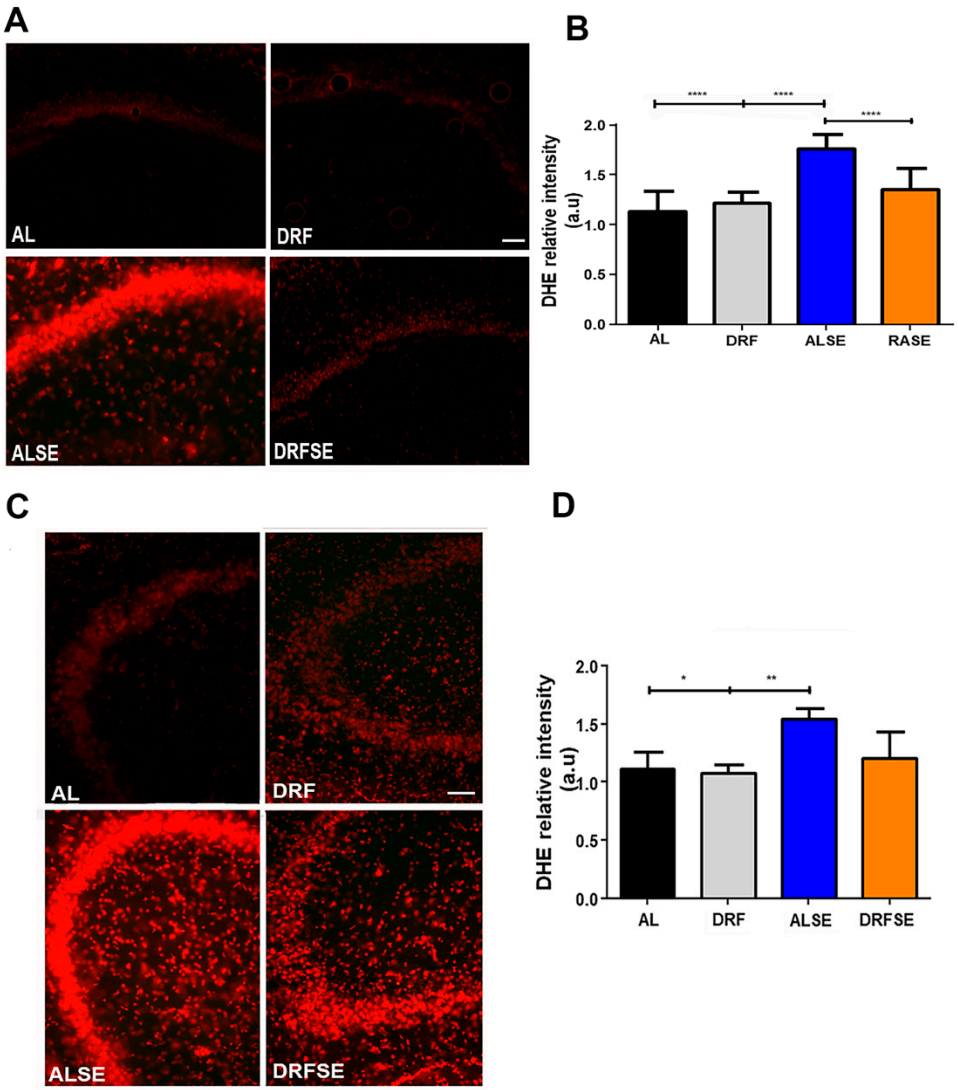

Figure 3. Effect of daytime-restricted feeding on superoxide radical DHE fluorescence in the CA1 and CA3 subfields of the dorsal hippocampus after SE. (**A**) Representative images of DHE-stained cells (red) in the experimental groups in the CA1 subfield. (**B**) Quantification of relative DHE intensity in the CA1 subfield. (**C**) Representative images of DHE-stained cells (red) in experimental groups in the CA3 subfield. (**D**) Quantification of relative DHE intensity in the CA3 subfield. Data are presented as the mean ± S.D. One-way ANOVA followed by Tukey's post hoc test or Kruskal–Wallis test followed by Dunn's post hoc test was used (n = 5 rats per group), * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. Scale bars: 50 μm.

3.3. Daytime-Restricted Feeding Modulates the Content of the Nrf2 Transcriptional Factor in Hippocampal Homogenates and Increases the Nrf2 Immunostaining in CA1 and CA3 Pyramidal Neurons in the SE Model

Since DRF induced a significant decrease in lipoperoxidation and a decrease in the relative density of DHE fluorescence, we evaluated whether the Nrf2 nuclear factor could be involved in the defense against cellular oxidative stress. The ALSE group showed a significant increase in cytoplasmic Nrf2 protein content compared to that in the AL and DRF groups ($p < 0.0001$, Figure 4A,B). Moreover, the restrictive diet increased the Nrf2 protein content after pilocarpine-induced seizures compared to the ALSE group ($p < 0.0001$, Figure 4A,B).

To further understand the protective role of the Nrf2 protein against oxidative stress, we studied its cellular distribution in the CA1 and CA3 hippocampal subfields since these brain regions become widely damaged in temporal lobe epilepsy [16]. As shown in Figure 4C, the relative intensity of Nrf2 in the CA1 hippocampal region was higher in animals subjected to SE than in the AL control or DRF groups. In particular, there was a high intensity in the perinuclear compartment (arrows) and a low intensity inside the nucleus ($p < 0.0001$, Figure 4C,D, arrowheads). Interestingly, the DRFSE group had higher Nrf2 relative intensity than the ALSE group ($p < 0.001$, Figure 4C,D). Furthermore, Nrf2 immunostaining in the DRFSE group was more intense in the perinuclear compartment (arrows) and showed medium intensity in the nucleus of the pyramidal cell layer (arrowheads).

Kim and Kang [17] recently showed that an Nrf2 activator prevents the loss of Nrf2 nuclear factor in astrocytes in rats with chronic epilepsy. Thus, we detected glial fibrillary acid protein (GFAP), a well-known astrocyte marker through immunofluorescence, to assess whether the restrictive diet might have a similar action. GFAP immunoreactivity was more abundant in the *stratum radiatum* and cells surrounding the pyramidal cell layer of CA1 in the DRFSE group than in the ALSE group (Figure 4B). Furthermore, we found a major overlapping of Nrf2 and GFAP signals in astrocyte processes in the DRFSE group versus the ALSE group (Figure 4B).

Similarly, in the hippocampal CA3 subfield, Nrf2 intensity was higher in the rats injected with pilocarpine compared to their respective controls ($p < 0.0001$, Figure 4E,F). Nrf2 immunostaining was localized in the perinuclear compartment, but unlike what was observed in CA1, the Nrf2 signal in this region was punctate and discontinuous. We also observed the Nrf2 signal in some nuclei of pyramidal neurons (Figure 4E). In the case of the DRFSE group, we did not observe significant changes in Nrf2 fluorescence intensity compared with the ALSE group, although there was a slight increase in the Nrf2 signal (Figure 4E,F). Similar results were obtained for GFAP immunoreactivity, in which the overlap of the Nrf2 and GFAP signals was more evident in the DRFSE group than in the ALSE group; however, such an observation was merely qualitative (Figure 4E).

3.4. Daytime-Restricted Feeding Increases the Content of Superoxide Dismutase 2 in Hippocampal Homogenates and the Immunostaining in CA1 and CA3 Pyramidal Neurons after the Acute Seizure Model

Due to the fact that superoxide dismutase 2 (SOD2) is a downstream protein of the Nrf2 signaling pathway [10], we evaluated the mitochondrial SOD2 levels in the hippocampal homogenates through Western blotting. In this regard, we observed that the ALSE group showed a significant increase in SOD2 protein content compared to the AL and DRF groups ($p < 0.0001$, Figure 5A,B). Notably, daytime-restricted feeding increased the SOD2 protein content after pilocarpine-induced seizures compared to the ad libitum pilocarpine-induced seizure ($p < 0.0001$, Figure 5A,B). Then, we evaluated the relative intensity of the SOD2 immunoreactivity of the pyramidal cell layer of the CA1 and CA3 subfields. Unlike the previous results in the SOD2 protein content in the hippocampal homogenates, we observed a significant decrease in the relative density of SOD2 immunostaining in pyramidal neurons of CA1 in the ALSE group compared to the AL group ($p < 0.001$, Figure 5C,D, arrows). Interestingly, the restrictive diet was able to significantly increase the immunoreactivity of SOD2 in the pyramidal cell layer of CA1 compared to the ad libitum pilocarpine-induced seizure ($p < 0.001$, Figure 5C,D, arrows). Similar results were observed in the CA3 region. There was a significant decrease in the relative density of SOD2 immunostaining in the pyramidal neurons of CA3 in the ALSE group compared to the AL- or DRF-alone groups

($p < 0.001$ and $p < 0.01$, respectively, Figure 5E,F, arrows). Notably, there was more of a tendency to increase the immunoreactivity of SOD2 in the DRFSE group than in the ALSE group; however, there were no statistically significant results (Figure 5E,F, arrows).

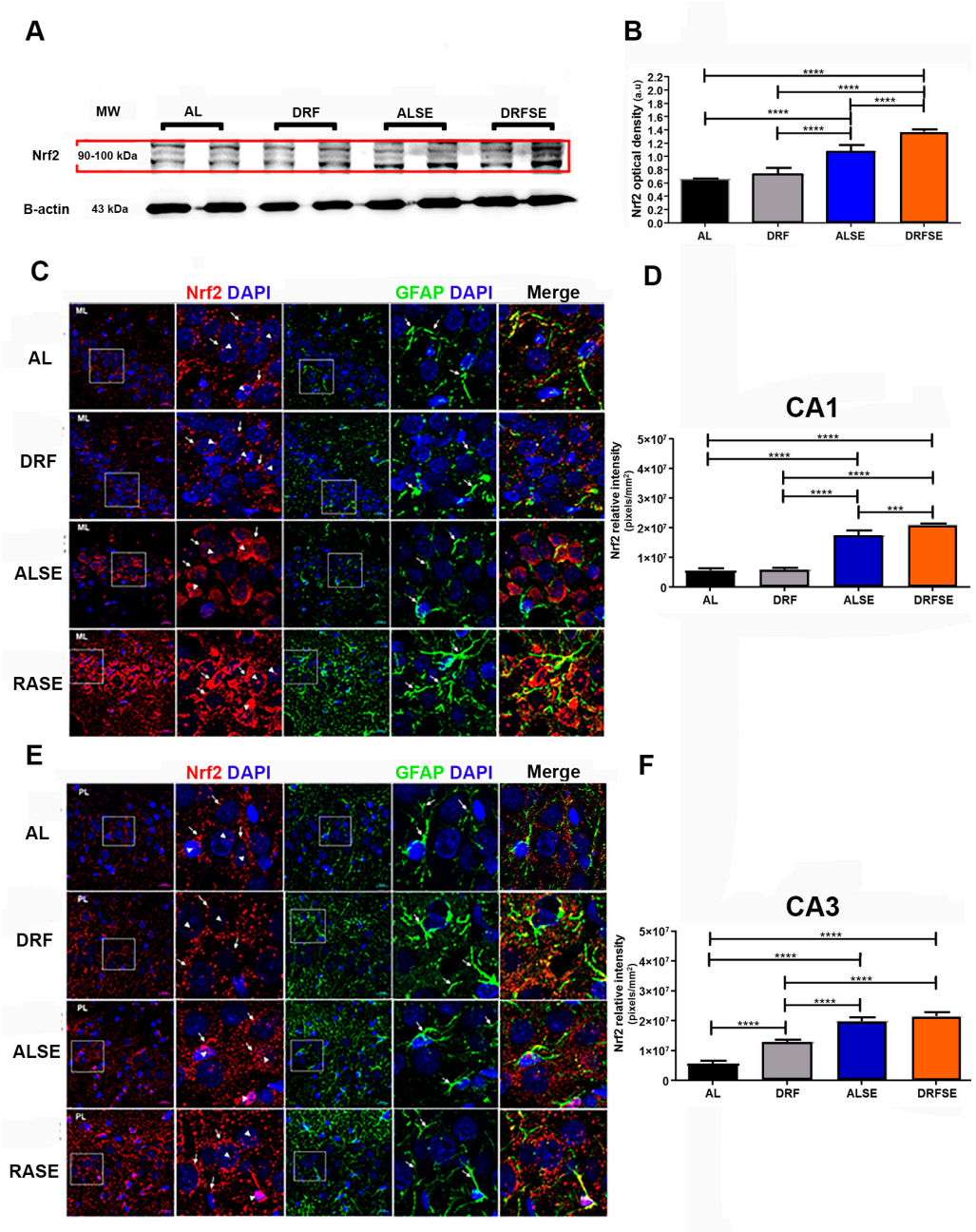

Figure 4. Effect of daytime-restricted feeding on the Nrf2 protein content and the Nrf2 and GFAP fluorescence in the CA1 and CA3 subfields of the dorsal hippocampus after SE induction. Representative immunoblots of Nrf2 in the AL, DRF, ALSE, and DRFSE experimental groups in the hippocampal homogenates (**A**). Quantification of the optical density of Nrf2 protein contents in hippocampal homogenates (**B**) Representative images of double immunofluorescence for Nrf2 (red) and GFAP (green) and counterstained with Hoechst (blue) in all experimental groups in the CA1 and CA3 subfields (**C**,**E**). Quantification of the relative intensity of Nrf2 in the CA1 subfield of all groups (**D**). Quantification of Nrf2 relative intensity in the CA3 subfield (**F**). Data are presented as the mean ± S.D, (n = 5 rats per group). Arrows show the cytoplasmic and head arrows show nuclear the distribution of Nrf2, respectively. One-way ANOVA followed by Tukey's multiple comparison test, *** $p < 0.001$, **** $p < 0.0001$. Scale bars: 20 µm; ML: molecular layer.

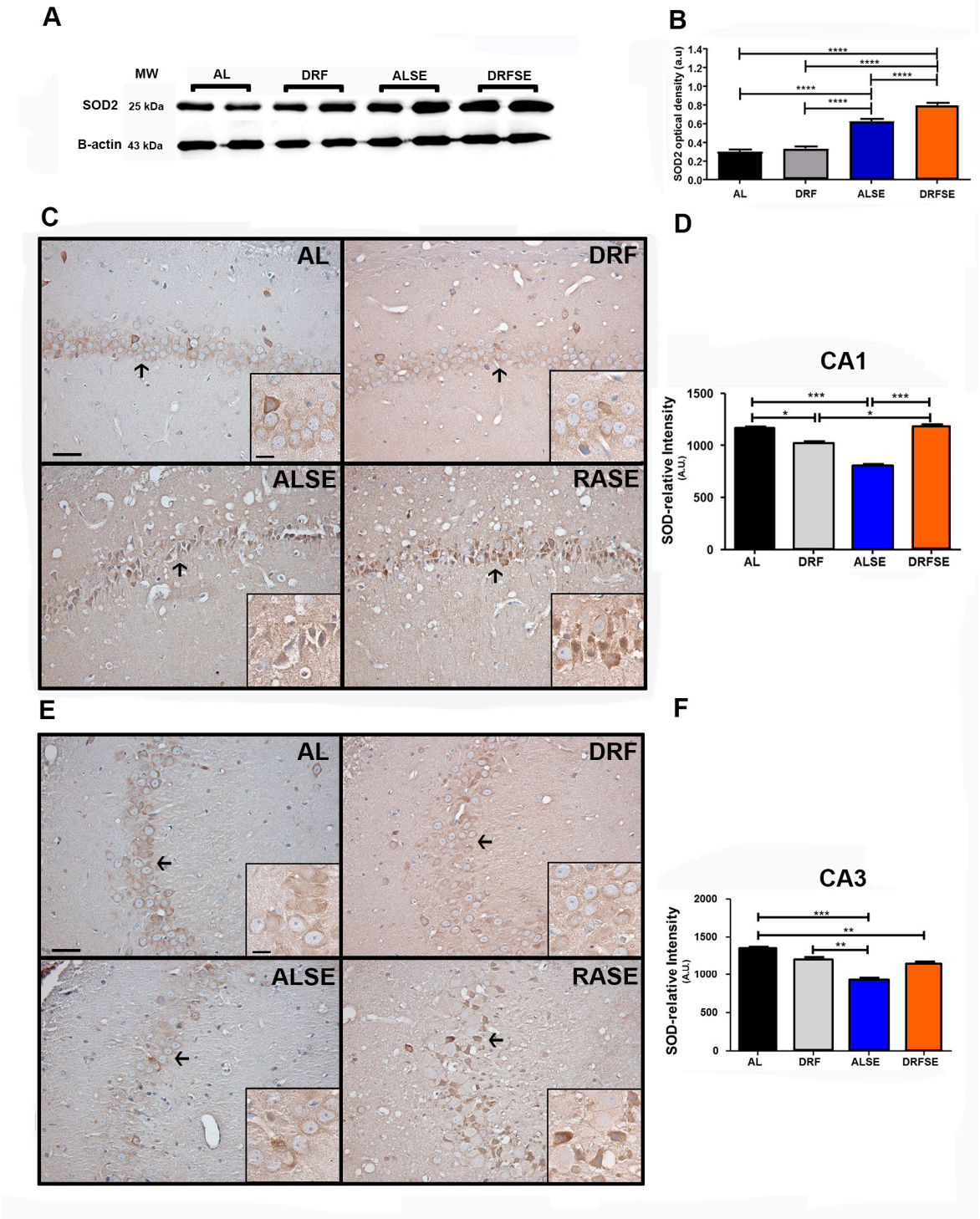

Figure 5. Effect of daytime-restricted feeding on the SOD2 protein content and the pyramidal distribution of SOD2 in the CA1 and CA3 subfields of the hippocampus after SE induction. Representative immunoblots of the SOD2 enzyme in the AL, DRF, ALSE and DRFSE experimental groups in the hippocampal homogenates (**A**). Quantification of the optical density of SOD2 protein contents in hippocampal homogenates (**B**). Representative photomicrograph of SOD2 immunoreactivity in the pyramidal cell layer of the CA1 subfield in all experimental groups (the arrows show the higher magnification image) (**C**) and the CA3 subfield (**E**). Quantification of SOD2 relative intensity in the CA1 (**D**) and CA3 subfields (**F**). Data are presented as the mean ± S.D, (n = 5 rats per group). One-way ANOVA followed by Tukey's multiple comparison test, * $p < 0.05$ ** $p < 0.01$, *** $p < 0.001$ **** $p < 0.0001$. Scale bars: 20 and 50 µm.

4. Discussion

Epilepsy is a global public health concern, and the development of new, effective pharmacological therapies has been limited. Therefore, addressing treatment efficacy is essential, especially for patients with drug-resistant epilepsy. Although many anticonvulsant drugs are available for epilepsy treatment, most target neurotransmitter systems or ion channels [5].

Oxidative stress in epilepsy results in cellular damage and the disruption of cellular function. Furthermore, it may cause cell death because neurons are particularly vulnerable to oxidant damage due to the high oxygen demand, poor repair capacity, and the presence of polyunsaturated fatty acids [18,19].

Several reports have shown that repeated seizures (SE) may induce peroxidation products due to the exacerbated production of reactive oxygen species. Likewise, numerous animal studies have demonstrated that antioxidants such as coenzyme Q10, vitamin C, N-acetyl-cysteine, and flavonoids reduce lipoperoxidation and restore the activities of different antioxidant enzymes, including superoxide dismutase, catalase, and glutathione [6].

In this study, we report that DRF ameliorates the oxidative stress induced by pilocarpine injection and that this antioxidant effect could be mediated by an increase in nuclear factor Nrf2 and its downstream protein SOD2. Our results indicate that daytime-restricted feeding can significantly reduce malondialdehyde levels in pilocarpine-injected rats (Figure 2). Furthermore, daytime-restricted feeding tends to decrease the production of superoxide radicals measured indirectly with DHE fluorescence (Figure 3). To our knowledge, this is the first report describing a potential antioxidant role for an intermittent fasting schedule such as daytime-restricted feeding in a pharmacological model of SE. However, another dietary intervention, a high-fat, low-carbohydrate diet (ketonic diet), has already been shown to display antioxidant potential in an epilepsy model [20,21].

Therefore, we focused on one of the main redox-sensitive transcription factors inducing antioxidant and detoxifying enzymes to protect cells against oxidative stress: nuclear factor erythroid 2-related factor 2 (Nrf2) [9]. Nrf2 mRNA levels are significantly upregulated in human epileptic hippocampal tissue and in the hippocampus of mice 72 h after pilocarpine injection, perhaps as an attempt to minimize the seizure-induced rise of free radicals [22]. In this regard, our results show that the seizures per se significantly increase the protein content of Nrf2 in hippocampal homogenates (Figure 4A,B). Moreover, Nrf2 immunostaining was also increased in the CA1 and CA3 hippocampal subfields (Figure 4C,E). These results are consistent with previous reports in which rats with electrically or pharmacologically induced epilepsy showed a substantial increase in Nrf2 mRNA levels and Nrf2 immunoreactivity [22–24]. Interestingly, recent data have shown that the activation of Nrf2 by different compounds can suppress mitochondrial oxidative stress, which mitigates seizure-induced damage [24–27]. In this regard, we hypothesized that daytime-restricted feeding could activate the Nrf2 nuclear factor. Our results indicate that daytime-restricted feeding induced a significant increase in Nrf2 protein content in the hippocampal homogenates in the pilocarpine-induced seizure group (Figure 4A). In agreement with this, the relative intensity of Nrf2 immunoreactivity in CA1 and CA3 pyramidal cells was mainly localized in the perinuclear compartment (Figure 4C,E, respectively). Importantly, the Nrf2 nuclear factor must translocate into the nucleus to bind to the antioxidant-responsive element (ARE) sequence in order to promote the transcription of downstream detoxifying enzymes [10]. In this regard, we observed a small part of the Nrf2 nuclear factor protein translocating into the nucleus in both the ALSE and the DRFSE group (Figure 4C), which correlated with Nrf2 immunostaining in some CA1 and CA3 pyramidal cell nuclei (Figure 4C,E, respectively). These results agree with previous work where kainic acid- or pentylenetetrazole-induced seizures activate an antioxidant enzyme regulated by Nrf2 [28].

Astrocytes have been widely recognized as the active partners of neurons because they modulate neuronal activity throughout the uptake and release of neurotransmitters [29]. Astrocytes also have an important role in epileptogenesis [30]. Furthermore, it is well

documented that after pilocarpine-induced status epilepticus, a population of astrocytes die while others are activated, promoting astrogliosis [31]. Thus, we hypothesize that astrocytes from animals subjected to DRF could contribute to the high Nrf2 expression to improve the oxidative stress produced by repeated seizures. Recently, Kim and colleagues showed that an analog of oleanolic acid induced Nfr2 expression in astrocytes in the CA1 region and prevented astrogliosis after SE induction. Accordingly, the increased colocalization of Nrf2 and the astrocyte marker in the CA1 and CA3 subfields (Figure 4C,E) suggests that daytime-restricted feeding could have a similar effect [31].

Recent evidence has shown the relationship between oxidative stress and mitochondrial dysfunction in epilepsy. As is known, mitochondria have several key cellular functions such as the generation of ATP, calcium homeostasis, neurotransmitter biosynthesis, and the control of cell death, and they are the primary site of reactive oxygen species (ROS) [32]. Experimental models of temporal lobe epilepsy have shown an increase in ROS levels [33,34]. Mitochondrial superoxide dismutase 2 (SOD2) is a major component of the antioxidative machinery that handles ROS in the mitochondrial matrix because it determines how much superoxide radical anion ($O_2^{\bullet -}$) is converted to hydrogen peroxide (H_2O_2) [35]. In this regard, Liang and colleagues showed that postnatal mutant mice lacking SOD2 exhibited frequent spontaneous motor seizures, providing evidence that oxidative stress-induced mitochondrial dysfunction may contribute to epileptic seizures [36]. Furthermore, it has been recently shown that specific neuronal deletion nSOD2 knockout mice develop epilepsy together with a selective loss of neurons [37]. According to our results, we observed that the SOD2 protein content increases in the hippocampal homogenates after pilocarpine-induced seizures (Figure 5A). Interestingly, DRF was able to further increase the protein content of SOD2, perhaps as an attempt to minimize the rise in ROS levels (Figure 5A). Unexpectedly, when we performed the immunohistochemistry technique to observe the cellular distribution of SOD2 protein in the pyramidal cell layer of the CA1 and CA3 subfields, we found that pilocarpine-induced seizures significantly reduced the immunoreactivity of SOD2 in both hippocampal regions (Figure 5C,E, respectively). These results could correlate with the increased levels of superoxide ion and hydrogen peroxide measured indirectly with the fluorescence of dihydroethidium (DHE) (Figure 3). Notably, DRF was able to recover the immunoreactivity of SOD2 in the pyramidal cell layer of the CA1 region (Figure 5C,D) and correlate with the reduction in the relative intensity of DHE in the same region (Figure 3A,B). Similar results were observed in the pyramidal cell layer of the CA3 region, where a pilocarpine-induced seizure decreased SOD2 immunoreactivity in neurons; however, DRF could not recover the basal levels of SOD2 after seizure induction (Figure 5E,F). These results show the crucial role of mitochondrial SOD in controlling the conversion of superoxide ion to hydrogen peroxide produced by seizures, and, most importantly, they show that DRF could downregulate the ROS levels by increasing antioxidant enzymes such as SOD2.

5. Conclusions

Our results suggest that daytime-restricted feeding reduces oxidative stress in the acute seizure model. This antioxidant effect could be partially mediated by the activation of the Nrf2 transcriptional factor, and the upregulation of enzymes involved in antioxidant cellular defense, such as SOD2 protein. Since ketone bodies like beta-hydroxybutyrate are produced in daytime-restricted feeding or the ketogenic diet [12,13], we cannot exclude the participation of other oxidative-stress resistance factors such as FOXO3A and metallothionein 2 A [38]. However, our data strongly support the idea that the Nrf2 protein could play a vital role in regulating oxidative stress through antioxidant enzymes such as SOD2.

In addition, we suggest the possible participation of astrocytes in modulating the expression of the Nrf2 nuclear factor to counteract the damage caused by seizure-induced oxidative stress. Our results support a possible use of DRF as an adjuvant treatment for drug-resistant epileptic patients that could improve the neurodegeneration observed in epilepsy.

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Data Availability Statement: The data generated in the present study may be requested from the corresponding author.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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Article

Dehydroepiandrosterone Attenuates Astroglial Activation, Neuronal Loss and Dendritic Degeneration in Iron-Induced Post-Traumatic Epilepsy

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Abstract: Iron-induced experimental epilepsy in rodents reproduces features of post-traumatic epilepsy (PTE) in humans. The neural network of the brain seems to be highly affected during the course of epileptogenesis and determines the occurrence of sudden and recurrent seizures. The aim of the current study was to evaluate astroglial and neuronal response as well as dendritic arborization, and the spine density of pyramidal neurons in the cortex and hippocampus of epileptic rats. We also evaluated the effect of exogenous administration of a neuroactive steroid, dehydroepiandrosterone (DHEA), in epileptic rats. To induce epilepsy, male Wistar rats were given an intracortical injection of 100 mM solution (5 µL) of iron chloride ($FeCl_3$). After 20 days, DHEA was administered intraperitoneally for 21 consecutive days. Results showed epileptic seizures and hippocampal Mossy Fibers (MFs) sprouting in epileptic rats, while DHEA treatment significantly reduced the MFs' sprouting. Astroglial activation and neuronal loss were subdued in rats that received DHEA compared to epileptic rats. Dendritic arborization and spine density of pyramidal neurons was diminished in epileptic rats, while DHEA treatment partially restored their normal morphology in the cortex and hippocampus regions of the brain. Overall, these findings suggest that DHEA's antiepileptic effects may contribute to alleviating astroglial activation and neuronal loss along with enhancing dendritic arborization and spine density in PTE.

Keywords: epilepsy; seizures; dehydroepiandrosterone; neuronal loss; astroglial activation; dendritic degeneration

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1. Introduction

Epilepsy is a common neurological disorder affecting about 65 million people worldwide [1]. The major characteristics of this neurological disorder are the onset of sudden and recurrent seizures resulting from changes in neuronal hyperexcitability and electrical discharge [2]. It has been estimated that among the total number of acquired epilepsy cases, 20% are of post-traumatic epilepsy (PTE) that occurs after a brain trauma [3]. The studies on human patients and animal models demonstrated that epileptogenesis involves a plethora of cellular and molecular changes, viz., alteration of brain circuitry, changes in the levels of neurotransmitters and receptors, aberrant gene expression, neurogenesis, and neuronal loss [4,5]. The mechanism of epileptogenesis in PTE is complex. It seems to be linked with multiple pathophysiological changes that can alter the balance between neuronal excitation–inhibition, making the brain more susceptible to producing recurrent and spontaneous seizures [6].

Seizures induced by intracortical injection of iron chloride ($FeCl_3$) in rodents reproduce characteristics of PTE in humans, resulting from severe head trauma [7]. This model has been characterized regarding electrophysiological, neurobehavioral, morphological,

biochemical, and molecular aspects. The model has also been extensively used to investigate novel therapeutic strategies for PTE [8–12].

Compelling evidence suggests that Mossy Fibers (MFs) sprouting, inflammation, astroglial activation, and neuronal degeneration are interesting disease-modifying targets of epilepsy [13,14]. The activation of glial cells is recognized to have a critical role in the onset and progression of epilepsy [15–17]. The activation and proliferation of astrocytes and microglia produce inflammatory cytokines that can influence neuronal hyperexcitability and degeneration [18,19]. Besides this, cognitive and behavioral impairments in epilepsy are the consequences of disruption of the neuronal network [20]. Thus, their attenuation can provide a strong rationale for investigating novel therapeutic agents with long-term anti-seizure potential.

Dendritic arbors and spines are important morphological entities of excitatory synaptic neurons, and changes in these entities are implicated in various neurological disorders. Particularly, any changes in dendritic arbors and spines have been reported to directly affect epileptogenesis and seizure onset [21]. Emerging evidence from animal models and human specimen suggest that dendritic pathologies such as abnormal arbors and loss of spines are reported in multiple forms of epilepsy [22–24]. Moreover, investigations conducted on patients with seizures, such as cardinal symptom, in pathological conditions like hippocampal sclerosis, tumors, and microdysgenesis, have also reported dendritic degeneration and spines loss [25,26]. However, the causes or consequences of PTE, especially in $FeCl_3$-induced experimental epilepsy, have not been determined till now.

Most currently available anti-seizure medications (ASMs) provide only symptomatic suppression of seizures without modifying the pathophysiology of epilepsy. The long-term use of these ASMs can also exert several adverse effects [27]. Hence, drugs with neuroprotective and disease-modifying properties are of particular interest since they may exert antiepileptic effects, reduce disease severity, and attenuate associated comorbidities. Research focusing on the therapeutic modulation of physiological and molecular changes can provide novel strategies with an effect beyond seizure suppression [28]. The development of efficient alternative therapeutics that might lessen the harmful changes occurring throughout the epileptogenic process is, therefore, urgently needed [29].

Dehydroepiandrosterone (DHEA) is an important circulating steroid hormone, synthesized novo by the adrenals, gonads, and brain. This steroid interacts with various receptors of neural growth factors and consequently exerts neuroprotective effects [30]. Evidence suggests that DHEA benefits various neurological disorders, including epilepsy [31]. Our earlier research revealed that DHEA's antiepileptic potential is accompanied by its antioxidative, antiapoptotic, and voltage-gated ion channels [8,9] modulatory properties. In addition, DHEA has been proven to diminish inflammation caused by microglia in mouse models of neuroinflammation and in vitro cultures of microglia [32], suggesting its anti-inflammatory potential. Given the above-mentioned characteristics of DHEA antiepileptic and disease-modifying effect of exogenous DHEA, treatment for 21 days was investigated by evaluating astroglial activation, neuronal loss, and dendritic morphology in the cortex and hippocampus of a rat model of experimental PTE.

2. Materials and Methods

2.1. Animals

Male Wistar rats (weighing 220–250 g) used for the experimentation were procured from Central Laboratory Animal Resources, Jawaharlal Nehru University, New Delhi, India. Throughout the experiment, the animals were housed in a pathogen-free standard environment (22 $\pm$ 2 °C) with a 12-h light/dark cycle at 50–60% humidity and had free access to food and water. All experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Jawaharlal Nehru University, New Delhi. Every effort was made to minimize the number of rats, their pain, and suffering.

2.2. Epilepsy Model

The experimental model of PTE was developed by administering $FeCl_3$ intracortically to rats as per the procedure described in previous reports [8,9]. Stereotaxic surgery was performed under an aseptic condition in a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA) using 4% volatile isoflurane (Baxter, Deerfield, MA, USA) as an anesthetic. An incision was made along the scalp midline, and peri-cranial muscles and fascia were gently removed to expose the skull. Then, a burr hole of 0.5 mm diameter was drilled for $FeCl_3$ injection (AP-1, ML-1, DV-1.5 mm). A total of 5 µL of 100 mM $FeCl_3$ solution (prepared in normal saline) was injected with the help of an injector cannula over 5 min (at a rate of 1 µL/min) via a microsyringe (Hamilton Company, Reno, NV, USA) fixed in a micro syringe pump controller (Stoelting, Wood Dale, IL, USA). Post injection, the cannula was ejected, and the burr hole was sealed with sterile bone wax. In the cortical area, four stainless steel surface electrodes were stereotaxically implanted (AP + 2 and −2, ML + 2 and −2, DV −1.5 mm from bregma). Moreover, at the CA1 area of the hippocampus, one intracerebral bipolar wire electrode was placed (AP-2.8, ML-2.5, DV-2.71 mm from bregma). Finally, the incision was stitched and Nebasulf® sprinkling powder (Pfizer Ltd., Bangalore, Karnataka, India) was applied to the surgical site for a few days to avoid infection.

2.3. Experimental Design

A total of 18 rats were randomly assigned to three experimental groups. (1) Control: (n = 6) rats received an intracortical saline injection as $FeCl_3$ solvent. (2) Epileptic: (n = 6) rats received an intracortical injection of $FeCl_3$ solution as described above. (3) Epileptic + DHEA: (n = 6) rats received an intracortical injection of $FeCl_3$ solution and DHEA treatment for 21 consecutive days.

After 20 days of $FeCl_3$ injection, DHEA, solubilized in 0.1% dimethylsulphoxide (Sigma Aldrich, St. Louis, MO, USA), was injected intraperitoneally (30 mg/kg b. wt.) for the next 21 days (Figure 1). DHEA dose and duration used in this study are consistent with our laboratory's earlier publications indicating its antiepileptic effect at 7, 14, and 21 days of treatment [8,33]. As 21 days of DHEA administration showed a stronger antiepileptic effect, the same was chosen.

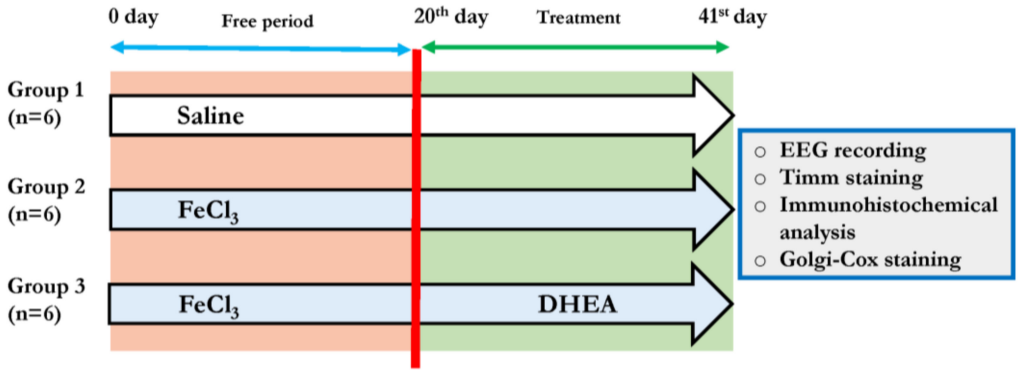

Figure 1. Schematic illustration of the treatment paradigm and assay parameters of the study.

2.4. EEG Recordings and Analysis

Electroencephalography (EEG) video recordings were used to measure epileptiform seizures in experimental rats. All rats were accustomed to the recording equipment and chamber for three days prior to the recordings beginning. The recordings were taken during the light phase, and animals were given at least 5 min to settle down. The EEG signals were filtered through 1 Hz to 100 Hz bandpass, amplified by an amplifier (P511 AC preamplifiers), and recorded using PolyVIEW 16 Data Acquisition System (Grass Technologies, West Warwick, RI, USA).

2.5. Tissue Preparation for Histopathology and Immunofluorescence Analysis

At the end of the treatment period, rats (n = 3) were anesthetized with ketamine: a xylazine mixture (100 mg/kg: 10 mg/kg) and transcardially perfused with saline (0.9% NaCl) and 2% paraformaldehyde (PFA). Intact brains were dissected out and post-fixed in 2% PFA overnight. Then, dehydrated by passing through 10, 20, and 30% of sucrose solutions. Coronal sections (10 µm thickness) of the brain were cut through the dorsal part of the hippocampus in a cryostat (Leica CM 1860, Leica Biosystems, Nussloch GmbH. Heidelberger Str., Germany) and collected on gelatin-coated slides for further analysis.

2.6. Timm Staining

Tissue sections were air dried, followed by incubation in Timm's working solution, consisting of 50% Arabic gum (120 mL), 2 M citrate buffer (20 mL), 0.5 M hydroquinone (60 mL), and 17% silver nitrate (1 mL) for 60 min in the dark. Following washes, sections underwent graded alcohol dehydration and xylene clearing. After that, sections were cover-slipped with DPX mounting media (Fisher Scientific, Mumbai, India), left to dry overnight, and photographed using a light microscope (Motic Instruments Co. Ltd., Chengdu, China). Timm staining intensity was quantified using densitometric analysis in the hippocampus (DG region) of the brain using FIJI software (Imagej 1.53t, NIH, Bethesda, MD, USA; http://fiji.sc/fiji accessed on 5 January 2022). The area and white background were used to normalize the grayscale staining's mean density. Then, using a linear scale, density ratings were nonparametrically graded.

2.7. Immunofluorescence Staining

The brain sections were washed in PBS and then treated with 5% Triton-X100 for 10 min. The non-specific antigens were then blocked with 3% normal goat serum (Abcam, Cambridge, UK). Subsequently, sections were covered with primary antibodies, such as mouse anti-GFAP (1:100, Invitrogen, Carlsbad, CA, USA) and rabbit anti-NeuN (1:100, Cell Signaling Technology, Danvers, MA, USA), and placed at 4 °C overnight. After three PBS washes, sections were incubated at room temperature with Alexa Fluor 488-conjugated goat anti-mouse (1:200, Invitrogen, Carlsbad, CA, USA) and Alexa Fluor 594-conjugated goat anti-rabbit (1:200, Invitrogen, Carlsbad, CA, USA secondary antibodies. Sections were washed again with PBS, and the nucleus was stained with 4′,6-diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, MO, USA). Next, sections were covered with Fluoromount^{TM} Aqueous Mounting Media (Sigma, St. Louis, MO, USA). Finally, images were taken under a fluorescent microscope (Nikon Eclipse 90iT, Tokyo, Japan).

2.8. Golgi-Cox Staining

Rats (n = 3) were perfused as described above. Isolated brains were rinsed with PBS and separated into two equal hemispheres. The staining was done according to the previously described procedure of Zhong et al. [34]. Briefly, 10 mL of impregnation solution was poured over the brain tissue and incubated for two days. Then, the solution was exchanged and incubated for the next two weeks. After impregnation, the excess solution was removed and wiped with tissue paper. Then, the tissue was transferred to 15 mL of chilled cryoprotectant solution with gentle shaking and stored at 4 °C. Next, the cryoprotectant solution was replaced after 24 h and again stored at 4 °C until the tissue sank into the bottom. After that, coronal sections measuring 80 µm thickness were cut using a cryostat and collected on slides coated with gelatin. Sections were then washed twice in distilled water for two min each, followed by a 10 min of soak in 20% ammonia solution in darkness. Then, sections were washed with distilled water, treated for 5 min with 1% sodium thiosulfate, and washed with distilled water again. Subsequently, sections were cleared with xylene after being dehydrated with graded alcohol solutions (50, 75, 95, and 100%). Finally, sections were cover-slipped with the aid of DPX and allowed to dry. Individual pyramidal neurons were imaged using a light microscope (Motic Instruments Co. Ltd., Chengdu, China) at 40× for arborization analysis and 60× for dendritic spines.

2.9. Analysis of Dendritic Morphology and Spine Density

A total of 10 pyramidal neurons from both cortex and hippocampus of each animal (n = 3) were captured under 40× objective and traced by the Simple Neurite Tracer (SNT) feature of Fiji software (https://imagej.net/plugins/simple-neurite-tracer/ accesed on 5 January 2022). The concentric ring method of Sholl [35] was used to analyze the branching pattern and complexity of dendrites. The number of dendritic intersections at each concentric ring from the center of the soma up to 200 µm of radial distance was used to calculate the complexity of neurons.

A total of 20 primary and secondary branches from the apical dendrites of five randomly chosen pyramidal neurons were photographed using a 60× objective lens to calculate spine density. Dendritic spines were counted using Fiji software. Primary and secondary dendritic branches chosen were 2 to 3 µm thick, at least 10 µm long, and focused on a single plane. Spines with two heads were counted as two spines. Two researchers unaware of group identities conducted individual counts, and the average results are represented as the number of spines/10 µm of dendritic length [36].

2.10. Statistical Analysis

Data were analyzed using one-way Analysis of Variance (ANOVA) and two-way ANOVA (for Sholl analysis) with Holm-Sidak post hoc test. Statistical analyses were conducted using SigmaStat 3.5 software (Systat Software Inc., San Jose, CA, USA). A probability value ≤ 0.05 was considered statistically significant.

3. Results

3.1. DHEA Treatment Alleviates Epileptiform Seizures in Epileptic Rats

EEG recordings from each group were examined to measure epileptiform seizure activity. EEG recordings from epileptic rats showed increased seizure activity in their cortex and hippocampus. As illustrated in Figure 2A, epileptic seizures can be easily distinguished in 20 s EEG stretches because of their distinct single spikes, polyspikes, and spike waves. DHEA treatment for 21 days significantly reduced spike waves, single spikes, and polyspikes associated with epileptiform activity (Figure 2A).

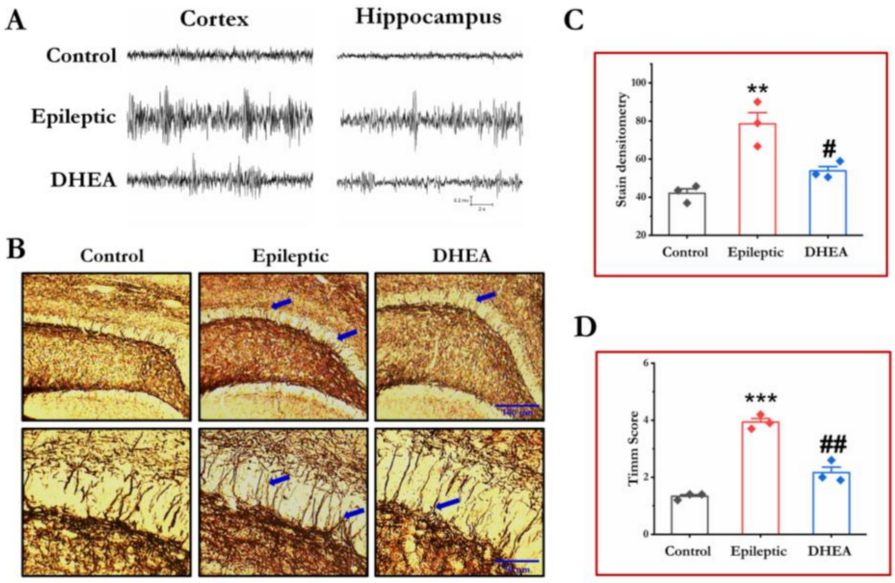

Figure 2. Representative EEG samples of 20 s duration from the cortex and hippocampus of control, epileptic, and DHEA-treated rats (**A**). Timm's stating images showing Mossy Fibers (indicated by arrows) in the dentate gyrus (DG) region of rats (**B**). Quantitative data analysis indicates lower stain density (**C**) and Timm's score (**D**) in DHEA-treated rats with respect to epileptic rats. ** $p \leq 0.01$, *** $p \leq 0.001$, significantly different from controls; # $p \leq 0.05$, ## $p \leq 0.01$, significantly different from epileptic rats. ANOVA F values for stain densitometry: 17.659 and Timm's score: 24.035.

3.2. DHEA Treatment Reduces MFs Sprouting in Epileptic Rats

Timm's staining was used to determine MFs sprouting in the DG region of the hippocampus. Stain intensity and Timm's score were calculated to assess the severity of MFs sprouting. We discovered that control rats showed no evidence of MFs sprouting, whereas epileptic rats had a clear band of Timm staining in the DG's molecular layer. DHEA-treated rats had significantly lower MFs sprouting than epileptic rats. Densitometric analysis of axonal sprouting and Timm's score confirmed that DHEA treatment could have significantly reduced the severity of MFs sprouting (Figure 2B–D).

3.3. DHEA Treatment Attenuates Activation of Astrocytes

GFAP is a well-known marker of reactive astrocytes, and we investigated the effect of DHEA on astroglial activation by evaluating its immunoreactivity in the experimental rats. Control rats showed a lower percentage of GFAP-positive cells in the cortex and hippocampus, and the cells resembled resting astroglia. In contrast, epileptic rats showed a higher percentage of GFAP-positive cells, morphologically similar to reactive astrocytes. The percentage of GFAP-positive cells was dramatically decreased in epileptic rats treated with DHEA. These findings imply a considerable diminution of activated astrocytes in the epileptic rats treated with DHEA (Figure 3).

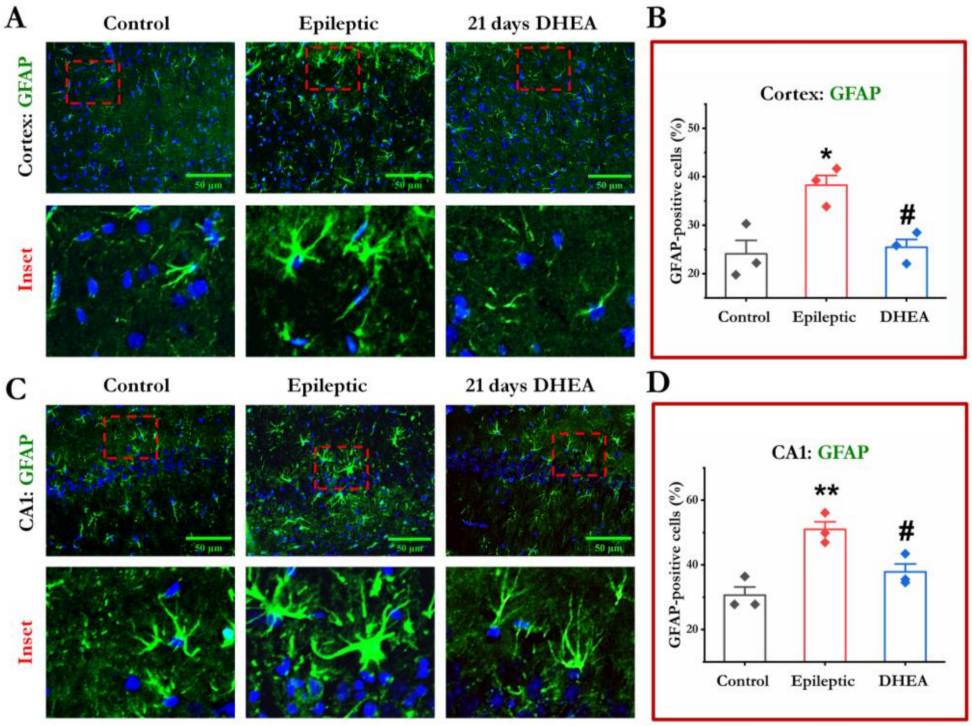

Figure 3. Immunofluorescence analysis of reactive astrocytes in the cortex and hippocampus regions of control, epileptic and DHEA-treated rats. Representative photomicrographs showing GFAP-positive cells (green) (**A**,**C**). Percentage of GFAP-positive cells in the cortex (**B**) and hippocampus (**D**) of experimental rats. Data are expressed as mean ± SD (n = 3 in each group). * $p \leq 0.05$, ** $p \leq 0.01$, significantly different from control group; # $p \leq 0.05$, significantly different from epileptic group. ANOVA F values for percentage of GFAP-positive cells: cortex 8.356; hippocampus 13.558.

3.4. DHEA Treatment Protects Loss of Neurons

To assess DHEA's neuroprotective effect, NeuN immunoreactivity was measured in the cortex and hippocampus of experimental rats. Control rats showed a higher percentage of NeuN-positive cells in the cortex and the hippocampus. The epileptic rats showed a significant decrease in NeuN-positive neurons in both regions of the brain. Interestingly, compared to epileptic animals, DHEA injection dramatically increased the percentage of

NeuN-positive cells. There was no significant difference in any brain regions between the control and DHEA-treated rats (Figure 4).

3.5. DHEA Treatment Rescues Dendritic Arborization

Dendritic arborization of pyramidal neurons seemed to be affected during the course of epileptogenesis. Morphological analysis of cortical and hippocampal CA1 pyramidal neurons in control rats showed long and extensively arborized dendrites. We discovered a considerable reduction in dendritic arbors, as well as disorientation of apical and basal dendrites of pyramidal neurons in epileptic rats. In contrast, DHEA treatment in epileptic rats significantly elevated dendritic arborization in both brain regions, suggesting the positive impact of DHEA in preventing impaired dendritic arborization in PTE (Figure 5).

3.6. DHEA Restores Dendritic Spines

Spine density analysis in apical dendrites of cortical and hippocampal pyramidal neurons showed a major population of mushroom-shaped spines in control rats. Further, the results revealed a considerably lower number of dendritic spines in epileptic rats both in the cortex and hippocampus regions. On the other hand, epileptic rats treated with DHEA showed a considerably higher number of dendritic spines in the apical branches of cortical and hippocampal pyramidal neurons (Figure 6).

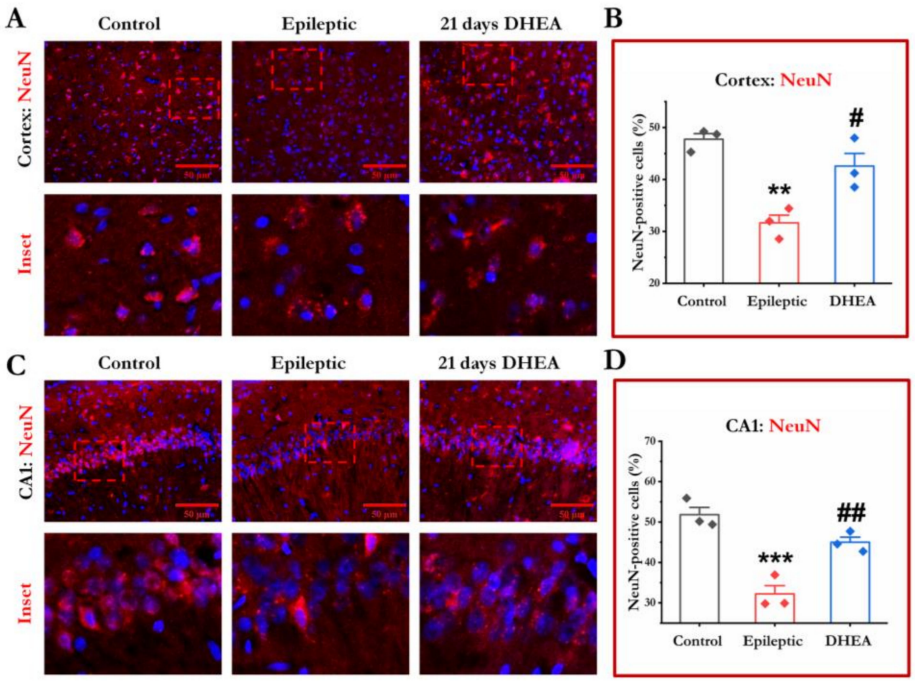

Figure 4. Immunofluorescence analysis of neuronal loss in the cortex and hippocampus regions of control, epileptic and DHEA-treated rats. Representative photomicrographs showing NeuN-positive cells (red) (**A**,**C**). Percentage of NeuN-positive cells in the cortex (**B**) and hippocampus (**D**) of experimental rats. Data are expressed as mean ± SD (n = 3 in each group). ** $p \leq 0.01$, *** $p \leq 0.001$ significantly different from control group; # $p \leq 0.05$, ## $p \leq 0.01$ significantly different from epileptic group. ANOVA F values for percentage of NeuN-positive cells: cortex 16.623; hippocampus 25.107.

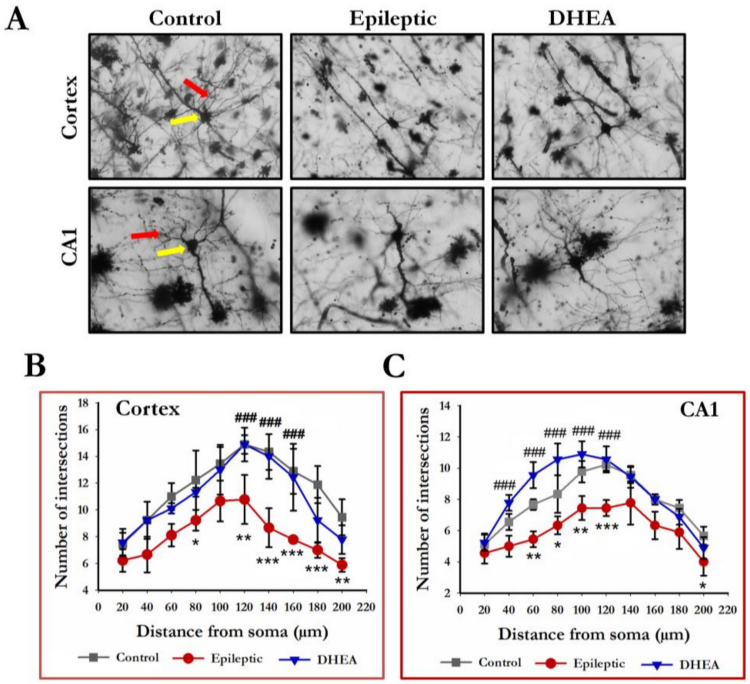

Figure 5. Dendritic arborization in the cortex and hippocampus of control, epileptic and DHEA-treated rats. Representative Golgi-Cox-stained neurons photomicrographs from the cortex and hippocampus captured under 40× magnification showing dendrites (red arrows) and soma (yellow arrows) (**A**). Quantification of dendritic arborization by evaluating the total number of intersections at each concentric ring away from the soma in the cortex (**B**) and hippocampus (**C**) of experimental rats. Data are expressed as mean ± SD (n = 3 in each group). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ significantly different from control group; ### $p \leq 0.001$ significantly different from epileptic group. ANOVA F values for the number of intersections: cortex 47.897; hippocampus 49.751.

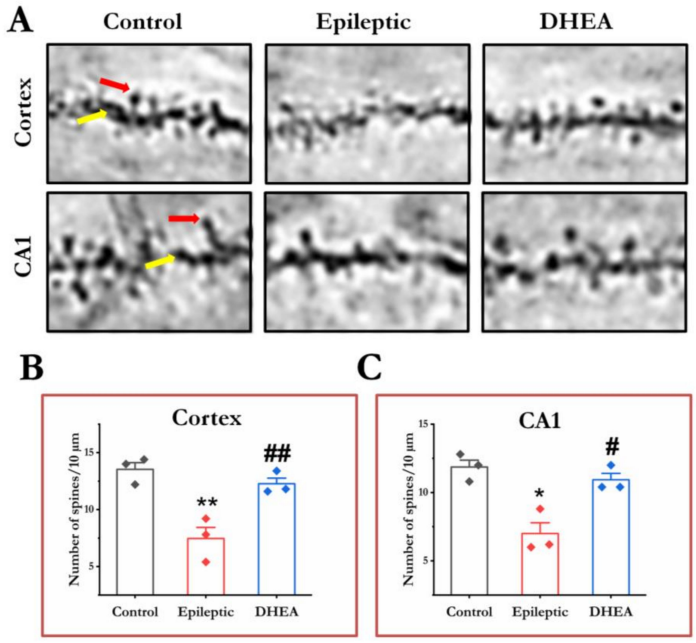

Figure 6. Morphological analysis of dendritic spines in the cortex and hippocampus of control, epileptic and DHEA-treated rats. Representative photomicrographs of Golgi-Cox-stained neurons from

the cortex and hippocampus captured under 60× magnification showing apical dendrites (yellow arrows) and spines (red arrows) (**A**). Average number of dendritic spines in pyramidal neurons of the cortex (**B**) and hippocampus (**C**) of experimental rats. Data are expressed as mean ± SD (n = 3 in each group). * $p \leq 0.05$, ** $p \leq 0.01$, significantly different from control group; # $p \leq 0.05$, ## $p \leq 0.01$ significantly different from epileptic group. ANOVA F values for several dendritic spines: cortex 15.260; hippocampus 12.070.

4. Discussion

Available evidence from clinical and pre-clinical studies indicates that epileptogenesis is a multifactorial process. In the case of PTE, seizures occur following head trauma. The possible pathophysiological mechanisms of PTE have been investigated in rodent models produced by intracortical $FeCl_3$ injection [7]. Several biochemical and molecular changes seem to be involved in the development of epilepsy after $FeCl_3$ injection, where cellular oxidative damage, apoptosis [8,37], levels of neurotransmitters and receptors [33], and voltage-gated ion channels [9,10,12] have major roles in the exacerbated excitability of neurons. Previous studies from our laboratory investigated the antiepileptic effects of DHEA along with its antioxidative and neuromodulatory role in PTE [8,9,33]. However, its effect on astroglial activation and dendritic degeneration remains unexplored.

The findings from this study indicate that the negative effects of $FeCl_3$-induced experimental PTE were reversed by exogenous treatment with DHEA. The results demonstrated that epileptic episodes in $FeCl_3$-injected rats were significantly reduced after 21 days of DHEA administration. DHEA treatment decreased the frequency and amplitude of high-amplitude of EEG events, which may have improved epileptiform seizures. The MFs are axons of dentate granule cells that, under normal conditions, create synapses with pyramidal cells of the hippocampus. In the epileptic brain, these axons lose their connecting targets, innervate the inner molecular layer of DG, and cause epileptogenesis via the formation of recurrent excitatory circuits [38]. Researches on both humans and animals suggest that the sprouting of MFs is one of the most common neuropathological features of epilepsy. In the present study, we discovered MFs' sprouting in the DG of rats with $FeCl_3$ injection. These consequences were ameliorated by DHEA treatment for 21 days, showing its antiepileptic potential, and are consistent with other research [8,9,33,37]. Suggesting the antiepileptic effect of DHEA, as evidenced by a considerable decrease of epileptic episodes in electrophysiological investigations.

The mechanism of epileptogenesis seems to be associated with the activation of glial cells, evident from the increased expression of astrocytic and microglial markers in experimental epilepsy [16,39]. According to a study by Lee et al. [40], astrogliosis is linked with glutamate overexpression in hippocampal sclerosis, which raises neuronal excitability and causes seizures. Moreover, the reactivation of astrocytes results in the rapid depletion of GABAergic neurons' synapses and cases of hyperexcitability of the hippocampal circuitry [41]. Hence, the possible mechanism by which reactive glial cells promote epileptogenesis seems modulated by neuronal excitability and inflammation [19]. A growing body of evidence from earlier studies provides insight into the importance of astroglial activation in epilepsy [19,42]. The current investigation reported significantly higher number of GFAP-positive cells in the cortex and hippocampus of epileptic rats. These results are consistent with previous reports [16,39] and indicate activation of astrocytes in both regions of the brain. Next, we evaluated the effectiveness of DHEA on astroglial activation and found that DHEA effectively reduced astrogliosis. This evidence supports the notion that neuroactive compounds, such as DHEA, can attenuate the process of astroglial activation in an experimental model of PTE.

PTE has also been reported to exhibit degeneration and death of cortical and hippocampal neurons [8]. Some studies reported that developing and matured neurons degenerate in patients and animal models of experimental epilepsy [43,44]. In this study, we observed that $FeCl_3$-induced epilepsy is linked with significant neuronal loss in the cortex and hippocampus regions, as evident from the lower number of NeuN-positive

cells in both regions. These findings agree with previous studies that show the extent of neuronal loss in different epilepsy models [8,45,46]. DHEA has been reported to act as a neurotropic or neuroprotective factor under different pathological conditions, including epilepsy [31]. Indeed, we found that DHEA administration results in an increased number of NeuN-positive neurons in both regions of the brain, which suggests the neuroprotective effect of DHEA in PTE. These findings are consistent with our prior report, indicating the neuroprotective effect of DHEA in $FeCl_3$-induced epilepsy through rescuing degenerative neurons and reducing apoptotic cell death [8].

The arborization of dendrites defines their connectivity and is pivotal for integrating information and synaptic plasticity. Numerous human and animal studies demonstrated structural abnormalities in dendrites that could contribute to neuronal dysfunction, cognitive and behavioral deficits, and epileptogenesis [21,47,48]. The degeneration of dendrites has been recognized as a common feature of epileptic tissues in both humans and animals [21,49]. The stabilization of dendritic structure could become a future therapeutic strategy for epilepsy. We examined the morphological changes of neurons using Golgi-Cox staining and found fewer arborized neurons in the cortex and hippocampus of epileptic rats. A line of studies performed on multiple forms of epilepsy reported abnormal morphological changes, viz. reduced dendrite arborization and length [14,22,24]. Interestingly, exogenous treatment of DHEA alleviated dendritic retraction caused in epileptic rats. Further, we observed that DHEA treatment rescued dendritic arborization, although some dendritic retraction persisted. Similarly, DHEA has also shown improved neuronal plasticity and facilitation of dendritic development in middle-aged rats exposed to chronic mild stress [50]. Overall, these results indicate that the antiepileptic potential of DHEA may also be accompanied by improved dendritic arbors leading to increased surface area and connectivity of dendrites.

The structural remodeling of synapses can be analyzed by counting the dendritic spines. Previously, various reports demonstrated significantly decreased dendritic spines in the pyramidal neurons of epilepsy patients and animal models [14,21–24]. These findings strongly suggest that abnormalities in dendritic spines can also play an important role in the pathophysiology of epilepsy. We also observed significantly decreased spine density in the cortex and hippocampus of epileptic rats, which is consistent with other results and suggests a correlation between dendritic spine abnormalities and seizures in PTE. However, DHEA treatment reduced the density of dendritic spines in both regions of the brain, thus, providing evidence that DHEA's antiepileptic effects may also be influenced by the recovery of dendritic spine density.

5. Conclusions

In conclusion, our results exhibit that DHEA possesses definite and substantial neuroprotective effects on the epileptic brain. The treatment of DHEA may have decreased the sprouting of MFs in the hippocampus, suggestive of a reduced incidence of seizures in PTE. Further, the steroid may have escorted an anti-seizure effect by ameliorating astroglial activation, neuronal loss, and dendritic degeneration in the cortex and hippocampus regions of the brain in PTE.

Author Contributions: C.P. and D.S. participated in the study concept. C.P. conducted the immunofluorescence assay and analysis. C.P., S.S.R. and J.T. contributed to Timm's and Golgi-Cox staining assays and analyses. C.P. and S.S.R. drafted the manuscript. D.S. provided critical revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The use of rats for the experiments was duly approved by the Institutional Animal Ethics Committee (IAEC) of Jawaharlal Nehru University, New Delhi, India (IAEC approval: 19/2016).

Informed Consent Statement: Not applicable.

Data Availability Statement: All the relevant data generated and analyzed during the current study are available upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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Review

A Comprehensive Review on Anti-Inflammatory Response of Flavonoids in Experimentally-Induced Epileptic Seizures

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Abstract: Flavonoids, a group of natural compounds with phenolic structure, are becoming popular as alternative medicines obtained from plants. These compounds are reported to have various pharmacological properties, including attenuation of inflammatory responses in multiple health issues. Epilepsy is a disorder of the central nervous system implicated with the activation of the inflammatory cascade in the brain. The aim of the present study was to summarize the role of various neuroinflammatory mediators in the onset and progression of epilepsy, and, thereafter, to discuss the flavonoids and their classes, including their biological properties. Further, we highlighted the modulation of anti-inflammatory responses achieved by these substances in different forms of epilepsy, as evident from preclinical studies executed on multiple epilepsy models. Overall, the review summarizes the available evidence of the anti-inflammatory potential of various flavonoids in epilepsy.

Keywords: epilepsy; seizures; inflammation; anti-inflammatory response; flavonoids

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1. Introduction

Epilepsy is an enduring brain disorder characterized by the prevalence of spontaneous and recurrent seizures (SRS). There are numerous types of epilepsies, some of which are influenced by genetic predispositions, some of which are brought on by brain damage, and some of which have an unknown underlying cause [1]. Epileptic seizures are the consequences of an unsynchronized and excessive electrical activity of a group of neurons. The spontaneous electrical discharges during seizures jeopardize the normal brain's electrical activity and can result in odd sensations or involuntary motions of various body parts [2]. People with epilepsy have a variety of psychological and social difficulties in daily life, including anxiety, sadness, sleep problems, emergency seizures, problems with thinking and memory, a lack of self-confidence, and poor social skills [3,4]. Recent estimates suggest that epilepsy affects almost 70 million individuals globally. The prevalence of epilepsy affects people worldwide, where more than 80% of cases are from low and middle-income countries [5].

The etiology of epilepsy seems to be influenced by several factors, including aging, genetic mutation, central nervous system (CNS) homeostasis, and brain insults, viz. oxidative stress, inflammation, and traumatic brain injury [6]. However, the pathophysiological mechanisms responsible for the onset and recurrence of epileptic seizures, pathological changes, and associated comorbidities remain largely unknown. The dynamic and neurophysiological changes of the brain accountable for ictogenesis can be studied in animal models of genetic and acquired epilepsy [7,8]. The neuroinflammatory pathways have been linked

with epileptogenesis and may be the target of disease-modifying therapies [9,10]. Moreover, evidence from human studies has demonstrated the involvement of neuroinflammation in the inception and development of various forms of epilepsy [11]. Thus, the evidence suggests that inflammation within the brain is intricated with the recurrence and precipitation of seizures. Moreover, repetitive seizures have also been reported to induce inflammatory mediators, which may increase brain excitability and neuronal degeneration [12,13].

Epilepsy can sometimes be efficiently treated and cured with the anti-seizure medications (ASMs) already on the market. However, these medications also have at least two significant drawbacks: First, 30% of patients have inadequate seizure control and develop intractable conditions even with excellent ASMs therapy. Second, because these drugs must be taken continuously to prevent seizures, they may have deleterious effects on cognitive development, as they work as general CNS depressants [14]. Thus, for the treatment of refractory epilepsy, novel therapeutic compounds with minimal adverse effects must be developed.

Polyphenols are plant constituents that exist as secondary metabolites and are stored in specialized tissues and vacuoles [15]. Some essential polyphenols are lignans, flavonoids, stiblins, phenolic alcohols, and phenolic acids, which are known to have several health benefits. They are potent antioxidants that are effective against oxidative stress-associated diseases and can alter various cell signaling pathways [16]. These compounds can interfere with inflammatory functions and are highly recommended to prevent inflammatory diseases [17–19].

Among the polyphenolic substances, flavonoids exhibit potent anti-inflammatory and antioxidant properties [20,21]. Numerous studies provide evidence that neuroprotective effects of flavonoids are accompanied by their anti-inflammatory properties through various mechanisms [22–24]. A growing body of evidence from preclinical studies has also demonstrated that numerous flavonoids exhibit anti-epileptic properties, which also seem to be contributed by their anti-inflammatory functions.

The aim of the present study was to highlight the current understanding of the potential anti-seizure action of flavonoids in diverse epilepsy models, with particular emphasis on the immunomodulatory effects of these compounds in the epileptic brain. Most of the paper comprises experimental investigations executed on animal models of epilepsy, and it details flavonoids' anti-seizure and immunomodulatory properties.

2. Neuroinflammation in Epilepsy

The inflammation process affecting nervous tissues is known as neuroinflammation, and it can be brought on by several exogenous or endogenous sources. Numerous conditions, including infection, toxic insult, autoimmune diseases, aging, traumatic brain injury, and spinal cord injury, can trigger neuroinflammation [25–27]. The primary mechanism of neuroinflammation is the activation of microglia and astrocytes by cytokines and chemokines [26]. Interleukins (ILs) are a group of cytokines expressed and released by leukocytes and other body cells. At least 40 different types of ILs are known to be linked with neuroinflammation [27]. Continuous microglial activation triggers the recruitment of peripheral immune cells, including macrophages, B and T lymphocytes, and other immune cells, thus regulating the innate and adaptive immune response [28]. Astrocytes are another type of cells that are activated during neuroinflammation; they have a close connection with the blood-brain barrier (BBB) and are capable of responding to signals released by damaged neurons or reactive microglia [29]. Their contribution to tissue repair can be significant, as seen in the case of glial scars that encourage axonal regeneration [30]. However, persistent, long-lasting insults may activate molecular pathways linked with the inflammatory response in the brain resident cells, leading to an unfavorable process that could impair the CNS [31].

The pathophysiology of epilepsy is complicated and seems to be associated with multiple factors, including neuroinflammation. Numerous published studies have linked neuroinflammation with neurological disorders like epilepsy [31–33]. Epilepsy is known

to originate from various structural or genetic changes or as secondary consequences of injuries to the brain. It can also have an unknown etiology or be caused by immunological, infectious, or metabolic disorders [34]. Certain chronic inflammatory diseases are known to promote epilepsy and other neurological manifestations. Recent estimates suggest about a five- and four-fold increased risk of epilepsy among children and non-elderly adults suffering from autoimmune disorders [35,36]. Although the altered inflammatory response in damaged neuronal tissue significantly contributes to the onset of epilepsy [32], it is still unclear how this imbalanced regulation of inflammation does so. Moreover, several studies have also shown that recurring epileptic seizures have long-term consequences on neuroinflammation, thus affecting the course and outcome of epilepsy [10,31,33].

Since the groundbreaking study of Goddard (1967), various mechanisms linking epilepsy to neuroinflammation have been proposed (Figure 1) [37,38]. Interestingly, different experimental models and patients displayed overexpression of the genes of the pro-inflammatory cascade [39]. Cytokines, the small secretory proteins produced by glial cells and neurons, are known to regulate the inflammatory cascades in the epileptic brain [40–42]. Interleukin-1β (IL-1β), its receptor (IL-1R), and the receptor antagonist are all known to be altered in the brain of various animal models of epilepsy. The innate immune response is brought on by the activation of toll-like receptors (TLRs), followed by the release of IL-1β from microglial cells [43]. The activation of TLRs is associated with epileptogenesis and a number of other disorders with a secondary epileptic phenotype [44–46]. The activation of TLRs can also be amplified by several hyperacetylated molecules, like high mobility group box 1 (HMGB1) [47], along with the development of ictogenesis in human and animal models of chronic epilepsy [48,49].

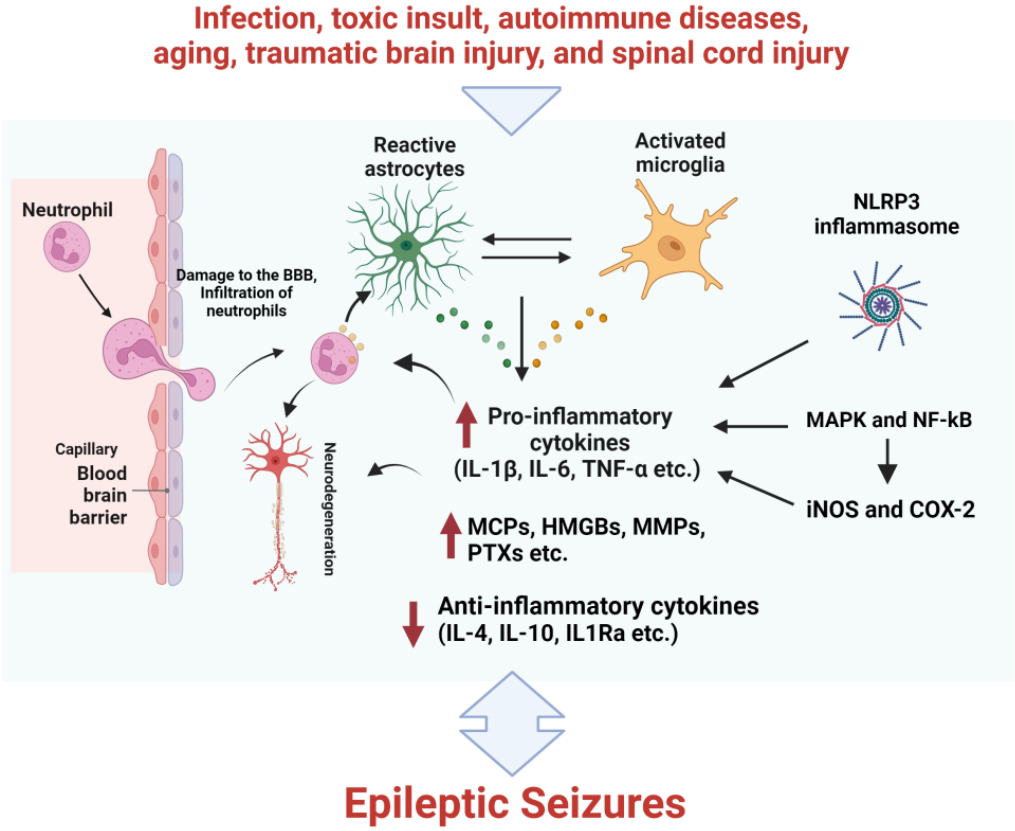

Figure 1. The neuroinflammatory process involved in epilepsy. Pathological insults caused by brain injuries, infections, genetic mutations, etc., stimulates neural cells. These cells produce and release inflammatory mediators in the brain, triggering a cascade of events that resulting in generation of epileptic seizures.

Reactive microglia may produce excessive levels of nitric oxide (NO) by inducible nitric oxide synthase (iNOS), which can disrupt neuronal mitochondria and activate nicotinamide adenine dinucleotide (NADPH) oxidase, and thus can produce superoxide anion radicals and pro-inflammatory molecules like tumor necrosis factor-α (TNF-α). Moreover, the release of inflammatory cytokines and iNOS is modulated by intrinsic glial pathways, including the mitogen-activated protein kinase (MAPK) and the nuclear factor- B (NF-B) signaling pathways [50]. Available evidence demonstrated that the levels of NO, iNOS expression, NF-B, and MAPK signaling are altered in various epilepsy models. Additional factors that contribute to neuroinflammation, including transforming growth factor-β (TGF-β), cyclo-oxygenase-2 (COX-2), and thrombospondin (TSP-1), have also been documented to alter in epilepsy [51–54].

It has recently been discovered that members of the pentraxin family (PTXs) proteins are important for inflammatory responses and have been linked to epilepsy [55]. PTX3 is expressed in the brain and released by white blood cells in response to inflammatory signals. Moreover, it can interact with the extracellular matrix, helps to remodel AMPA receptors, and regulates circuit excitability [56,57]. Matrix metalloproteases (MMPs) are calcium-dependent zinc containing endopeptidases and are known to modulate inflammation. The MMP-2 and MMP- 9 are proteases that function extracellularly and control various cellular processes, including neuroinflammation [58]. Studies have shown that neuroinflammation can increase the levels of MMP-2 and MMP-9 in the epileptic brain [58,59]. Changes in the extracellular matrix affect the equilibrium between excitation and inhibition and synaptic plasticity after MMP-9 stimulates the receptor for advanced glycation end-products, which eventually results in the production of numerous cytokines [60].

The nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome is a multiprotein complex that activates caspase-1, cleaves pro-IL-1β to form mature IL-1β, and has been identified as a key mediator of IL-1β functions [61,62]. The NLRP3 inflammasome has been shown to regulate innate immunity and inflammation in the CNS [63]. Accumulated evidence from recent studies strongly suggests that NLRP3 inflammasome-mediated inflammation is associated with epilepsy [64–66].

3. Flavonoids

Flavonoids are the most common group of natural polyphenolic compounds in dietary foods and vegetables. These substances share a C6-C3-C6 phenylbenzopyran backbone, which comprises two phenyl rings (A and B) linked by a three-carbon heterocyclic ring (C). Evidence from preclinical research demonstrates flavonoids' antioxidant, anti-cancer, anti-diabetic, and neuroprotective properties [67–69]. Additionally, flavonoids have reduced the risk of neurodegenerative diseases and alleviated neuroinflammation. These compounds can influence inflammatory pathways by inhibiting glial cell activation, cytokine release, NO generation, NADPH oxidase activity, and iNOS expression [21,70]. Flavonoids have been shown to improve human health by influencing key immune system components such as T cells, B cells, mast cells, NK cells, and neutrophils [21,70].

3.1. Classification of Flavonoids

Per their chemical makeup, flavonoids can be characterized into six classes, determined by the degree of C ring oxidation and unsaturation as well as the carbon in the C ring to which the B ring is linked. The benzene and phenyl rings are designated as the A and B rings, respectively, while the oxygen-containing-pyrone ring is referred to as the C ring [71]. Figure 2 represents the major classes of flavonoids, which include flavones, flavonols, flavanones, flavanols, isoflavones and anthocyanidins, as well as their members and sources.

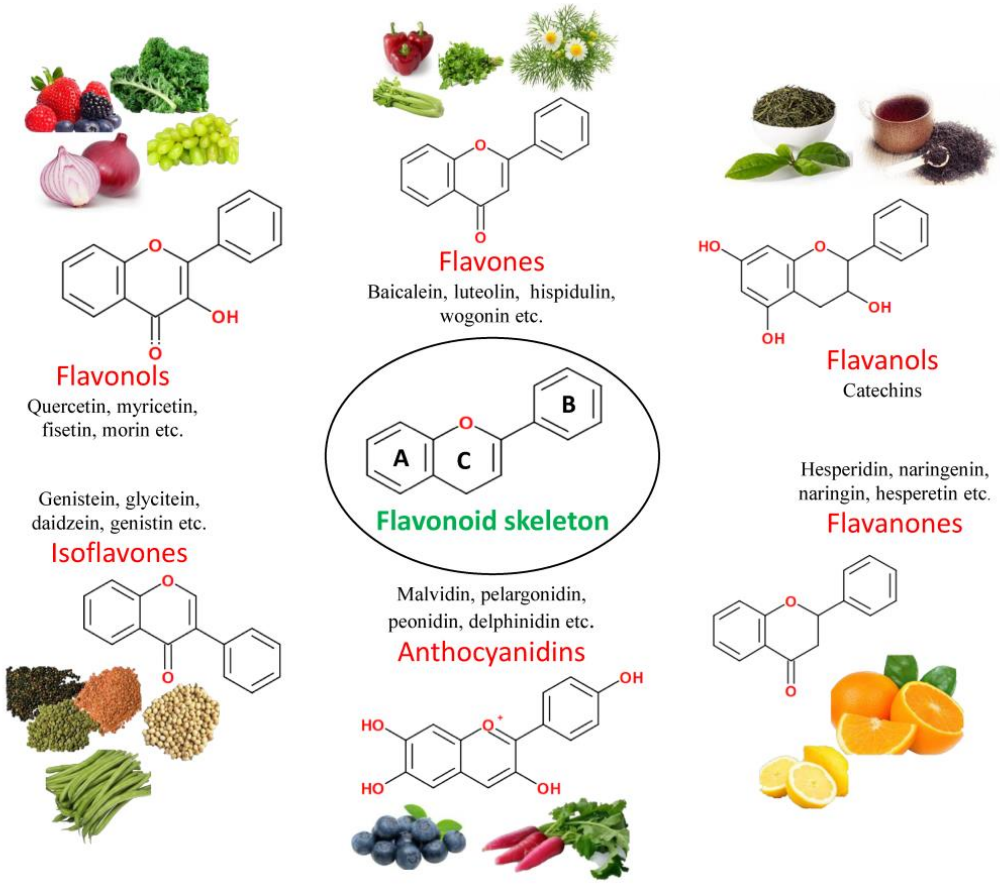

Figure 2. Structure and classification of flavonoids and their dietary sources.

3.1.1. Flavones

One of the major classes of flavonoids is flavones. Compounds in this class have a ketone in position 4 of the C ring and a double bond between positions 2 and 3. Additionally, the majority of flavones found in fruits and vegetables have a hydroxyl group in position 5 of the A ring. The hydroxyl group in other places, like position 7 of the A ring or positions 3 and 4 of the B ring, differs based on the taxonomic classification of the particular fruit or vegetable [72]. Apigenin, luteolin, baicalein, tangeretin, jaceosidin, and eupatilin are all members of this class of flavonoids [70,73].

3.1.2. Flavonols

Flavonols and flavones share a similar structural makeup, except that flavones have an additional hydroxyl group (C-3) that can be glycosylated. Flavonols exhibit a wide range of methylation and hydroxylation as well as distinct glycosylation patterns. When it comes to flavonols, quercetin and kaempferol are the most prevalent in plant foods and continue to be bound to sugar molecules rather than being free [74]. Bioactive compounds like quercetin, kaempferol, fisetin, myricetin, and rutin are important flavonols exhibiting several biological activities, e.g., anti-oxidative, anti-inflammatory, and neuroprotective properties [50,70].

3.1.3. Flavanones

Flavanones, also known as dihydroflavanones, differ from other flavonoids: they lack a double bond between positions 2 and 3 and have a chiral center in position 2 [75]. Flavanones are distinguished from flavones by the absence of a double bond between C-2 and C-3, resulting in a saturated C ring [76]. Among the most researched flavanones are hesperidin, naringenin, eriodictyol, narirutin, and neohesperidin, which have been

reported to exert multiple health benefits in oxidative stress and inflammation-linked diseases [70].

3.1.4. Anthocyanidins

Anthocyanidins are sugar-free anthocyanins with an aromatic A ring bound to a heterocyclic C ring with oxygen. The C ring is linked to a third aromatic ring B via a carbon-carbon bond [77]. The absence of a ketone group at position 4 on the C ring distinguishes anthocyanidins from flavonols and flavanones. Anthocyanins are the primary plant pigments, and the pH as well as methylation or acylation at the hydroxyl groups on the A and B rings determines their color [78]. Some of the most significant anthocyanidins are natural flavonoids such as malvidin, pelargonidin, peonidin, cyanidin, petunidin, and delphinidin [21,50].

3.1.5. Isoflavones

Isoflavones are a class of flavonoids with structural similarities to estrogens and 17-β-estradiol that can bind to estrogen receptors [79]. In contrast to other classes, the basic structure of these molecules includes a B ring attached to position 3 of the C ring via aryl migration, rather than position 2 [78]. This class's important and well-studied bioactive flavonoids include genistein, glycitein, daidzein, daidzin, and genistin [70,79].

3.1.6. Flavanols

Flavanols are a type of flavonoids also known as flavan-3-ols due to the presence of a hydroxyl group at position 3 on the C ring. Moreover, the ketone group at position 4 of the C ring and the C-2 and C-3 do not have a double bond in these molecules [80]. Catechins and epicatechins are the major representatives of this class of flavonoids. The most important catechins are catechin, gallocatechin-3-gallate (GCG), epicatechin (EC), epigallocatechin-3-gallate (EGCG), and epigallocatechin (EGC). All of the aforementioned compounds are created either with or without allyl substituents like epicatechin, catechin, gallocatechin, and epigallocatechin [21,70].

4. Flavonoids with Anti-Inflammatory Response in Epilepsy

Flavonoids are a special group of bioactive substances with distinct therapeutic properties. These compounds have been recognized as major constituents of the Indian Traditional Medicine system and the Chinese Herbal Medicine system from time immemorial and are known to work as neuromodulators. The therapeutic potential of these compounds is attributed to their interaction with the immune system and antioxidant effects [81]. A growing body of research has investigated the effectiveness of various flavonoids against diverse forms of epilepsy. Moreover, the flavonoids' structure is vital and is responsible for their anti-inflammatory action. The positions of the hydroxyl groups are critical to imparting this feature, since they have a planar ring structure with unsaturation at C2–C3. In order to retain their anti-inflammatory effect, the hydroxyl groups at the 3′ and 4′ positions of the B ring of flavonoids are crucial [21]. Thus, it is plausible to speak of the anti-inflammatory response of flavonoids in connection with the treatment of epilepsy. Hereunder, we reviewed the beneficial effect of these flavonoids in epilepsy with particular emphasis on their anti-inflammatory properties (Table 1).

Table 1. Summary of anti-inflammatory properties of flavonoids in epilepsy.

Flavonoid	Model Used	Doses	Effects	References
Baicalein	Pilo-induced epilepsy in rats	40 mg/kg, i.p.	Reduces pro-inflammatory cytokines levels (TNF-α and IL-1β)	[82]
	Pilo-induced epilepsy in rats	20, 40, and 80 mg/kg, orally	Downregulates the expression of cytokines (IL-1β, IL-6, and TNFα), and IGF1R	[83]
	Tremor rats	10, 20, and 40 mg/kg, i.p.	Suppresses release of cytokines (TNF-α, IL-1β, IL-6, and IL-10), and p-JNK and p-p38 levels, while increasing pERK level	[84]
	PTZ-kindled rats	50 and 100 mg/kg, orally	Decreases levels of IL-1β and IL-6 and inhibits TLR4/MYD88/Caspase-3 pathway	[85]
Luteolin	KA-injected rats	10 and 50 mg/kg, i.p.	Suppresses microglial activation in hippocampus	[86]
	PTZ-induced seizures in rats	10 mg/kg, i.p.	Reduces iNOS and MMP-2 activity, and increases eNOS activity	[87]
Hispidulin	KA-induced seizures in rats	10 and 50 mg/kg, i.p.	Suppresses microglialosis, pro-inflammatory cytokines production (IL-1β, IL-6, and TNF-α), c-Fos expression and MAPK activation	[88]
Schaftoside	PTZ-induced seizures in zebrafish	100, 200, 400 μM	Decreases IL-1β, IL-6, NF-B, and c-fos expression	[89]
Vitexin	Neonatal hypoxic ischemia-induced seizures in rats	45 mg/kg, i.p.	Decreases neutrophil infiltration and IL-1β, IL-6, and TNF-α expression	[90]
Wogonin	KA-induced TLE in rats	100 mg/kg, orally	Decreases IL-1β, TNF-α, and NF-kB expression	[91]
Rhoifolin	Hippocampal neuronal cell culture (HT-22 cell line)	5, 10, and 20 μM	Reduces IL-1β, IL-6 and TNF-α levels, and inhibits NF-B/iNOS/COX-2 pathway	[92]
Amentoflavone	PTZ-kindled mice	25 mg/kg, orally	Decreases IL-1β, IL 18, and TNF-α expression, and inhibits NLRP3 inflammasome activation	[93]
	Pilo-kindled mice	25 mg/kg, orally	Decreases NO, PGE2, IL-1β and IL-6 production, inhibits NF-B p65 activation	[94]
Quercetin	Febrile seizures in prenatally stressed rats	10 mg/kg, i.p.	Decreases levels of IL-1β, IL-6, and TNF-α	[95]
	KA-induced seizures in mice	100 mg/kg, i.p.	Reduces microglial activation, and levels of TNF-α, IL-1β, and activates NF-B	[96]
	PTZ-kindled mice	25 and 50 mg/kg, i.p.	Reduces astrocytes activation	[97]
	Neonatal hypoxic ischemia-induced seizures in rats	25, 50, and 100 mg/kg, i.p.	Reduces IL-1β, IL-6, TNF-α, MCP-1 and iNOS levels and TLR4/NF-B signaling in hippocampus	[98]
	PTZ-kindled rats	100 mg/kg, orally	Suppresses TNF-α, IL-6, IL-1β and NF-kB expression, and increases IL1Ra, IL-4, and IL-10 expression	[99]
Rutin	KA-kindled rats	50 and 100 mg/kg, orally	Suppresses astrocytes activation, downregulates IL-1β, IL-6, TNF-α, HMGB1, IL-1R1, and TLR-4 expression, and upregulates IL-10 expression	[100]
Fisetin	PTZ-kindled mice	5, 10, and 20 mg/kg, orally	Decreases HMGB1, TLR-4, IL-1R1, IL-1β, IL-6, and TNF-a levels, and NF-kB and COX-2 expression	[101]

Table 1. *Cont.*

Flavonoid	Model Used	Doses	Effects	References
Kaempferol	PTZ-kindled rats	100 mg/kg, orally	Downregulates TNF-α, IL-6, IL-1β and NF-kB expression and upregulates IL1Ra, IL-4, and IL-10 expression	[99]
Morin	PTZ-kindled rats	10 mg/kg, i.p.	Suppresses TNF-α expression, mitigates astrocyte activation, and IL-6/p-JAK-2/p-STAT3 signaling	[102]
	KA-kindled mice	20, 40, and 80 mg/kg, orally	Decreases microglial activation and IL-1β, TNF-α, and iNOS levels and inhibits mTORC1 pathway	[103]
Myricetin	PTZ-kindled mice	100 and 200 mg/kg, orally	Downregulate MMP-9 expression	[104]
Myricitrin	KA-induced TLE in rats	5 mg/kg, i.p.	Decreases TNF-α concentration	[105]
Galangin	PTZ-kindled mice	30 mg/kg, i.p.	Decreases microglial and astrocytic activation	[106]
Naringin	PTZ-kindled rats	20, 40, and 80 mg/kg, i.p.	Reduces TNF-α levels	[107]
	KA-kindled mice	80 mg/kg, i.p.	Decreases TNF-α expression in activated microglial cells	[108]
	KA-induced status epilepticus in rats	20, 40, and 80 mg/kg, i.p.	Decreases TNF-α expression	[109]
Naringenin	KA-kindled mice	50 and100 mg/kg, i.p.	Reduces IL-1β, and TNF-α levels in microglial cells and inhibits mTORC1 pathway	[110]
Hesperetin	KA-induced TLE in mice	5, 10, and 20 mg/kg, orally	Reduces TNFα, IL-1β, and iNOS levels	[111]
Hesperidin	Febrile seizure in rat pups	100 mg/kg, orally (Maternal administration)	Decreases TNF-α, IL 10, and TLR4 expression	[112]
	PTZ-induced seizures in Zebrafish larvae	1, 5, and 10 μM (Preincubated)	Reduces c-fos and IL-10 expression	[113]
Silibinin	Lithium-Pilo-induced TLE in rats	50 and 100 mg/kg, orally	Inhibits TNF-α, IL-1β, IL-6, and HIF-1α expression	[114]
	KA-kindled mice	50, 100, 200 mg/kg, i.p.	Inhibits TNF-α and IL-1β expressions and mTORC1 pathway	[115]
Genistein	PTZ-kindled rats	5 and 15 mg/kg, i.p.	Reduces astrocytes and microglial activation, and TNF-α, IL-1β, p-JAK2, p-STAT3 expression	[116]
Catechin	PTZ-kindled rats	100 mg/kg, orally	Suppresses TNF-α, IL-6, and IL-1β levels, and NF-kB 4 expression, and upregulates IL1Ra, IL-4, and IL-10 expression	[99]
Epigallocatechin-3-gallate	Lithium-Pilo-induced TLE in rats	25 mg/kg, i.p.	Decreases TLR4, NF-kB, and IL-1β expression	[117]
	KA-induced kindling TLE in mice	30 mg/kg, i.p.	Inhibits astrocytes and microglial activation	[118]

4.1. Baicalein

Baicalein (5,6,7-trihydroxyflavone) is a flavone isolated from the roots of *Scutellaria baicalensis* and *Scutellaria lateriflora*. Baicalein has shown beneficial effects against dozens of diseases, including neurological disorders, and inflammation. Its therapeutic efficacy has also been reported in multiple epilepsy models, and it improves seizure propensity and cognitive deficits [84,119]. According to a study by Qian et al. [82], baicalein treatment after the onset of SRS helps temporal lobe epilepsy (TLE) rats to improve cognitive

deficits and protects their hippocampal neurons. Additionally, the research has shown that baicalein reduces oxidative stress and inflammation markers (TNF-α and IL-1β) in the sera and hippocampus of TLE rats. Oral administration of baicalein (20, 40 and 80 mg/kg) improved epilepsy symptoms, attenuated microglial proliferation, decreased the expression of Insulin-like growth factor 1 receptor (IGF-1) and inhibited inflammation, as evident from the reduced expression of IL-1β, IL-6, and TNF-α in the brain of pilocarpine-induced epileptic rats [83]. Baicalein (10, 20 and 40 mg/kg) pretreatment for 14 days in tremor rats (TRM) reduced epileptiform activity and improved cognitive impairments. Furthermore, decreased oxidative stress and inflammatory responses were observed, along with changes in the HSP70 and MAPK cascades [84]. Baicalin (50 and 100 mg/kg) showed an anti-seizure effect and improved cognitive dysfunctions in PTZ-induced epileptic rats. Additionally, it decreased the production of IL-1β and IL-6 and activated the TLR4/MYD88/Caspase-3 pathway while reducing neurodegeneration in the CA3 region of the hippocampus [85]. These findings suggest that the anti-epileptic effects of baicalin may be accompanied by a reduction of neuroinflammation and an activation of the TLR4/MYD88/Caspase-3 pathway.

4.2. Luteolin

Luteolin (3′,4′,5,7-tetrahydroxyflavone) is a flavone found in various foods and beverages, including pepper, celery, broccoli, thyme, and chamomile tea. This bioactive compound can cross the brain and have various biological effects, including neuroprotection. Lin et al. [86] investigated the anti-inflammatory effect of luteolin in rats with KA-induced seizures. The study showed that luteolin protects neuronal loss, inhibits glial activation, and boosts Akt activation in the hippocampus of KA-injected rats. Pretreatment with luteolin (10 mg/kg i.p.) in PTZ-injected rats showed a reduction in the frequency of seizures, a decrease in iNOS and MMP-2 activity, and an increase in eNOS activity [87]. Together, these studies indicate that suppressing the activation of glial cells, iNOS, and MMP-2 may contribute to the anti-inflammatory response of luteolin in epilepsy.

4.3. Hispidulin

Hispidulin (4′,5,7-trihydroxy-6-methoxyflavone) is a flavone that occurs naturally and is an active ingredient in various traditional Chinese medicinal herbs, including Artemisia and Salvia species [120]. Its powerful anti-oxidative, anti-fungal, anti-inflammatory, anti-cancer, and anti-neoplastic capabilities have been proven by several in vitro investigations [121–123]. It can cross the BBB, modulates the GABA receptors, and has anti-convulsant characteristics [124]. In a study by Lin et al. [88], it was found that giving hispidulin intraperitoneally (10 and 50 mg/kg) to rats before KA injection reduces the severity of their seizures and the expression of pro-inflammatory cytokines like IL-1β, IL-6, and TNF-α, as well as microglial activation. The study suggests that the anti-seizure effect of hispidulin may be led by its anti-inflammatory properties.

4.4. Schaftoside

Schaftoside, also known as apigenin 6-C-glucoside-8-C-arabinoside, is a flavonoid found in many Chinese medicinal herbs, including *Eleusine indica*, *Dendrobium nobile*, *Lysimachia christinae Hance*, and *Capsicum annuum* [125]. Previous research revealed that schaftoside has various pharmacological effects, including anti-inflammatory, anti-melanogenic, and anti-oxidative properties [126]. In zebrafish, schaftoside pretreatment alleviated PTZ-induced seizures by suppressing apoptosis and decreasing the expression of inflammatory cytokines like IL-1β, IL-6, and NF-B [89]. These results indicate that schaftoside ameliorates seizure progression mainly by an NF-B mediated inflammatory response and apoptosis.

4.5. Vitexin

Vitexin (5,7-dihydroxy-8-methoxyflavone), a c-glycosylated flavone, is an essential constituent of traditional Chinese medicines. This flavonoid is mainly found in passion

flowers, bamboo leaves, wheat leaves, pearl millet, mosses, Passiflora, etc. Vitexin has recently gained popularity due to its antioxidant, anti-inflammatory, anti-hyperalgesic, anti-cancer, and neuroprotective properties [127,128]. According to Luo et al. [90], vitexin effectively alleviated spontaneous seizures in neonatal rats with hypoxic ischemia-induced seizures (HINS). Further, the study reported that vitexin decreases neutrophil infiltration and the expression of IL-1β, IL-6, and TNF-α in the rat brain, indicating that the cytokines' neuroinflammatory response may also be responsible for vitexin's potential anti-seizure effects.

4.6. Wogonin

Wogonin is an O-methylated flavone that can be found in a variety of plants, including the roots of *Scutellaria baicalensis*. Wogonin and its derivatives possess various health benefits, including antiviral, anti-cancer, antioxidant, anti-inflammatory, and neuroprotective properties [129,130]. Recently, Guo et al. [91] investigated the anticonvulsant effect and underlying mechanism of wogonin in KA-induced TLE rats. The study found that wogonin treatment improves cognitive functions and reduces oxidative stress, cellular apoptosis, and inflammatory mediators such as IL-1β, TNF-α, and NF-B in the hippocampus. Overall, the findings indicate that wogonin's neuroprotective effect in epilepsy may also be mediated by its immunomodulatory properties.

4.7. Rhoifolin

Rhoifolin (apigenin 7-O-neohesperidoside) is a well-known apigenin family trisubstituted flavone. It is found in various foods and plants, including grapefruits, bitter oranges, lemons, grapes, tomatoes, and bananas [131]. Preclinical evidence suggests that rhoifolin has significant biological effects, including antioxidant, anti-inflammatory, anti-arthritic, and anti-cancer properties [132–134]. The effectiveness of rhoifolin was evaluated in acquired epilepsy using hippocampal neuronal cultures. The study found that rhoifolin increases cell viability, decreases apoptosis, alleviates oxidative stress, and lowers levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) along with p-p65, p-Ikb, iNOX, and COX-2 [92]. Thus, the study suggests that the neuroprotective effect of rhoifolin in acquired epilepsy may be attributed to its anti-oxidative and anti-inflammatory potential via inhibition of the NF-B/iNOS/COX-2 axis.

4.8. Amentoflavone

Amentoflavone is a biflavonoid that is created when two apigenin molecules are joined together through oxidation and form a bond at positions C-3 of the hydroxyphenyl ring and C-8 of the chromene ring. This biflavonoid can be isolated and identified in over 120 plants, some of which have been used for hundreds or even thousands of years as traditional folk medicines in different parts of the world [135]. Amentoflavone has a wide range of biological effects, including antioxidant, anti-inflammatory, anti-senescence, anti-tumor effects, and is advantageous against cardiovascular and CNS diseases [135–140]. The intragastric administration of amentoflavone (25 mg/kg) to pilocarpine-kindled mice for three days reduced the severity of epileptic seizures and decreased prostaglandin E2 and NF-B expression, IL-1β and IL-6 production, and neuronal loss and apoptosis in the hippocampus [94]. Similarly, a study by Rong et al. [93] demonstrated that amentoflavone decreases seizure susceptibility, cognitive impairments, and the loss of hippocampus neurons in PTZ-kindled mice. Moreover, amentoflavone reduced inflammatory cytokines (IL-1β, IL-18, and TNF-α) and inhibited NLRP3 inflammasome activation. These findings showed that amentoflavone subdues epileptogenesis, exerts neuroprotective benefits in PTZ-induced kindling mice via suppressing pro-inflammatory molecules and the NLRP3 inflammasome, and modulates the inflammatory process.

4.9. Quercetin

Quercetin (3,3′,4′5,7-pentahydroxyflavone) is a widespread flavonol found in fruits, vegetables, seeds, nuts, flowers, bark, and leaves. It is a highly recommended natural component for treating inflammatory and metabolic diseases. A thorough analysis of the literature reveals that quercetin has various pharmacological effects, including antioxidant, anti-inflammatory, neuroprotective, and anti-epileptic actions [141–143]. According to a study by Mkhize et al. [95], quercetin has the ability to treat febrile seizures, as it down-regulates the expression of several pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 in the presence of prenatal stress. Furthermore, a recent study discovered that quercetin inhibits the production of pro-inflammatory cytokines such as TNF-α and IL-1β and activates NF-B in the glial cells [96]. Similarly, Ahmed et al. [99] found that quercetin treatment in PTZ epileptic mice reduces the severity of seizures, improves behavioral issues, and repairs cellular damage. The study revealed that quercetin decreases the expression of pro-inflammatory cytokines, e.g., TNF-α, IL-1β, IL-6, and NF-B, while increasing the expression of anti-inflammatory cytokines, e.g., IL-1Ra, IL-4, and IL-10. Recently, Wu et al. [98] demonstrated that quercetin attenuates later-life seizure susceptibility, anxiety-related behavior, and memory impairments in the rat model of hypoxia-induced neonatal seizure (HINS). Additionally, the study exhibited that quercetin decreases the expression of TNF-α, IL-6 MCP-1, IL-1β, and iNOS in the serum and hippocampus, as well as the protein levels of TLR4, and p-NF-kB p65 in the hippocampus. The effectiveness of quercetin-loaded magnetic nanoparticles was evaluated in PTZ-kindled mice [97]. The research showed that quercetin nanoparticles reduce epileptic seizures and neuronal death in rats by decreasing astrocytic activation. The study also suggested that the anti-seizure effects of quercetin nanoparticles may have been enhanced due to quercetin's improved bioavailability. These findings suggest that quercetin's anti-inflammatory response in epilepsy may be aided by inhibiting astrocyte and microglia activation and downregulating NF-B and pro-inflammatory cytokines, along with upregulating anti-inflammatory molecules.

4.10. Rutin

Rutin (quercetin-3-rutinoside), also known as rutoside and sophorin, is an integral derivative of quercetin, which is abundantly present in peaches, oranges, lemons, grapes, apples, berries, nuts, and other fruits and vegetables. This bioactive compound is quercetin and rutinose-containing flavonol glycoside [144]. Accumulating evidence indicates that rutin effectively prevents seizures in animal models of epilepsy [143,145]. A recent study looked into rutin's possible anti-seizure mechanism in KA-treated rats. The researchers discovered that pretreatment with rutin (50 and 100 mg/kg) for 7 days reduces seizure severity, reverses neuronal loss, and increases glutamate levels in the hippocampus. Rutin also inhibited activation of astrocytes, decreased the protein levels of pro-inflammatory molecules such as IL-1β, IL-6, TNF-α, HMGB1, IL-1R1, and TLR-4, and increased the level of anti-inflammatory molecules IL-10 [100]. Overall, the study found that rutin reduces KA-induced seizures and neuronal loss in rats by suppressing the IL-1R1/TLR4-related neuroinflammatory cascade.

4.11. Fisetin

Fisetin (7,3′,4′- flavon-3-ol) is a tetra hydroxy flavone found in a variety of fruits and vegetables, including strawberries, apples, grapes, onions, cucumbers etc. This flavonoid has marked antioxidant activity that makes it a potent therapeutic agent for various health issues [146,147]. Growing evidence suggests the anti-oxidative and neuroprotective effects of fisetin in epilepsy [148,149]. According to Khatoon et al. [101], fisetin reduced myoclonic jerks and seizures in PTZ-induced mice by reducing neuroinflammation and apoptosis, as evident from the decreased expression of HMGB1, TLR-4, IL-1R1, IL-1β, IL-6, and TNF-α in the cortex and hippocampus regions of the brain. Hence, the findings suggest that fisetin's anti-epileptic potential is implicated in experimental epilepsy and may be accompanied by suppressing the release of pro-inflammatory and apoptotic molecules.

4.12. Kaempferol

Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a natural flavonol found in fruits and vegetables, including broccoli, apples, strawberries, and beans [150]. Evidence from the available literature suggests that kaempferol has potential therapeutic properties to treat various human diseases including cancer, inflammatory diseases, cardiovascular diseases, neurological diseases etc. [151–154]. Recently a study by Ahmed et al. [99] found that kaempferol treatment in PTZ epileptic rats attenuates seizure severity, improves behavioral impairments, and restores cellular damage. Further, the study revealed that kaempferol decreases the expression of pro-inflammatory cytokines e.g., TNF-α, IL-1β, IL-6 and NF-B, while increasing the expression of anti-inflammatory cytokines e.g., IL-1Ra, IL-4 and IL-10. Hence, the anti-epileptic potential of kaempferol may be accompanied by its role in suppressing the pro-inflammatory molecules, while enhancing the anti-inflammatory ones.

4.13. Morin

Morin (3,5,7,2′,4′-pentahydroxyflavone) is a yellow flavonol found in various plants, most notably the Moraceae family [155]. Morin has a wide range of pharmacological benefits, including antioxidant and anti-inflammatory properties [156]. The flavonoid's beneficial effects have been investigated in different neurological disorders, including epilepsy [157–159]. The oral administration of morin hydrate to KA-injected mice reduced seizures susceptibility and inhibited granule cell dispersion (GCD) and the activation of the mammalian target of rapamycin complex 1 (mTORC1), along with the restoration of apoptotic proteins and pro-inflammatory molecules (IL-1β, TNF-α, and iNOS) in the hippocampus [103]. Similarly, morin (10 mg/kg, i.p.) reduced seizure severity in PTZ-kindled rats, improved cognitive deficits, and diminished neuronal loss and astrogliosis. The study further showed that the pro-inflammatory cytokines and receptors like TNFR-1, TNF-α, IL-1β, and IL-6 were diminished, and the hippocampal IL-6/p-JAK2/p-STAT3/GFAP cue was significantly decreased [102]. Together, these immunomodulatory effects provide evidence for morin's promising role in the treatment of epilepsy.

4.14. Myricetin

Myricetin (3,3′,4′,4,5,5′,7 hexahydroxyflavone) is another important flavonoid classified as a flavonol [160]. It is a key component of many human meals and beverages, including vegetables, teas, and fruits. It is best known for its iron-chelating, antioxidant, anti-inflammatory, and anti-cancer properties [160,161]. Myricetin supplementation has been proven to have therapeutic effects on a number of nervous system disorders, including cerebral ischemia, Alzheimer's disease, Parkinson's disease, epilepsy, and glioblastoma [162]. Myricetin (100 or 200 mg/kg) administered orally for 26 days, prior to each PTZ injection to mice, reduced seizure and mortality rates, downregulated the expression of apoptotic proteins, and restored GABA and glutamate levels. Additionally, it has downregulated the expression of MMP-9 following PTZ-kindling [104], thus suggesting that myricetin's anti-seizure potential is attributed to its MMP-9-mediated immunomodulatory properties.

4.15. Myricitrin

Myricitrin (myricetin-3-O-a-rhamnoside) is a flavonol made up of myricetin linked to an alpha-L-rhamnopyranosyl at position three. The antioxidant activity of myricitrin was demonstrated to be significant, with greater free radical scavenging activity than that of other flavonol rhamnosides or quercetin. This flavonoid is abundant in the dry bark of *Myrica rubra* (Lour.) and exerts many bioactivities, including anti-inflammatory, antioxidative, and anti-fibrotic effects [163,164]. According to Keikhaei et al. [105], pretreatment with myricitrin prevented seizures, improved spatial learning and memory, and restored oxidative stress and inflammatory markers (TNF-α) in rats with KA-induced acute and

chronic epilepsy. Overall, these findings suggest that myricitrin's antioxidant and TNF-α-mediated anti-inflammatory potential may help to alleviate seizures in epilepsy.

4.16. Galangin

Galangin (3,5,7-Trihydroxy-2-phenyl-4H-1-benzopyran-4-one) is a flavonol found in honey, *Alpinia officinalis*, *Helichrysum aureonitens*, and propolis in substantial concentration. Galangin shows a variety of pharmacological activities, such as anti-cancer, anti-mutagenic, anti-clastogenic, and anti-oxidative properties [165–167]. Moreover, numerous studies have exhibited the neuroprotective potential of galangin in various neurological disorders [168,169]. Recently, a study by de Zorzi et al. [106] has exhibited that galangin reduces the risk of seizure severity by regulating multiple neurochemical alterations like activation of microglia and astrocytes in PTZ-induced seizures in mice. Thus, the study gives a hint that galangin's anti-seizure potential may also be accompanied by its anti-inflammatory properties.

4.17. Naringin

Naringin (4′,5,7-trihydroxyflavanone-7-rhamnoglucoside) is a flavone glycoside found in citrus fruits such as grapefruits, pummelos, and sour oranges. Naringenin is one of the active compounds used in Chinese herbal medicine. In recent years, the bioactive compound has attracted the interest of researchers, as it possesses a plethora of biological and pharmacological properties [151]. Naringin is one of the most effective flavonoids for suppressing TNF-α production in PTZ-induced seizures [107,109]. Treatment with naringin in male C57BL/6 mice after KA injection postponed the start of seizures and reduced the frequency of SRS. Additionally, naringin therapy reduced autophagic stress, preserved hippocampal CA1 neurons, and decreased a rise in TNF-α in activated microglia [108]. These findings imply that the anti-autophagic stress and anti-neuroinflammatory properties of naringin may help to prevent epileptic seizures and neuronal death in the hippocampus.

4.18. Naringenin

Naringenin (4′5,7-trihydroxyflavanone) is a naringin metabolite found in grapefruit and other citrus fruits. It is a deglycosylated naringin variant and readily crosses the BBB. The flavonoid has demonstrated antioxidant, anti-inflammatory, and neuroprotective properties in experimental models of brain diseases [170]. Naringenin has also been shown to lessen the severity of seizures in pilocarpine and PTZ-induced mouse models; thus, it can be a probable therapeutic agent for epilepsy. Furthermore, Park et al. [110] found that naringenin delayed the onset of seizures and reduced GCD by inhibiting the activation of the mTOR1 in both neurons and reactive astrocytes, as well as reducing the level of pro-inflammatory cytokines like TNF-α and IL-1β in the dentate gyrus of KA-injected mice. Overall, these findings indicate that naringenin may help to prevent epileptic-seizure-induced damage in the hippocampus of the TLE model by mitigating neuroinflammation.

4.19. Hesperetin

Hesperetin (4′-methoxy-3′,5,7-trihydroxyflavanone) is a dihydroflavone derived from the breakdown of hesperidin and found in young citrus fruits such as lemons and sweet oranges [171]. Accumulating evidence from preclinical research showed encouraging results for hesperetin in the treatment cancer, cardiovascular and neurodegenerative diseases, and other health issues. Clinical research has confirmed that hesperetin has potential cardioprotective and neuroprotective properties [172]. According to Baradaran et al. [173], hesperetin (50 mg/kg) reduces seizures and oxidative stress in a PTZ-induced seizures model. Furthermore, Kwon et al. [111] discovered that hesperetin reduces GCD and inhibits the expression of the pro-inflammatory molecules produced by activated microglia in the hippocampus of KA-treated mice.

4.20. Hesperidin

Hesperidin (3,5,7-trihydroxyflavanone 7-rhamnoglucoside) is a -7-rutinoside of hesperetin found in citrus fruits like lemons, sweet oranges, and grapefruits. It has numerous pharmacological effects, including anti-cancer, anti-inflammatory, anti-hyperlipidemic, anti-hypertensive, diuretic, antiviral, and calcium channel blocking actions [174]. Hesperidin and its aglycone can cross the BBB and exhibit neuroprotective effects in in vitro and in vivo experiments [175]. Recently, Sharma et al. [113] have also explored the possibility that hesperidin therapy improves seizure latency, reduces hyperactive responses in PTZ-mediated seizures in zebrafish larvae, and modulates the expression of BDNF and IL-10. Hesperidin's affinity for several receptors, including IL-10, was also demonstrated via in-silico research. Hesperidin (100 mg/kg) given to pregnant rats reduced TNF-α, IL-10, and TLR4 protein expression and improved cognition in male offspring with febrile seizures [112]. Overall, these findings suggest that hesperidin has anti-seizure effects, possibly due to its anti-inflammatory properties and TLR4 downregulation.

4.21. Silibinin

Silibinin, commonly known as silybin, is a natural flavanone derived from *Silybum marianum* fruits and seeds. It is silymarin's principal active component and has been demonstrated to provide a range of pharmacological health advantages, including antioxidative, anti-inflammatory, anti-cancer, and neuroprotective benefits. According to Kim et al. [115], silibinin therapy decreased the susceptibility to and frequency of SRS in KA-injected mice. The study also showed that silibinin decreases the abnormally high levels of apoptotic and pro-inflammatory molecules (TNF-α and IL-1β) in mice with KA injections. Similarly, Wu et al. [114] found that silibinin has anti-inflammatory and anti-apoptotic effects in the rat model of TLE, and inhibits overexpression of TNF-α, IL-1β, IL-6, caspase-3, cleaved caspase-3, and HIF-1α in the hippocampus. These findings suggest that silibinin's anti-epileptic and neuroprotective role may be connected with its inhibition of neuroinflammation.

4.22. Genistein

Genistein (5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one) is a flavonoid that belongs to the isoflavone class. It is a phytoestrogen derived primarily from legumes like lupines, fava beans, soybeans, kudzu, and Psoralea. Preclinical evidence suggests that genistein has a variety of pharmacological actions, including antioxidant, anti-inflammatory, anti-microbial, angiogenesis, anti-cancer, and neuroprotective properties [176]. The administration of genistein (5 or 10 mg/kg) to PTZ-induced epileptic rats reduced seizure intensity and duration, inhibited astrocytes and microglial activation, and decreased the mRNA and protein expression of p-JAK2, p-STAT3, TNF-α, and IL-1β in the hippocampus [116]. These findings suggest that the anti-seizure effect of genistein may be accompanied by the inhibition of astrogliosis and the JAK2/STAT3 inflammatory pathway.

4.23. Catechin

Catechin is a class of flavonoids produced by the plant *Camellia sinensis* var. Sinensis. Growing research has shown that catechin has several preventive properties for the treatment of fever, inflammatory diseases, wounds, and malignancies [177,178]. It is well known to reduce inflammation through the inhibition of NF-B and pro-inflammatory cytokines [179]. Recently, a study by Ahmed et al. [99] investigated the way that catechin treatment in PTZ epileptic rats attenuates seizure severity, improves behavioral impairments, and restores cellular damage. Further, the study revealed that catechin decreases the expression of pro-inflammatory cytokines, e.g., TNF-α, IL-1β, IL-6, and NF-B, while increasing the expression of anti-inflammatory cytokines, e.g., IL-1Ra, IL-4, and IL-10. These results indicate that catechin exerts its anti-epileptic potential by modulating pro-and anti-inflammatory cytokines.

4.24. Epigallocatechin-3-Gallate

(-)-Epigallocatechin-3-gallate (EGCG) is the major catechin found in green tea. EGCG has been linked to a variety of potential health advantages, such as antioxidant effects, cancer chemoprevention, reduced weight loss, improved cardiovascular health, and protection against ionizing radiation-induced damage to the skin [180–182]. EGCG alleviated SRS frequency, cognitive impairment, synaptic dysfunction, and loss of hippocampal neurons, along with the expression of TRL-4, NF-B, and IL-1β levels in the lithium-pilocarpine treated rats [117]. Similarly, free EGCG and EGCG-loaded PEGylated-PLGA nanoparticles reduced the number and severity of seizures, neuronal death, and glial activation in the hippocampus of KA-injected mice [118]. Together, these studies imply that EGCG can suppress seizures and improve cognitive function and neuronal loss in epilepsy through its anti-inflammatory action.

5. Mechanism of Action of Flavonoids in Epilepsy

The anti-inflammatory action of flavonoids is known to be regulated by numerous pathways. The available research focusing on flavonoids and their mechanisms in the context of epilepsy indicated that flavonoids have a distinctive way of modulating of neuroinflammation. Hereunder, we discuss the neuromodulatory mechanism of action of flavonoids in different experimental models of epilepsy.

The anti-inflammatory activity of baicalein was accompanied by the activation of ERK, while inhibiting p-JNK and extracellular signal-regulated kinase like MAPK, and by NF-B activation in TRM rats [84]. Baicalein has also suppressed the activation of the TLR4/MYD88/caspase-3 signaling pathway in PTZ-kindled rats [85]. Furthermore, it can ameliorate the inflammation by inhibiting IGF1R, a tyrosine kinase receptor that activates PI3K and promotes Akt and mTOR pathways in pilocarpine-induced epilepsy in rats [83]. Similar to baicalein, the anti-inflammatory action of hispidulin was also mediated by suppressing the activation of MAPK pathway [88]. Both NOS and MPPs are important mediators of inflammation, and luteolin interferes with these mediators in PTZ-kindling [87]. Vitexin reduces hypoxia-ischemia-induced seizures by inhibiting neutrophil infiltration and IL-1β, IL-6, and TNF-α expression in rats [90]. Wogonin reduces the level of IL-1β and TNF-α by decreasing the expression of NF-kB in TLE [91]. Rhoifolin was observed to modulate the NF-κB/iNOS/COX-2 signaling cascade in the acquired epilepsy [92]. Amentoflavone influences epileptogenesis and exhibits neuroprotective effect by inhibiting the NLRP3 inflammasome, a mediator of the caspase1/IL-1β cascade of inflammatory process, in PTZ-kindled mice [93]. It can also inhibit NF-B signal pathway and inflammatory mediators like NO and PGE2, and pro-inflammatory cytokines like IL-1β and IL-6 in pilocarpine-induced epilepsy [94].

Quercetin showed its anti-inflammatory action by suppressing the TLR4/NF-kB signaling pathway in the animal models of experimental epilepsy [98,99]. One of its derivatives, rutin, reduced the release of inflammatory molecules like IL-1β, IL-6, TNF-α, and HMGB1 and inhibited IL-1R1 and TLR4 expression in KA-injected rats [100]. In a study, fisetin was observed to exhibit its anti-inflammatory action by inhibiting the NF-B/COX2, IL-1R1/TLR4, and AkT/mTOR signaling cascades in PTZ-kindled mice [101]. Morin was found to inhibit neuroinflammation by downregulating the IL-6/p-JAK2/p-STAT3/GFAP cue, as evidenced by the decreased expression of p-JAK2, p-STAT3, and GFAP, along with the other inflammatory mediators [102]. The flavonoid has also prevented GCD and suppressed the mTORC1 pathway in KA-induced seizures [103]. Naringenin was found to inhibit the microglia-derived neuroinflammation in the DG of KA-treated mice, probably by inhibiting GCD and mTORC1 activation [110]. Similarly, hesperetin was shown to alleviate the neuroinflammatory response by suppressing GCD by inhibiting mTORC1 activation [111], while the anti-inflammatory action of hesperidin was accompanied by TLR4 expression [112] and c-fos [113] in experimentally induced seizures. Similar to that of morin and hisperetin, the anti-inflammatory response of silibinin has also been associated with the inhibition of GCD by suppressing mTORC1 activation [115] and HIF-α expres-

sion [114] in the hippocampus of epileptic rodents. Genistein can attenuate the activation of microglia and astrocytes and combat inflammation by suppressing the JAK2/STAT3 pathway [116]. The anti-inflammatory potential of EGCG, observed on lithium-pilocarpine-induced epilepsy, is mediated by inhibiting the TLR4/NF-kB signaling pathway [117]. Hence, the available evidence from preclinical data suggests that the anti-inflammatory action of various flavonoids may have been contributed by the modulation of signaling pathways like TLR4/MYD88/caspase-3 signaling, iNOS/NF-B/COX-2, JAK2/STAT3, IL-1R1/TLR4 and AkT/mTOR, NLRP3-mediated caspase1/IL-1β, HIF-α, IGF1R, etc.

6. Conclusions and Future Prospects

The rising incidence of epilepsy cases, combined with the limited efficacy of the available anti-epileptic drugs, affects a significant number of people worldwide each year, hurting personal, social, and economic aspects of their lives. Adopting a targeted approach to combating both the disease-promoting risk factors and the pathomolecular processes that drive the development and progression of epilepsy may be more effective in reducing the symptoms associated with this neurological disorder. Considering that epileptic seizures are accompanied by a heightened central and systemic inflammatory response, inflammation may be a potential therapeutic target for the management of epilepsy. In addition, it is now known that neuroinflammation, or increased brain inflammation, interacts with various neurological components and processes associated with the pathophysiology of epilepsy.

In this review paper, we present the preclinical research findings that used flavonoids to target neuroinflammatory pathways in epilepsy. Thus, the review provides insight into a promising therapy for managing epilepsy by targeting neuroinflammation. Preclinical findings showed that flavonoids could reduce the production of pro-inflammatory cytokines by directly affecting inflammatory regulators like NF-kB and NLRP3 inflammasomes and altering the activity of resident immune cells in the brain, along with the proteins and signaling pathways that affect their activation. In these studies, flavonoids also prevented a long-term neuroinflammatory response by increasing the levels of anti-inflammatory cytokines and decreasing the levels of pro-inflammatory mediators such as COX-2, NOS, and others. Neuroinflammatory pathways are linked to the process of epileptogenesis, and flavonoids could be a promising therapy for treating epilepsy, given their considerable anti-neuroinflammatory potential. Despite the substantial number of preclinical studies showing that the anti-epileptic effects of flavonoids are strongly linked to the regulation of neuroinflammatory pathways, epilepsy patients are rarely examined for their anti-neuroinflammatory potential. To prove flavonoids' effectiveness in treating epilepsy and comprehend entirely their anti-neuroinflammatory modes of action, which affect the molecular pathways regulating neuronal homeostasis in epilepsy, thorough preclinical and clinical investigations must yet be done.

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Review

Therapeutic Strategies to Ameliorate Neuronal Damage in Epilepsy by Regulating Oxidative Stress, Mitochondrial Dysfunction, and Neuroinflammation

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Abstract: Epilepsy is a central nervous system disorder involving spontaneous and recurring seizures that affects 50 million individuals globally. Because approximately one-third of patients with epilepsy do not respond to drug therapy, the development of new therapeutic strategies against epilepsy could be beneficial. Oxidative stress and mitochondrial dysfunction are frequently observed in epilepsy. Additionally, neuroinflammation is increasingly understood to contribute to the pathogenesis of epilepsy. Mitochondrial dysfunction is also recognized for its contributions to neuronal excitability and apoptosis, which can lead to neuronal loss in epilepsy. This review focuses on the roles of oxidative damage, mitochondrial dysfunction, NAPDH oxidase, the blood–brain barrier, excitotoxicity, and neuroinflammation in the development of epilepsy. We also review the therapies used to treat epilepsy and prevent seizures, including anti-seizure medications, anti-epileptic drugs, anti-inflammatory therapies, and antioxidant therapies. In addition, we review the use of neuromodulation and surgery in the treatment of epilepsy. Finally, we present the role of dietary and nutritional strategies in the management of epilepsy, including the ketogenic diet and the intake of vitamins, polyphenols, and flavonoids. By reviewing available interventions and research on the pathophysiology of epilepsy, this review points to areas of further development for therapies that can manage epilepsy.

Keywords: epilepsy; oxidative stress; mitochondrial dysfunction; inflammation; antioxidants; antiepileptic drugs; antiseizure medications; neuromodulation; keto diet; nutrients

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1. Introduction

Epilepsy is a chronic neurological disorder characterized by unprovoked and repeated seizures that occurs in millions of people globally [1–9]. Epilepsy has serious cognitive, social, psychological, and economic consequences [10,11]. Epileptic seizures can seriously lower the quality of life when uncontrolled. Epilepsy arises from an increased frequency and synchrony of neuronal firing and an imbalance of excitatory neurotransmitters over inhibitory neurotransmitters [12]. Focal-onset seizures most frequently occur in the temporal lobe, making temporal lobe epilepsy (TLE) the most common form of epilepsy, which is also marked by impaired learning and memory [13–15]. Recurring and unpredictable partial complex seizures occur in TLE, which comprises 60% of all cases of epilepsy [16]. Status epilepticus (SE), another form of epileptic seizure defined by convulsive seizure activity lasting more than 5 min, results in high morbidity and mortality [17,18].

Epileptic seizures can lead to the death of neurons, which in turn promotes epileptogenesis and the occurrence of seizures [19–21]. The proposed mechanisms of epileptogenesis involve alterations in synapses, neurotransmitters, receptors, oxidative stress, mitochondrial dysfunction, cytokine signaling, and apoptosis [22,23]. A growing body of evidence links the development of epilepsy to the presence of oxidative stress and overproduction of reactive oxygen species (ROS) [24–26]. Prior to the onset of seizures, oxidative stress

induces neurological changes, including inflammation, neurodegeneration, and a lowered seizure threshold, resulting in epileptogenesis [24,27]. By altering Ca^{2+} homeostasis, oxidative stress hastens seizure onset, neurodegeneration, and neuronal excitability [28,29]. Experimental evidence indicates that inflammation in the brain is also associated with epilepsy [30,31]. Neuroinflammation has been observed in both animal models of epilepsy and patients with epilepsy [32–34]. Chronic neuroinflammation causes peripheral immune cells, astrocytes, microglia, and endothelial cells in the blood–brain barrier (BBB) to produce inflammatory molecules [35].

The discovery of novel anti-epileptic therapies necessitates understanding contributors to the onset of epilepsy to identify therapeutic targets [36]. This review focuses on the roles of oxidative stress, mitochondrial dysfunction, inflammation, NADPH oxidase (NOX), neuronal excitotoxicity, and BBB dysfunction in the pathogenesis of epilepsy. Anti-inflammatory medications, antioxidants, anti-epileptic drugs (AEDs), and anti-seizure medications (ASMs) are used to treat epilepsy and manage its progression [37–39]. Current knowledge on the treatment of epilepsy with AEDs and ASMs is presented in this review, along with information on the potential nutritional and pharmacological regulation of antioxidant capacity and inflammation in patients with epilepsy. We discuss the use of antioxidants, ASMs, and AEDs, including acetyl-L-carnitine (ALC), melatonin, N-acetylcysteine (NAC), baicalein, coenzyme Q10 (CoQ10), astaxanthin, curcumin, valproic acid, levetiracetam, cannabidiol (CBD), brivaracetam, and ursolic acid. Although a wide number of medications against epilepsy are available, approximately one-third of patients do not respond to currently available pharmaceuticals [40,41]. In addition, we discuss the use of non-pharmacological interventions such as neuromodulation, including vagus nerve stimulation (VNS), and surgery to treat epilepsy. We also present the role of diets, including the ketogenic diet, and nutrients, including vitamins, polyphenols, and flavonoids, in epilepsy treatment. A search was conducted in the PubMed/Medline database using appropriate keywords (epilepsy, oxidative stress, mitochondrial dysfunction, inflammation, antioxidants, antiepileptic drugs, antiseizure medications, neuromodulation, keto diet, and nutrients). This search revealed a rapid expansion of the literature on the role of oxidative stress, mitochondria dysfunction, and inflammation on the pathogenesis of epilepsy, as well as treatment for epilepsy. Around 1000 articles were finalized for this review, and of those, 358 articles were used in this manuscript.

2. Epilepsy and Oxidative Stress

Oxidative stress, which can contribute to the onset of diseases such as epilepsy, describes an imbalance between the generation and removal of ROS/reactive nitrogen species (RNS) [42–44]. Aerobically active organs are particularly susceptible to the generation of free radicals and ROS because of the premature leakage of electrons from the electron transport chain [45–47]. One product of the transfer of electrons to O_2 is superoxide anions ($O_2^{\bullet -}$). To counteract this, superoxide dismutase (SOD) converts $O_2^{\bullet -}$ to H_2O_2, which is converted by glutathione peroxidase (GPX) and catalase (CAT) to water and oxygen [48–53]. At baseline, 1–5% of a cell's oxygen consumption is used to generate ROS, but this rate can be elevated by altered mitochondrial homeostasis, such as that in the setting of Ca^{2+} overload [47]. Oxidative stress ultimately causes cellular damage through lipid, DNA, and protein oxidation [54–62]. Oxidative damage can occur, especially in the iron–sulfur clusters in complexes I and III of the electron transport chain [56]. Due to its high metabolic requirements, the brain actively conducts aerobic metabolism, which makes it uniquely vulnerable to oxidative stress [63]. In addition, iron is abundant in the brain because of its necessity for neurological functioning, although its presence also increases the susceptibility to oxidative stress [64]. Seizures have been observed to induce ROS/RNS production, resulting in oxidative stress and subsequent cellular damage [65,66]. Inhibiting ROS production has been indicated to prevent the neuronal damage that accompanies epileptic seizures [67,68].

Clinical and experimental studies indicate that oxidative stress is both a cause and consequence of the progression of epilepsy [69,70]. Various models of epilepsy are asso-

ciated with increases in the levels of oxidative stress biomarkers [71]. For example, in chemical convulsion models of epilepsy induced by the administration of pentylenetetrazol (PTZ), kainic acid (KA), or pilocarpine, the levels of F2-isoprostanes, which are markers of lipid peroxidation, were increased in brain areas, including hippocampal regions [71]. At the same time, the activities of antioxidant enzymes, including SOD, CAT, and GPx, were reduced [62,71]. Patients with TLE also displayed greater levels of peripheral blood markers of oxidative damage [72,73]. Patients with SE exhibited decreased plasma activities of SOD, CAT, and glutathione (GSH) and decreased serum total antioxidant capacity [74].

Epileptic seizures induce oxidative stress, which can cause further neuronal damage and lead to the development of subsequent seizures in a chain reaction [75]. Acute seizures result in excess ROS formation through increased mitochondrial dysfunction and increased NOX activity [76–78]. Additionally, glutamate receptor activation and excitotoxicity, which are two mechanisms of brain injury in epilepsy, contribute to oxidative stress [79]. The persistent neuronal firing that accompanies epilepsy can lead to the formation of free radicals, which can leak from the electron transport chain and react with oxygen to cause oxidative stress [43]. Consistent with this, persistent epileptic seizures have been found to result in nucleic acid, lipid, and protein oxidation, leading to cellular damage [70].

Both animal models and genetic studies support that oxidative and nitrosative stress induced by recurrent seizures leads to neuronal death [18,80]. The development of epilepsy is associated with neuronal loss through apoptosis [69]. For instance, patients with epilepsy exhibit a progressive decline in hippocampal size, resulting in additional severe seizures and cognitive deficits [69]. A single instance of SE in animal models produces long-standing changes within mitochondria, including mitochondrial DNA (mtDNA) damage and excess hydrogen peroxide production in the inner mitochondrial membrane [70]. One mechanism by which oxidative stress has a causative role in epilepsy is by inducing neuronal hyperexcitability, which is a key feature of epilepsy [70]. Moreover, mtDNA mutations that cause metabolic dysfunction in neurons can give rise to genetic epilepsy, further indicating that oxidative stress can contribute to epileptogenesis [68,70]. Intracellular damage induced by ROS is frequently observed in epileptic brain samples following surgical resection, which is consistent with the potential causative role of oxidative stress in epileptic processes, including neurodegeneration and neuronal hyperexcitability [73,79]. Figure 1 illustrates the potential interactions between seizures, oxidative stress, mitochondrial dysfunction, neuroinflammation, antioxidants, antiseizure medications, antiepileptic drugs, anti-inflammatory agents, nutrients, and the keto diet.

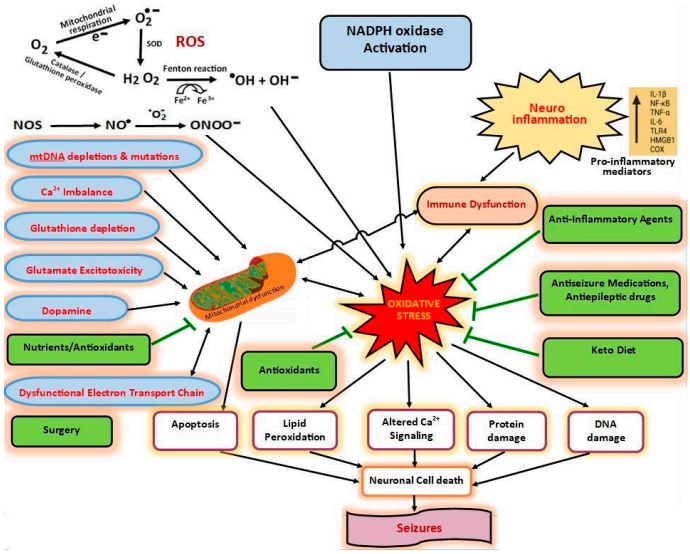

Figure 1. Potential interactions between seizures, oxidative stress, mitochondrial dysfunction, neuroinflammation, antioxidants, ASMs, AEDs, anti-inflammatory agents, nutrients, and keto diet.

3. Epilepsy and Mitochondrial Dysfunction

Mitochondria are organelles that function in energy generation, which is crucial for neuronal activity [81]. The brain's high energy requirements make it dependent on mitochondria, which are involved in neurotransmitter synthesis, Ca^{2+} sequestration, redox signaling, and cell death [81]. Mitochondria are essential in ATP synthesis through oxidative phosphorylation, as well as fatty acid oxidation, glutamate and urea metabolism, and antioxidant activity regulation [19,82,83]. Mitochondrial dysfunction leads to altered neurotransmission and neuronal excitability [84,85]. Because mtDNA is close to the site of ATP synthesis, its 37 genes are especially susceptible to oxidative damage [86]. ROS can leak from the mitochondrial electron transport chain, thereby contributing to oxidative damage in the mitochondria, mitochondrial dysfunction, and subsequent tissue injury [82,87,88].

Several forms of epilepsy are associated with impaired mitochondrial function and increased ROS generation [77,89,90]. Moreover, mitochondrial dysfunction has been proposed as one cause of seizure occurrence in epilepsy [79,91]. This is supported by epileptic seizures being a symptom of genetic mitochondrial diseases involving mtDNA and nuclear DNA mutations [92]. Specifically, mtDNA damage has been suggested to contribute to the development of epilepsy [93]. mtDNA oxidative damage and increased mitochondrial hydrogen peroxide were observed in a KA-induced TLE model [94]. Studies in rats treated with KA and pilocarpine also indicated that mitochondrial oxidative stress results in oxidative damage to DNA during epileptogenesis [95]. Similarly, animal models of epilepsy induced by homocysteic acid were observed to have mitochondrial dysfunction [91].

One manner in which mtDNA damage from oxidation can cause epileptogenesis is through inhibiting mitochondrial base excision repair, leading to neuronal apoptosis [94,96]. Additionally, ROS can promote the opening of the mitochondrial permeability transition pore (MPTP), which leads to an efflux of ions and mitochondrial molecules that ultimately cause cell death [73]. Decreases in neuronal ATP and increased mitochondrial Ca^{2+} levels have been observed during seizures [83]. An excess of mitochondrial Ca^{2+} can lead to the generation of ROS through xanthine oxidase activation and through other pathways, as well as to the production of RNS [97].

4. Lipid Peroxidation

Epileptic seizures can also cause oxidative damage to intracellular lipids [98,99]. Polyunsaturated fatty acids within phospholipid bilayers surrounding cells and organelles are particularly vulnerable to oxidation [98]. Similar to protein oxidation and mtDNA damage, the brain is also at risk of lipid peroxidation following seizures [98]. After seizures, Ca^{2+} can activate phospholipase A_2, which releases arachidonic acid [98]. The metabolism of arachidonic acid can lead to the further formation of ROS. The peroxidation of arachidonic acid leads to the generation of F2-isoprostanes and isofurans through catalysis by free radicals [100]. After KA administration, seizures were observed to increase F2-isoprostane and isofuran levels in several hippocampal regions [101]. The appearance of additional lipid peroxidation markers, including 4-hydroxy-2-(E)-nonenal and malondialdehyde, indicated oxidative damage to lipids occurring within 4 h into an SE episode and up to 24 h afterward [102]. This suggests that lipid peroxidation is a consequence of seizure activity, and it may be a component of epileptogenesis.

5. Epilepsy and Inflammation

Inflammatory molecules can bind to surface receptors on neurons and other brain cells to activate signaling pathways [103]. Accumulating evidence suggests that inflammation contributes to seizure onset and epileptogenesis [104–106]. Signaling downstream of inflammation can lead to neuronal damage, which contributes to the clinical manifestations of pathology [107,108]. Meanwhile, seizures can induce neuroinflammation, and repetitive seizures might result in chronic inflammation [109]. This can lead to a disruption of the brain's cytokine balance, further contributing to the progression of epilepsy. The production of inflammatory cytokines induces the generation of free radicals and alters glutamater-

gic synaptic transmission in a manner that promotes excitotoxicity [110,111]. In chronic epilepsy, long-term hyperexcitability and impaired synaptic transmission are observed in central nervous system (CNS) tissue following persistent inflammation [112,113]. In addition, neuroinflammation attributable to brain injury from repetitive seizures can lead to glial activation, which contributes to the occurrence of secondary seizures [31].

Several studies indicated that repetitive epileptic seizures are associated with increased levels of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6; additionally, they are associated with increases in the protein expression of caspase-3, BAX, and BH3, which are involved in apoptosis and neurodegeneration [39,64,114,115]. After seizures, patients with epilepsy were observed to have elevated serum and cerebrospinal fluid TNF-α, IL-1β, IL-6, and IL-1 receptor antagonist levels [116,117]. The onset and spread of seizures were also found to induce rapid regional inflammatory responses in animal models of epilepsy [118]. Chronic neuroinflammation can contribute to epilepsy through mechanisms such as elevated TNF-α expression, promoting hyperexcitability and the activation of AP-1, which regulates apoptosis through pathways including JNK signaling [114,115]. This is consistent with apoptosis being a cause of neuronal death during the progression of epilepsy [119]. Cytokine production after seizures is observed in various cell types in areas of seizure onset, including glia, myeloid cells, and neurons [120,121]. The release of cytokines from microglia is suggested to support epileptogenesis by aggravating oxidative stress in the mitochondria [114,122].

Anti-inflammatory drugs hold promise for treating epilepsy, with favorable clinical evidence supporting inflammation suppression as a strategy for ameliorating the pathology of epilepsy [108,123]. Therefore, anti-inflammatory drugs may be beneficial in managing epilepsy, preventing seizure progression, and protecting against cognitive deficits [38,39].

6. Epilepsy and NOX

NOX is an enzyme complex that generates cellular ROS and promotes neurodegeneration, neurotoxicity, and memory deficits. Therefore, it has been suspected to be involved in epileptogenesis [24,124]. The NOX family includes seven isoforms (NOX1–5, DUOX1, DUOX2) that generate H_2O_2 by transferring an electron from NADPH to oxygen [125]. A mouse model of epilepsy induced by PTZ treatment displayed oxidative stress, altered neurotransmission, memory deficits, and anxiety-like and depression-like behavior, which were alleviated by NOX inhibition [42].

Accumulating evidence indicates that NOX is a mediator of epilepsy progression [10]. In animal models of epilepsy, NOX has been shown to be a key source of ROS during seizures and a contributor to neuronal death and neurodegeneration [24,77,126,127]. In particular, NOX2 is a major source of ROS generated in the presence of seizure activity [83]. NOX activation after PTZ treatment, along with mitochondrial damage, leads to ROS/RNS formation, decreased antioxidant enzyme levels, lipid peroxidation, elevated nitrite levels, and ultimately limbic neurodegeneration [128,129]. In addition, NOX2 elevation has been observed in neurons and glia of surgically resected sites where seizure activity originates in patients with refractory epilepsy. This further suggests that NOX2 activity is involved in epileptogenesis [130]. NOX2 activation has been observed in early epileptic seizures attributable to hyperactivated NMDA receptors [77,131,132].

Inhibition of NOX2 activity can suppress neuronal death caused by seizures in different models of epilepsy [77,133–135]. In one study investigating treatment with gp91ds-tat, a competitive inhibitor of NOX2, gp91ds-tat prevented cellular changes downstream of in vitro seizure-like activity, including Ca^{2+} oscillation, ROS formation, mitochondrial depolarization, and neuronal loss [24]. Additionally, gp91ds-tat treatment in a rat model 1 h after KA-induced SE led to reduced NOX2 expression and decreased cortical and hippocampal NOX activity [24]. Continuous intracerebroventricular injection of gp91ds-tat also decreased the occurrence of seizures in a rat model of epilepsy [24]. Overall, the anti-seizure activity of gp91ds-tat suggests that NOX2 can contribute to epileptogenic processes, including seizure development, oxidative stress, and ROS formation [24].

7. Epilepsy and Excitotoxicity

A fundamental feature of the pathogenesis of epilepsy is an imbalance between excitatory and inhibitory neurotransmission [136]. The levels of glutamate, an excitatory neurotransmitter, have been reported to be unusually elevated in both patients with epilepsy and animal models of epilepsy [10]. One consequence of excessive glutamatergic neurotransmission and glutamate receptor activation is oxidative stress, which leads to excitotoxicity, one form of neuronal apoptosis [62]. Glutamate promotes the activation of NMDA receptor-mediated Ca^{2+} influx into neurons [10]. Accumulated Ca^{2+} leads to neuronal depolarization, ROS formation through the arachidonic acid cascade, and eventual apoptosis [135,137]. ROS downstream of Ca^{2+} influx can further alter glutamate receptors, damage glutamate transporters, and contribute to oxidative stress by reducing GSH production [138,139]. This leads to a state of hyperexcitability and eventual neuronal death. Excessive ROS generation is a prerequisite for neuronal excitotoxicity, which is a well-characterized feature of epilepsy [10].

Meanwhile, GABA is the major inhibitory neurotransmitter of the CNS [136]. GABA can bind to $GABA_A$ receptors, which are heteromeric ligand-gated Cl^- channels. Therefore, GABA stimulates these receptors to permit an influx of Cl^- [136]. These ions decrease depolarization in neurons to dampen the effects of excitatory signals [140]. When the inhibitory input from GABA binding to $GABA_A$ receptors is inhibited, neurons undergo hyperexcitability and apoptosis [141]. ROS modulates both synaptic and extrasynaptic inhibition by GABA at hippocampal and cerebellar $GABA_A$ receptors [142,143]. Because of GABA's role in epilepsy, GABA receptors are targets of several anti-seizure drugs.

8. BBB Dysfunction

The BBB consists of endothelial cells that limit the transfer of molecules and pathogens between the bloodstream and brain tissue [144]. The BBB's tight junctions protect the brain against infection and maintain homeostasis by strictly regulating the influx and efflux of substances [144]. Leakage of the BBB is proposed to be both a cause and consequence of epileptic seizures [145]. Glutamate signaling in seizures can increase the expression of matrix metalloproteinase, a tissue-remodeling enzyme that degrades extracellular matrix components. It can also cause reduced tight junction protein expression [146]. These two mechanisms contribute to BBB leakage triggered by seizures. Conversely, BBB leakage can also aggravate epilepsy [147]. Blood leakage through the BBB can increase the extracellular levels of glutamate and potassium, which increase neuron excitability and reduce the seizure threshold, increasing the likelihood of seizures [148]. The entry of albumin and other serum proteins also induces neuronal hyperexcitability and inflammation through cytokine production [147]. A disrupted BBB could also permit more leukocytes to enter the brain, potentially contributing to epileptogenic neuroinflammation [145]. Another way in which the BBB can affect the course of epilepsy is by blocking the entry of ASMs and AEDs and increasing their efflux from the brain, which can result in treatment-resistant epilepsy [145].

9. Epilepsy and Antioxidants (Antioxidant Therapies)

Antioxidants are molecules that counteract ROS, which, if uncontrolled, can lead to oxidative stress [149–152]. There are many substances with antioxidant properties, including vitamins A, C, and E; polyphenols; and GSH, the functions of which are aided by several antioxidant enzymes [40]. Antioxidants can balance ROS by a number of molecular mechanisms. For instance, they can restrict ROS generation either physically or by binding metal ions. Once ROS are generated, antioxidants function to neutralize ROS, chemically quench their activity, or otherwise catalyze their neutralization [153]. They can also disrupt radical chain reactions, scavenging ROS before they are able to cause cellular damage [153].

Due to their major neuroprotective role, antioxidant therapy has increasingly been considered a promising approach for treating diseases involving neurodegeneration [149,154]. Research in this area has suggested that antioxidants such as vitamin C, vitamin E, polyphenols,

melatonin, lipoic acid, and NAC effectively limit oxidative-stress-associated neurodegeneration in drug-resistant epilepsy [99]. Therefore, antioxidant therapy aimed at decreasing oxidative stress can be helpful in alleviating seizures in patients with drug-resistant epilepsy [155]. Specifically, recent studies demonstrated that antioxidants protect cells from the neurotoxic effects of seizures [156,157]. For instance, vitamin E has been shown to effectively inhibit ferroptosis, one method of neuronal death, following epileptic seizures [10]. In another study, Alzoubi et al. investigated the effect of vitamin E supplementation on epileptic seizures by feeding rats with control, a high-fat diet (HFD), vitamin E, or vitamin E combined with an HFD over 6 weeks [149]. They found that although the HFD normally increased susceptibility to PTZ-induced seizures, this effect could be prevented by vitamin E supplementation, likely through its strengthening of the hippocampal antioxidant mechanism [149]. Although antioxidants have multiple forms and sources, medicinal plants have been increasingly studied as sources of natural antioxidants, including phenolic acids, carotenoids, and flavonoids, which exhibit particularly strong antioxidant properties [158].

9.1. Acetyl-l-carnitine

ALC is a modified amino acid that naturally occurs in the body and can cross the BBB, allowing it to exert neuroprotective effects by inhibiting oxidative stress and apoptosis, as well as glial activation and neuroinflammation [159,160]. Research has demonstrated that through these mechanisms, ALC can effectively attenuate SE. In 1 study using a KA model of TLE, rats treated with 100 mg/kg ALC showed reduced neuronal loss and seizure intensity and attenuated a higher incidence of SE [29].

9.2. Melatonin

Melatonin has been shown to have neuroprotective effects in human epilepsy and in various animal models [161–163]. For instance, prior studies demonstrated that melatonin reduced the incidence of iron-induced seizures and increased the initial seizure latency in pilocarpine- and penicillin-induced seizure models [164]. Some researchers reported the therapeutic effects of melatonin in the PTZ model, which potentially involved the regulation of GABA receptors and the inhibition of neuronal nitric oxide synthase activity to interfere with glutamatergic pathways. Similarly, studies using the KA model found that melatonin prevented the neurotoxic effects of seizures, including ROS production, mtDNA damage, lipid peroxidation, hippocampal cell loss, and decreased GSH and mitochondrial complex II activity [165–167]. It is worth noting that one study found no significant neuroprotective effects of melatonin in PKZ and KA models [168]. Overall, research suggests that melatonin is an effective component of strategies for treating epilepsy.

9.3. NAC

NAC is a precursor to GSH that is used clinically to prevent the oxidative stress-induced depletion of GSH [169,170]. NAC also counters oxidative stress through its own antioxidant properties, including donating sulfhydryl groups to directly scavenge free radicals [171]. A study in which NAC was administered at 500 mg/kg twice daily along with 5 mg/kg sulforaphane daily in a rat model of SE observed a substantial neuroprotective effect [171]. NAC and sulforaphane treatment led to a 70% decrease in seizure frequency, a 30% increase in the time to the onset of epileptic seizures, and the amelioration of cognitive impairments accompanying epileptogenesis [172]. In another study of a fluid percussion injury model of epilepsy in rats, chronic NAC treatment reduced the seizure threshold to a level comparable to that of PTZ-induced seizures as opposed to what would be expected following brain injury [173]. Additionally, patients with Unverricht–Lundborg disease, a form of genetic epilepsy, tolerated NAC well, and they had a reduced seizure burden after several months of treatment [174].

9.4. Baicalein

Baicalein is another compound with bioactive properties relevant to protection against neurodegeneration in various brain disorders [175,176]. One study examined the effects of baicalein injections in rats with spontaneous recurrent seizures. Although there was no apparent reduction in the frequency of these spontaneous recurrent seizures, rats treated with baicalein showed better cognition and reduced mossy fiber sprouting and hippocampal cell loss [1]. These results were attributed to baicalein's antioxidant and anti-inflammatory properties, the regulation of synapse-associated proteins, and the recovery of glucocorticoid pathway function, all of which were observed in this study [1]. These findings indicate that baicalein is a beneficial adjuvant therapy in epilepsy.

9.5. CoQ10

CoQ10 is a potent endogenous antioxidant that protects against ROS generation and oxidative damage [177,178]. CoQ10 both directly scavenges free radicals and indirectly regenerates other antioxidant compounds, including vitamin E, to exert antioxidant effects [179]. CoQ10 deficiency can contribute to the clinical manifestations of epilepsy [177]. Supporting this, one study found that patients with epilepsy had significantly lower CoQ10 levels than healthy controls [177]. In this study, decreased serum CoQ10 levels were correlated with more frequent seizures and a longer duration of epilepsy. CoQ10 has also shown promising effects when used in combination with traditional anti-epileptic drugs. In one study, CoQ10 and valproic acid reduced oxidative stress and prevented histopathological damage to the brain and liver more effectively than valproic acid alone [26]. This suggests that the administration of CoQ10 and valproic acid in combination can prevent the hepatotoxicity of valproic acid while potentiating its anti-epileptic activity [26]. Another study examined the efficacy of CoQ10 along with the ASM phenytoin in rats with pilocarpine-induced seizures. In this study, CoQ10 reduced the severity of seizures and alleviated oxidative stress [180]. Together, these studies suggest that CoQ10 can also be an effective and well-tolerated adjuvant therapy for epilepsy.

9.6. Astaxanthin

Astaxanthin is a carotenoid found in microalgae, yeast, and marine organisms, including salmon, shrimp, krill, and crayfish [181]. Astaxanthin can easily cross the BBB without causing toxicity [182]. This strong antioxidant decreases ROS generation and prevents oxidative damage [183–185]. Moreover, astaxanthin has anti-apoptotic, anti-inflammatory, and immune-enhancing activity [186–188]. In various neurological disorders, astaxanthin was found to mitigate brain damage and cognitive deficits [189]. A study of rats treated with astaxanthin starting shortly after SE onset found that treatment improved cognitive performance in a test of spatial memory [181]. Astaxanthin treatment reduced the inflammation observed in the brains of these rats, and this anti-inflammatory mechanism might be responsible for its neuroprotective effects [181].

10. Epilepsy and AEDs

More than two dozen AEDs are currently available for the treatment of epilepsy [190,191]. Pharmacologic strategies achieve seizure remission in an estimated 65–80% of patients with epilepsy [192,193]. AEDs can be used alone or in combination, although they are often used as monotherapy to prevent toxicity [194]. Classical AEDs such as valproic acid, levetiracetam, and benzodiazepines are frequently used as a first-line treatment against myoclonic seizures [195].

10.1. Valproic Acid

Valproic acid is widely used with considerable efficacy in treating simple and complex seizures during epilepsy. [196–199] It can be used as either monotherapy or polytherapy. In one study, valproic acid treatment in PTZ-treated mice exhibited neuroprotection, including reduced histopathological alterations, improved behavioral symptoms, increased

antioxidant levels, and decreased inflammation, as evidenced by reduced TNF-α expression [183]. Furthermore, co-administration with astaxanthin offered greater benefits against epilepsy [183]. It is important to note that chronic valproic acid administration can increase ROS levels within cells, inducing the occurrence of seizures. Another risk of valproic acid is its reported hepatotoxicity, as evidenced by marked increases in serum levels of the aminotransferases AST, ALT, and ALP in rats treated with valproic acid [200]. Interestingly, co-administration of ellagic acid reduced valproic acid-induced hepatic injury in these rats [200].

10.2. Levetiracetam

Levetiracetam is a more recent AED that is effective in the control of partial-onset seizures [201–205]. Levetiracetam's proposed mechanism of action is its ability to bind to synaptic vesicle protein 2A (SV2A), which prevents Ca^{2+} release from presynaptic neurons [206,207]. In this manner, levetiracetam can act as a neuromodulator. Compared to the characteristics of older AEDs, levetiracetam is thought to be more efficacious with lower toxicity [208,209]. In 1 study involving 145 people in a group receiving levetiracetam, it was found that SE resolved and functioning was enhanced in 47% of patients [210]. There was 1 meta-analysis on levetiracetam in children with focal seizures that found a 55% median reduction in seizure occurrence [194]. There was 1 group that conducted a randomized, double-blind study of 114 children and adults who had at least 12 seizures in the previous year despite pharmacological treatment [194]. The group that was provided levetiracetam as an adjunctive therapy had a 38.7% reduction in seizure frequency, compared to 14.3% in the group provided a placebo [194]. Notably, levetiracetam was effective in alleviating refractory epilepsy in both adults and children [194]. Similarly to other AEDs, levetiracetam might also be effective as one element of polytherapy. In PTZ-injected rats, the combination of levetiracetam and sodium selenite was more protective than levetiracetam monotherapy in delaying epilepsy progression and improving performance on behavioral tests [211].

11. Epilepsy and ASMs

The majority of currently available ASMs reduce neuronal excitability and seizure occurrence, although they might not treat the underlying etiology of epilepsy [212–215]. Many ASMs exert an anti-convulsive effect by repressing excitatory neurotransmission through their targeting of ion channels [216–218].

11.1. CBD

CBD is a cannabinoid without psychoactivity that has been investigated as an adjuvant for AEDs [219–221]. This is due to CBD's anti-inflammatory properties, including its ability to prevent microglia activation and the release of inflammatory factors from astrocytes [222,223]. The efficacy of CBD in reducing seizure frequency has been demonstrated in both humans and animal models [224,225]. For instance, CBD has proven beneficial in clinical trials for medically refractory epilepsy syndromes [226]. A survey of 117 parents of children with epileptic spasms or Lennox–Gastaut syndrome found that 85% of participants felt CBD improved seizures, and 14% observed a complete absence of seizures when CBD was used [223]. Furthermore, a study analyzing 580 children and adults with drug-resistant epilepsy found that 12 weeks of CBD treatment reduced the median convulsive seizure frequency per month by 51% and total seizure frequency by 48% [223]. Additional evidence supporting the use of CBD in epilepsy comes from an open-label study of 162 patients with epilepsy originating in childhood. CBD treatment for 12 weeks reduced the monthly seizure frequency by an average of 36.5% [227].

CBD has also been shown to be effective as an adjunctive therapy alongside other ASMs. This is supported by both case studies and clinical trials. In one report, three pediatric patients with medically refractory epilepsy from Rasmussen encephalitis were provided adjunctive CBD along with their ASMs [228]. The inclusion of CBD offered clinical benefits beyond what would be expected from including an additional ASM in the

treatment regimen [228]. Moreover, in four randomized clinical trials, CBD administered as an adjunctive therapy more effectively reduced seizure frequency than a placebo in patients with Lennox–Gastaut syndrome and Dravet syndrome [229].

11.2. Brivaracetam

Brivaracetam is a recently approved ASM that is being used as an adjunctive therapy for patients with focal seizures [230–234]. Brivaracetam has a similar mechanism of action as levetiracetam in that it exhibits high-affinity binding to SV2A vesicles. Additionally, it shows linear pharmacokinetics [235]. Some evidence indicates that brivaracetam is also effective in pediatric patients with focal seizures [236]. In 1 study analyzing 34 such patients aged 3–17 years, 16 patients responded significantly after 3 months of brivaracetam treatment. Ten of these patients had complete resolution of focal seizures [235]. A study of 200 adults with medically refractory epilepsy who were treated with brivaracetam found that 23% experienced at least a 50% reduction in seizure frequency [231]. Other research indicated that 50 mg/day of brivaracetam is an effective dose to significantly reduce seizure frequency [237]. This dose was also well tolerated, with rare adverse effects. Another use of brivaracetam and levetiracetam is SE treatment, allowing the two drugs to be used in emergency cases [238]. Although the two drugs have a similar mechanism of action, brivaracetam is suggested to be less likely than levetiracetam to cause adverse behavioral effects [239,240]. Therefore, some patients would benefit from switching from levetiracetam to brivaracetam [239,240].

11.3. Ursolic Acid

Ursolic acid has been demonstrated to prevent oxidative stress by inhibiting ROS generation [241–244]. It has also been shown to have anti-inflammatory effects, including inhibiting MAPK signaling to prevent NF-κB translocation and subsequent secretion of inflammatory compounds [245]. Through its attenuation of oxidation and inflammation, ursolic acid can exert a substantial neuroprotective effect [216,246]. In one study, these properties of ursolic acid allowed it to decrease seizure susceptibility and improve cognitive dysfunction in rats injected with pilocarpine [216]. During SE, GABAergic interneurons are often damaged or lost, which removes inhibitory signals by GABA from the neural circuitry [247]. Notably, ursolic acid has been observed to preserve GABA levels by inhibiting GABA transaminase [248]. Moreover, ursolic acid was found to prevent the loss of GABAergic interneurons in the previously described pilocarpine-induced rat model [216]. This suggests enhanced inhibitory neurotransmission as a possible mechanism by which UA dampens the cellular effects of SE.

11.4. Curcumin

Curcumin, which is produced by the herb *Curcuma longa*, possesses a broad range of activities, and it has been used as a traditional remedy for seizures [249–252]. The antioxidant properties of curcumin have been demonstrated in various epilepsy models, including KA, amygdala kindling, and post-kindled models [149,253–255]. Moreover, curcumin was found to prevent the spread of electrical activity to form generalized seizures in an iron-induced epilepsy model [256]. Similarly, *C. zedoaria* extracts were used as a treatment in rats kindled with PTZ injection [257]. *C. zedoaria* extract, which contains compounds including curcumin, elevated the tonic seizure threshold and decreased mortality [257]. Moreover, *C. zedoaria* extract improved performance in learning and memory among these rats, with one potential mechanism for this benefit being the extract's enhancement of GABAergic signaling [257].

12. Epilepsy and Neuromodulation

Neuromodulation is a palliative treatment for patients with chronic drug-resistant seizures [40,258–261]. It encompasses the application of direct or induced electric currents to alter neural activity. Neuromodulation has been pursued as a strategy to reduce the

occurrence and duration of seizures in patients with epilepsy who do not respond well to medication [262,263]. Neuromodulation consists of both invasive and non-invasive therapies. Invasive methods include VNS, deep brain stimulation, which uses implanted electrodes, and responsive neurostimulation, which is activated when a seizure is detected [264–268]. Less-invasive treatment options include transcutaneous VNS, transcranial direct current stimulation, and trigeminal nerve stimulation [269–275]. As a whole, neuromodulation strategies can induce a 30%–40% decrease in seizure occurrence after 3 months of treatment [258]. Only a small fraction of people maintain a total absence of seizures for at least 1 year after neuromodulation, but the majority have over a 50% decrease in the frequency of seizures [258].

12.1. VNS

VNS entails the use of a pulse generator to administer periodic electrical impulses to the vagus nerve [40,276,277]. This method can be especially beneficial in patients with medically refractory epilepsy who would also not be indicated for curative surgical treatment [278,279]. VNS achieved a greater than 50% reduction in seizure frequency in half of the patients, although fewer than 5% experienced total resolution of seizures [280,281]. VNS is effective even over a long period, and its ability to control seizures can improve over time [40]. The vagus nerve may inhibit the formation of seizures in more excitable regions of the brain, including the thalamus, thalamocortical projections, and limbic system [258]. This presents one mechanism of action for VNS in epilepsy. In addition, VNS increases serotonin and norepinephrine release through its activation of the raphe nuclei and locus coeruleus. Increased serotonin and norepinephrine transmission can be preventive against epilepsy [282,283].

12.2. Epilepsy and Surgery

Surgical interventions for epilepsy include curative procedures, palliative procedures such as corpus callosotomy, and implantation of devices for neuromodulation [284]. In its curative form, surgery can limit seizure spread and reduce seizure frequency by removing cortical areas that are necessary for the generation of seizures [41,285]. However, curative surgery prioritizes the preservation of normal cognitive abilities [40]. The ability of curative surgery to completely eliminate epilepsy is influenced by many variables, including epilepsy type, etiology, and the extent of resection [286]. Overall, surgery is a highly safe and efficacious option for treating epilepsy, although it has been underutilized [287,288]. Some evidence indicates that surgery can be more effective than medication for some patients with TLE [289]. In one study, patients with medically refractory TLE were randomized to either receive temporal lobe resection or continue drug therapy [290]. In total, 58% of patients who underwent surgery experienced complete elimination of seizures at a 1-year follow-up, compared to 8% of patients on AEDs [290]. Surgical removal of the sites of seizure origination may be a necessary strategy for patients with multidrug-resistant epilepsy [18]. For the third of patients with focal epilepsy who cannot find symptom control with medications, surgery offers an opportunity to alleviate or resolve seizures [284].

13. Epilepsy and Diet Therapy

The ketogenic diet consists of high fat content, sufficient protein levels, and extremely low carbohydrate intake [291–294]. It has classically been used as a dietary treatment for epilepsy [295–298]. Several trials described the efficacy of ketogenic diets in patients with pediatric epilepsy. A randomized controlled trial found that 38% of pediatric patients on a ketogenic diet had at least a 50% reduction in seizure frequency after 3 months, compared to only 6% of controls [299]. Furthermore, 7% of the ketogenic diet group had a near-total seizure reduction of at least 90%, which was not observed in any controls [193]. In another study evaluating 6 months of ketogenic diet consumption, the overall seizure frequency in pediatric patients was reduced by 70.79%, and the seizure severity was decreased by 35% [300]. The ketogenic diet is especially beneficial as a treatment option for medically

refractory epilepsy when pharmacological strategies do not provide sufficient seizure control [301]. In trials of pediatric patients with drug-resistant epilepsy, the ketogenic diet can decrease the seizure frequency by more than 50% in up to half of the participants [302,303]. For instance, 1 study of 90 children <6 years old included controls, patients with refractory epilepsy treated with AEDs, and patients with refractory epilepsy on a ketogenic diet [301]. Compared to the group on AEDs, the group on a ketogenic diet had a lower seizure frequency and severity, as well as higher total antioxidant capacity [301].

Consistent with this finding, the ketogenic diet is believed to function in part through its antioxidant mechanism [304]. It can increase the GSH availability within cells and protect mtDNA from oxidative damage while reducing ROS formation within mitochondria [3,98,305]. The ketogenic diet can result in the formation of ketone bodies, which can generate acetyl-CoA for ATP synthesis and reduce ROS generation [306,307]. This also prevents the opening of the MPTP and subsequent release of excess Ca^{2+} [308,309]. These activities of the ketogenic diet contribute to its protective effect against oxidative stress. The ketogenic diet has additionally been demonstrated to have anti-inflammatory activity in an animal model of spinal cord injury [310]. The ketogenic diet may modulate neuroinflammatory pathways that cause seizure-induced neuronal loss [311,312]. The ketogenic diet might also enhance GABA production and inhibit glutamate synthesis, thereby exerting effects against epilepsy [313]. This is corroborated by evidence that patients on a ketogenic diet have higher cerebrospinal fluid levels of GABA [306].

14. Epilepsy and Nutrients

Nutrients with known antioxidant or anti-inflammatory activity include vitamin A, vitamin C, omega-3 fatty acids, polyphenols, and carotenoids [314–319]. Vitamins can offer benefits against epilepsy, especially when used as an adjunctive therapy [320]. Multivitamin therapy, including vitamin B6, vitamin B9, vitamin D, vitamin E, and CoQ10, administered adjunctively, reduced the average monthly seizure frequency from nine to two [321]. After 6 months of treatment, 63% of individuals had at least a 50% decrease in seizure occurrence [321]. Although vitamin B6 has specifically been demonstrated to lead to better outcomes in epilepsy, it is important to note that it does not provide benefits for all patients [322–325].

Vitamin D supplementation is also promising for epilepsy treatment, particularly because several investigations have observed vitamin D deficiencies among patients with epilepsy [326–329]. In 1 clinical trial, a treatment arm investigated 4 weeks of treatment with 4000 IU/day of vitamin D3, followed by 4 weeks of treatment with 16,000 IU/day of vitamin D3 [213]. This treatment group had nearly a 70% decrease in the average seizure frequency [213]. Among individuals with medically refractory epilepsy and vitamin D3 deficiency, administering vitamin D3 was found to reduce seizure frequency by up to 40% [330]. In a pediatric study of 648 children with epilepsy, vitamin D supplementation also led to more effective seizure control [329]. The findings from clinical trials were corroborated by animal models of epilepsy, in which vitamin D administration had anti-seizure activity and vitamin D receptor knockout mice had more frequent seizures [331].

A study in which 400 IU of vitamin E were administered to patients with epilepsy for 3 months recorded a nearly 60% reduction in seizure frequency [323]. Vitamin E has been especially promising as long-term adjunctive therapy in refractory epilepsy [332,333]. As an antioxidant, vitamin E promotes the clearance of ROS and prevents oxidative damage to proteins and lipids [334,335]. In rats with pilocarpine-induced seizure, vitamin E provided neuroprotection, evidenced by its ability to increase CAT levels and mitigate the increase in free fatty acid levels in the brain [336].

Another vitamin that could offer benefits for patients with epilepsy is vitamin C, especially because this group has been observed to have lower serum vitamin C levels [337]. In several models of epilepsy, including pilocarpine-, PTZ-, and penicillin-induced epilepsy, vitamin C improved seizure control and outcomes, such as mortality and seizure latency [338].

Studies of animal models of epilepsy revealed that vitamin C could mitigate oxidative stress, which might explain its ability to control seizures [339,340].

14.1. Fish Oil and Fatty Acids

Another nutrient that has reduced seizure frequency among patients with epilepsy is fish oil [341–343]. Because fish oil is established to be safe within a dose of 4 g/day, its administration as an adjunctive supplement could offer benefits in managing epilepsy with little risk of adverse effects [344]. There was 1 study that found that 0.6–2 g/day of fish oil decreased seizure frequency and duration. Conversely, some trials did not find an effect of fish oil and omega-3 polyunsaturated fatty acids on seizure suppression [345–347]. Short-chain fatty acids are also promising as part of an epilepsy treatment regimen because they have antioxidant and anti-inflammatory effects [348].

14.2. Magnesium and Zinc

Patients with epilepsy display reduced levels of magnesium, which has been proposed to be a cause of seizures [349–351]. Consistent with this, the severity of epilepsy is correlated with the degree of magnesium deficiency [352]. Zinc supplementation might also be beneficial for epilepsy, as evidenced by the administration of zinc in a PTZ-induced rat model of epilepsy. Zinc was found to mitigate epileptogenesis, prevent oxidative stress, and reduce neuroinflammation [353].

14.3. Polyphenols and Flavonoids

Polyphenols are compounds that can cross the BBB and serve as neuromodulators. Therefore, they are being considered for their potential to ameliorate CNS diseases [136,354,355]. In particular, polyphenols might be able to disrupt the course of epileptogenesis that gives rise to recurrent seizures [38]. For instance, the polyphenol resveratrol was found to prevent neurodegeneration in a KA model of SE and reduce oxidative stress and neuroinflammation [356]. Another study of a KA rat model of epilepsy found that 10-day resveratrol (15 mg/kg once daily) treatment prevented neuronal loss and decreased the frequency of seizures [357].

Flavonoids, as a class, are promising nutritional treatments for epilepsy and CNS disorders because of their antioxidant properties [136]. Additionally, flavonoids can increase the activity of GABA receptors, increasing the strength of inhibitory neurotransmission [136]. Some evidence suggests that the flavonoid quercetin can improve outcomes in epilepsy, especially because it was found to reduce inflammation in KA-induced epilepsy models [358]. As part of its anti-inflammatory effect, quercetin blocks microglial activation and pro-inflammatory cytokine secretion. Quercetin might especially be helpful as an adjunctive therapy because when administered with levetiracetam, it alleviated depression that was comorbid with epilepsy [358].

15. Conclusions

Epilepsy is a CNS disorder with a high prevalence that carries a significant burden through the presence of recurring seizures. Because the brain has the highest demand for oxygen consumption among all organs, it is especially vulnerable to oxidative stress and subsequent damage. ROS generation and oxidative stress can contribute to epileptogenesis and eventual neuronal death. In addition, oxidative stress can increase neuronal hyperexcitability and increase the likelihood of seizure occurrence. Another process contributing to epilepsy pathophysiology is mitochondrial dysfunction, which can induce neuronal death, a feature that has also been observed in epilepsy. Furthermore, neuroinflammation is proposed to be a key contributor to the onset and progression of epileptic seizures. In addition, seizures can contribute to processes such as oxidative stress and inflammation, leading to the progression of epilepsy.

Various therapeutic strategies are available to treat epilepsy, including AEDs, ASMs, and antioxidants. By preventing the accumulation of ROS and free radicals and guarding

against oxidative stress, antioxidants can address one aspect of epilepsy pathophysiology. Most ASMs that are presently available seek to target seizures rather than epilepsy pathophysiology, and they work by decreasing neuronal excitability. Although many medications are available to control epilepsy, approximately one-third of patients continue to have seizures that cannot be resolved with medication. These patients with medically refractory epilepsy can have a lower quality of life, cognitive deficits, and low mood. In these cases, other treatment options include surgery, neuromodulation, and dietary strategies. Understanding the consequences of diet therapies such as the ketogenic diet and specific nutritional supplements such as antioxidant vitamins can support the further development of nutritional strategies in epilepsy treatment. Targeting processes underlying epileptogenesis, such as oxidative stress, inflammation, and mitochondrial dysfunction, may be a fruitful area of investigation for new antiepileptic therapies. Increasing the range of available interventions may provide alternative treatment options for medically refractory epilepsy. This review provides an overview of several causative processes in epilepsy and how they correspond to specific treatment strategies. Through a discussion of epilepsy pathogenesis and promising therapeutic strategies, this review can provide insight into avenues for the future development of clinical interventions for epilepsy.

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Clinical, Sociodemographic, and Psychological Factors Associated with Transition Readiness in Patients with Epilepsy

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Abstract: Background: The transition to adult care for patients with epilepsy is a complicated clinical issue associated with adverse outcomes, including non-adherence to treatment, dropout of medical care, and worse prognosis. Moreover, youngsters with epilepsy are notably prone to emotional, psychological, and social difficulties during the transition to adulthood. Transition needs depend on the type of epilepsy and the epileptic syndrome, as well as on the presence of co-morbidities. Having a structured transition program in place is essential to reduce poor health consequences. A key strategy to optimize outcomes involves the use of transition readiness and associated factors assessment to implement the recognition of vulnerability and protective aspects, knowledge, and skills of these patients and their parents. Therefore, this study aims to provide a comprehensive framework of clinical and psychosocial aspects associated with the transition from pediatric to adult medical care of patients with epilepsy. Methods: Measures examining different aspects of transition readiness and associated clinical, socio-demographic, psychological, and emotional factors were administered to 13 patients with epilepsy (M_{age} = 22.92, SD = 6.56) with (n = 6) or without (n = 7) rare diseases, and a respective parent (M_{age} = 56.63, SD = 7.36). Results: patients showed fewer problems in tracking health issues, appointment keeping, and pharmacological adherence as well as low mood symptoms and moderate resiliency. Moreover, they referred to a low quality of sleep. Notably, parents of patients with rare diseases reported a lower quality of sleep as compared to the other group of parents. Conclusions: Increasing awareness around transition readiness is essential to promote self-management skills of patients with epilepsy and their parents. Anticipating the period of transition could be beneficial, especially to prevent problematic sleep patterns and promote independence in health care management. Parents of patients with epilepsy and rare diseases should be monitored for their mental status which can affect patients' well-being.

Keywords: transition readiness; epilepsy; psychological; parents

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1. Introduction

Epilepsy is a highly common chronic neurologic disease presenting unpredictable and recurrent seizures due to anomalous electrical activity in different areas of the cerebral cortex, resulting in a neuronal hypersynchrony or insufficient electrical inhibition [1]. The Task Force of the International League Against Epilepsy (ILAE) defined epilepsy as being characterized by one of the following criteria: (1) at least two unprovoked seizures in the past 24 h, (2) one unprovoked seizure and a ≥60% probability of having an additional

seizure over the next 10 years, or (3) reporting an epilepsy syndrome [2]. Epilepsy is one of the most common neurological diseases affecting all ages, with a worldwide prevalence rate of 6.38 per 1000 persons [3]. In Europe, the prevalence of epilepsy has been estimated with ranges varying from 3.3–7.8/1000 inhabitants in the general population [4] to 3.4–5.8/1000 in youngsters, with results largely depending on age-specific populations [5].

Consistently, as compared to other lifetime periods, higher prevalence rates have been observed in adolescents (e.g., 14%) and young adults (e.g., 6.3%) when compared to other ages [6]. As a result, researchers' interest in studying epilepsy's implications on the quality of life during these transitional ages increased. The World Health Organization Expert Committee conceptualized adolescence as the period between 10 and 19 years, characterized by profound biological changes and culminating in the maturation of complex cognitive and behavioral abilities [7]. This life phase is characterized by the search for independence and the establishment of a growing consolidation of identity and autonomy, with a parallel strengthening of physical and cognitive competence and subsequent decline in relatedness with parents [8]. The development of autonomy consistently continues during emerging adulthood (19–25 years), another developmental age in terms of identity explorations consisting of dramatic changes in life circumstances (e.g., finishing school, entering the workforce, leaving parental home, marriage) [9]. Notwithstanding the expected evolutive enhancement of self-reliance, emerging adults still tend to perceive their family members as the main sources of assistance [10]. This evidence is critical considering that many studies have shown that parents' support of autonomy is associated with a range of positive outcomes for their offspring (e.g., high academic performance and low levels of depression [11,12]).

Young people's need to overcome age-specific transitional challenges to increase autonomy encounters several barriers and lifestyle complications when living with a chronic condition such as epilepsy [13]. This disease can hinder the achievement of key developmental tasks, negatively impacting independence, peer socialization, and self-esteem [13]. Studies have suggested that epilepsy-related aspects such as exposure to a higher number of anti-seizure medications (ASM) [14], greater seizure severity, and comorbidity [4] may be risk factors for poor a quality of life in youngsters. Moreover, a lack of perceived independence and autonomy in managing their medical condition contributes to this risk [15,16]. Besides that, young people with epilepsy also experience a drastic interruption in their continuity of care when they are transferred from the pediatric to the adult healthcare system [17]. In this regard, the literature indicated that 40–50% of adolescents continue to present with epilepsy throughout life [18], and thus require ongoing specialist healthcare delivery into adulthood [19]. During this process, patients could experience mental distress, fear, and apprehension and even stop their medication [20]. Consequently, it is essential to plan the transition of care for youths with epilepsy, conceived as a coordinated process of transfer from a pediatric environment to an adult-centered care setting [20]. The transition to a care process is different from the mere transfer to care as it focuses on the individual experiences and needs of the patient and includes the multisystemic coordination among different health professionals involved in the education of a patient and his/her family [20]. The consequences of poor transition planning can include increases in healthcare expenditures and a decreased quality of life [21]. The American Epilepsy Society [22] recommends introducing transition to patients of around 10–13 years of age; notwithstanding, empirical research on the best practices to assist with youth transition is limited [23]. Transition is an individualized process, and its successful execution depends not only on a patient's epilepsy and associated condition (e.g., seizure severity and treatment adherence) but also on the individual level of transition readiness [24].

Transition readiness has been defined as "indications that a patient and those in their support system (e.g., parents and providers) can successfully transition from child-centered to adult-oriented healthcare" ([25], p. 12). These indications help clinicians address self-management of health and improve the health-related skills of young patients and their

caregivers [24]. Multi-wave assessment of transition readiness can be crucial to evaluate a patient's ability to transition successfully in terms of disease knowledge, self-management skills, and expectations from healthcare providers [21]. The literature showed that young patients with epilepsy experiencing high transition readiness also reported a high quality of life [26–28] and that certain demographic variables (e.g., socio-economic status, race/ethnicity) may be associated with a higher likelihood to successfully utilize transition readiness skills [29]. The level of transition readiness could be influenced by parents' agreement and support for initiating independent care [30]. Since parents are the principal healthcare agents for their sons affected by epilepsy, the examination of their perceptions of the transition from pediatric to adult healthcare is needed to develop effective and orchestrated plans for healthcare management [31]. A previous study showed that caregivers and patients differently perceive transition readiness, suggesting the importance of evaluating both transition experiences [32]. Some authors suggested that families of children with epilepsy experience high levels of stress during the parental role transition [33]. This negative impact of the transition process on caregivers' mental adjustment may also depend on the specific epilepsy characteristics reported by their offspring. For instance, high seizure control of children was associated with better parental quality of life [34], suggesting that the severity of epilepsy may influence parental psychological experiences of the disease. A variable of particular interest in this context is sleep [35,36]. It is well known that in children and adolescents, seizures promote disruption of sleep patterns, and affect the quality, quantity, and architecture of sleep [37]. For instance, in a recent meta-analysis, children with epilepsy were shown to sleep, on average, 34 min less than healthy controls across self-report, actigraphy, 24 h video EEG, and polysomnography measures [38]. Interestingly, the quality and duration of sleep may be associated with quality of life and clinical outcomes (e.g., depression) in children and adolescents with epilepsy [39]. To the best of our knowledge, however, whether sleep may be associated with transition readiness in this population remains to be explored.

Taken together, the literature indicates that transition readiness is associated with a high perceived quality of life in patients and their caregivers, yet little is known about the levels of specific psychological and personal characteristics associated with this vulnerable phase. Therefore, the present study aims to identify sociodemographic, clinical, and psychological factors presented by young patients with epilepsy and epilepsy syndrome, formally transferred to adult care and their parents.

2. Materials and Methods

2.1. Participants and Study Design

Patients affected by epilepsy and epilepsy syndrome, accompanied by their parents, participated in the present study. Participants were admitted to the Transition Clinic and transferred from the Child Neurology and Psychiatry Unit to the Neurology Unit of the University Hospital of Rome "Tor Vergata" in the period between October 2022 and May 2023. Patients and their parents completed a series of questionnaires in the presence of pediatric neuropsychiatry and neurology specialists. Patient and parent forms of each questionnaire were presented to the corresponding participant. Patients completed the questionnaires without consultation with their parents. The diagnoses of epilepsy and epilepsy syndrome were made in accordance with the ILAE recommendations [22] (see above). All the procedures were approved by the ethics committee of University Hospital of Rome Tor Vergata (R.S. 191/17-192/17; Eudract 2017-000990-35).

2.2. Measures

Participants completed a socio-demographic form including questions on age, sex, educational level, socioeconomic status, and clinical information regarding their medical issues and psychological comorbidities. Moreover, a series of questionnaires were administered (see below).

Transition readiness. The Epilepsy Transition Readiness Assessment Questionnaire (EpiTRAQ) [40] is a 20-item measure assessing transition readiness in patients and their caregivers. The questionnaire assesses five areas of transition: managing medications, appointment keeping, tracking health issues, talking with providers, and managing daily activities. Responses ranged from 1 to 5, with higher scores indicating greater transition readiness. This scale showed an excellent internal reliability ($\alpha = 0.953$).

Medical adherence. The Self-reported Medication Taking Scale (SRMTS) [41] was completed by patients to measure their levels of medication adherence. An adapted version was provided to caregivers. The SRMTS is composed of seven items rated with a 5-point Likert scale from never (=0) to always (4). Scores ranged from 1 to 13, with higher scores indicating less difficulty in medication assumption. The SRMTS showed very good psychometric properties ($\alpha = 0.959$).

Quality of life. The Pediatric Quality of Life Inventory Epilepsy Module (EpiPedsQL) [42] was used to evaluate the health-related quality of life of patients. It consists of 29 items assessing five domains: impact, cognition, sleep, executive functions, and behavior/mood. An adapted version was employed to assess the parental experience of their offspring's level of quality of life. Respondents recorded their answers using a 4-point scale ranging from 0 (=never) to 4 (=always). The scale was highly reliable ($\alpha = 0.905$). The Short Form 12 (SF-12) [43] consists of 12 items that assess eight dimensions of health (physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, and mental health). Two overall factors were derived from the SF-12, the physical component score and the mental component score. High scores indicate a lower life quality. This scale demonstrated low internal reliability ($\alpha = 0.552$).

Depression. The Patient Health Questionnaire (PHQ-9) [44] consists of nine items that reflect the nine diagnostic criteria for major depression as defined by the DSM-IV. Each item evaluates symptoms occurring over the past 2 weeks and is scored from 0 (absence of symptoms) to 3 (presence of symptoms nearly every day). The total scores ranged from 0 to 27 and higher scores suggest a high risk of presenting with depression (0–4: absence of depression; 5–9: subthreshold depression; 10–14: moderate depression; 15–27: severe depression). The internal reliability was acceptable ($\alpha = 0.767$).

Anxiety. The General Anxiety Disorder (GAD-7) [45] is a screening tool composed of seven items investigating anxiety-related symptoms as experienced during the past two weeks. Responses are rated on a 4-point Likert scale. The total scores ranged from 0 to 21 and higher scores suggest a high risk of presenting anxiety (0–4: absence of anxiety; 5–9: subthreshold anxiety; 10–14: moderate anxiety; 15–21: severe anxiety). The internal reliability was good ($\alpha = 0.864$).

Resiliency. The Resiliency Scale [46] is a 25-item scale that measures the ability to cope with adversity. Respondents rate items on a Likert scale from 0 to 7. Higher scores indicated a higher level of resilience. This scale showed good psychometric properties ($\alpha = 0.741$).

2.3. Statistical Analysis

First, descriptive statistics were computed to characterize the sample in terms of sex, age, epilepsy onset, epilepsy type, seizure frequency, seizure type, and anti-seizure medications (ASMs). The normality of the data was assessed through the Shapiro–Wilk test. Mann–Whitney *U* tests were used to assess the statistical significance of differences in each psychological aspect investigated between parents of patients with epilepsy and parents of patients with epileptic/rare syndromes. The Mann–Whitney *U* test is a rank-based procedure which is more appropriate with ordinal and not-normally distributed data [47].

3. Results

3.1. Description of the Sample of Patients

3.1.1. Demographic and Clinical Characteristics

A total of 13 patients (61.5% F; $M_{age} = 25.92 \pm 6.56$; range: 20–40) were included in this study. Of these, seven (57.2% F; $M_{age} = 21.71 \pm 1.70$; range: 20–25) were affected by

epilepsy, and six patients (66.7% F; M_{age} = 30.9 ± 6.79; range: 24–40) were affected by epilepsy syndrome and rare diseases with epilepsy. In total, 53.8% of patients reported that they live with at least one parent, whereas 46.2% reported that they also live with brothers and/or sisters. Each patient has a total of 13 years of education, and the majority (77.8%) reported a below to middle socio-economic status. As regards clinical characteristics, five patients had an epilepsy syndrome (tuberous sclerosis complex), one patient had a rare disease with generalized epilepsy, and seven patients were affected by generalized epilepsy. The mean age of onset was 9.15 (SD = 7.19), and the etiology of epilepsy was genetic and structural (n = 5), unknown (n =3), genetic (n = 2), and suspected genetic (n = 3). The frequency of seizures was less than one per year for most patients (n = 7), while the remaining showed one episode per year (n = 3), one–two episodes per year (n = 1), one episode per month (n = 1), and multi-daily episodes (n = 1). Patients did not present with seizures at night, as recorded by the parents or by the epilepsy diary (however, no long-term EEG monitoring was performed at the time of the study). Patients were prescribed one (n = 5), two (n = 7), and three (n = 1) ASMs. More specifically, patients were treated with Levetiracetam (LEV) (n = 4), highly purified Cannabidiol and LEV (n = 1), Everolimus (n = 1), Lacosamide and Everolimus (n = 1), Lamotrigine and Topiramate (n = 1), Valproic Acid (VPA) and LEV (n = 1), VPA and Carbamazepine (n = 1), VPA and Clonazepam (n = 1), VPA and Everolimus (n = 1), and Oxcarbazepine and LEV (n = 1).

Eleven patients presented multiple comorbidities with psychiatric, neurological, and cardiovascular conditions. Table 1 shows the distribution of these aspects in the epilepsy and epilepsy syndrome groups.

Table 1. Clinical characteristics of patients with epilepsy (n = 7) and patients with epilepsy syndrome or epilepsy in comorbidity with rare diseases (n = 6).

Variable	Patients with Epilepsy n (%)	Patients with Epilepsy Syndrome/Rare Diseases n (%)
Epilepsy type		
Generalized	7 (100%)	1 (16.7%)
Focal		4(66.6%)
Focal/generalized		
Epilepsy Syndrome		1 (16.7%)
Seizure type		
Generalized	7 (100%)	2 (33.3%)
Focal		2 (33.3%)
Combined		2 (33.3%)
Aetiology		
Genetic	5 (71.4%)	1 (16.7%)
Unknown	2 (28.6%)	
Combined genetic and structural		5 (83.3%)
Age at epilepsy onset	M = 11.4 ($\pm$5.02)	M = 6.4 ($\pm$8.89)
Seizure frequency		
Seizure type: generalized		
<1/year	4 (57.1%)	1 (16.7%)
$\geq$1/year	3 (42.8%)	
Seizure type: focal		
<1/year		2 (33.3%)
$\geq$1/year		2 (33.3%)
Seizure type: combined		
<1/year		1 (16.7%)
$\geq$1/year		
Concomitant ASMs		
1 (%)	4 (57.1%)	1 (16.7%)
2 (%)	3 (42.8%)	4 (66.66%)
$\geq$3 (%)		1 (16.7%)

3.1.2. Psychological Characteristics

A total of four patients from the rare diseases outpatient clinic were not asked to complete the questionnaires as they presented moderate to severe intellectual disability. Therefore, a total of nine patients completed the questionnaires investigating levels of transition readiness (EpiTRAQ), medical adherence (SRMTS), quality of life (EpiPedsQL), depression (PHQ-9), anxiety (GAD-7), and resiliency (RS). Considering cutoff scores of PHQ-9, two patients (15.4%) reported mild depressive symptoms and one patient (7.7%) reported moderate depression, whereas the majority (n = 6; 66.7%) did not report depressive symptoms. Mild anxiety symptoms assessed through GAD-7 were detected among three patients (23.1%), whereas one patient (7.7%) presented clinically severe anxiety. Other patients (n = 5; 55.6%) did not report any symptoms. Table 2 summarizes the mean scores for each domain examined.

Table 2. Psychological characteristics of patients.

Questionnaires Completed by Patients (n = 9)	Mean	SD	Min	Max
Managing medication (EpiTRAQ)	3.19	0.60	2	4
Appointments keeping (EpiTRAQ)	2.58	0.86	1	4
Tracking health issues (EpiTRAQ)	2.72	1.19	0	4
Tracking with providers (EpiTRAQ)	3.39	0.70	2	4
Daily activities (EpiTRAQ)	2.94	0.82	1	4
Medical adherence (SRMTS)	4.11	4.08	0	13
Cognitive (EpiPedsQL)	0.80	0.82	0	2
Sleep (EpiPedsQL)	0.92	0.40	0	1
Executive function (EpiPedsQL)	4.44	5.22	0	1
Mood (EpiPedsQL)	1.06	0.81	0	2
Depression (PHQ-9)	4.44	2.83	1	10
Anxiety (GAD-7)	5.22	3.42	1	10
Resiliency (RS)	58.89	5.88	51	68
Mental component (SF-12)	48.35		43	59
Physical component (SF-12)	52.68		35	58

3.2. Description of the Sample of Parents

3.2.1. Demographic and Clinical Characteristics

The parents of the patients included in the study were asked to complete questionnaires regarding their children's transition and some aspects of their quality of life. In total, 12 parents (75% F; M_{age} = 56.6 ± 7.36; range: 46–74) were recruited (one subject was the parent of two patients included in the study). Half of the parents had secondary education, 25% (n = 3) completed middle school, and 16.7% (n = 2) had post-secondary education. More than half (53.84%) were employed, married (55.5%), and the majority (90%) reported a below to middle socio-economic status.

3.2.2. Psychological Characteristics

A total of 12 parents completed the questionnaires investigating levels of transition readiness (EpiTRAQ), medical adherence (SRMTS), quality of life (EpiPedsQL, SF-12), depression (PHQ-9), and anxiety (GAD-7) (Table 3). Considering cutoff scores of PHQ-9, three parents (23.1%) reported mild depressive symptoms, two parents (15.4%) reported moderate depression, whereas the majority (n = 6; 54.5%) did not report depressive symptoms. Mild and clinically severe anxiety symptoms assessed through GAD-7 were detected in two parents (15.4%), whereas the majority (n = 7, 63.6%) did not report any anxiety symptoms.

Table 3. Psychological characteristics of parents.

Questionnaires Completed by Parents (n = 12)	Mean	SD	Min	Max
Medication (EpiTRAQ)	2.91	0.99	1	4
Appointments (EpiTRAQ)	2.65	1.18	1	4
Tracking (EpiTRAQ)	2.79	1.15	1	4
Providers (EpiTRAQ)	3.38	1.13	1	4
Daily activities (EpiTRAQ)	2.90	1.05	0	4
Medical adherence (SRMTS)	7	9.81	0	28
Impact (EpiPedsQL)	3.56	0.78	0	3
Cognitive (EpiPedsQL)	1.31	1.42	0	4
Sleep (EpiPedsQL)	1.36	0.99	0	4
Depression (PHQ-9)	4.55	3.86	0	10
Anxiety (GAD-7)	4.73	4.38	1	13

3.2.3. Group Differences

Mann–Whitney *U* tests were used to examine differences between each psychological variable among parents of patients with epilepsy and parents of patients with epilepsy syndrome/rare diseases. The results indicated significant differences in the domains EpiPedsQL-Impact ($U = 39.500$, $p = 0.005$), and EpiPedsQL-Sleep ($U = 39.500$, $p = 0.007$). More specifically, higher scores in sleep difficulties and in the impact of epilepsy on quality of life were observed in the group of parents of patients with epilepsy syndrome/rare diseases as compared to those observed in the group of parents of patients with epilepsy. No other significant results were observed for the other domains ($p > 0.05$).

4. Discussion

This study aimed to understand the complex healthcare transition process for patients with epilepsy by examining transition readiness, demographic, clinical, and psychological variables in a sample of patients with epilepsy or epilepsy syndrome/rare diseases, and their parents. This topic is of particular relevance considering the lack of a comprehensive understanding of the experiences of youngsters and parents approaching this ongoing process. Results can support the upskilling of health professionals in the delivery of age-appropriate care and educational programs. A recent systematic review highlighted that transitional plans for the transfer from pediatric to adult care are not often available and evidence on the efficacy of transition programs for young people is limited [48]. Notwithstanding, several psychological problems are frequent (e.g., anxiety, depression) [49] and adversely affect the transition process [50]. Transition programs are not adapted to the age-specific needs of patients and there is no consensus on the evaluation of adequate transitions, the impact on patient experience, and population costs [48]. Therefore, the need to perform a standardized evaluation of patient experience to identify key features of the transitional process and the outcomes defining successful transitional care recently emerged [21,48]. Notwithstanding, the recommended age for an effective transition has been identified as between 12 and 14 years old [51]. Notably, in the present study, the mean age of patients who underwent transitions was 25.92 ± 6.56 years, which was later than recommended. This evidence aligns with previous research [52] and confirms that young adult patients with epilepsy tended to remain in the pediatric clinics, suggesting that the discussion of transition before reaching the age of maturity may not be routinely delivered [53]. This gap in the healthcare system could be seen in the present study by the evidence that patients seemed to report lower levels of readiness in all the domains measured by the EpiTRAQ, as compared to those reported by young adult patients with a wide variety of other medical diseases [54]. Indeed, this finding suggested that epilepsy may be a particularly stressful and challenging condition, even more than other illnesses, due to its unpredictable nature [48], and thus encouraged the expansion of transitional readiness models and theories. We are also aware that these results were obtained in Italy, where the guidelines for transition are probably still lacking and further studies should be published for helping patients, parents, and clinicians in this very sensitive time of patients' lives.

Young people with epilepsy often report psychosocial, emotional, and behavioral difficulties [16] due to the interaction between neurological (e.g., neurotransmitter-based pathogenic mechanisms), sociodemographic (e.g., financial difficulties), psychological (e.g., social stigma), and epilepsy-related aspects (e.g., use of certain antiepileptic drugs) [16]. In our sample, three patients (33.3%) reported mild to moderate depressive symptoms, whereas four patients (44.4%) presented mild to severe anxiety symptoms. Moreover, patients presented higher scores for both depressive and anxiety symptoms as compared to those reported by studies on young adults without epilepsy [55].

These findings prioritized the need to anticipate the transition to adult care in order to prevent the deterioration of mental health in youngsters, by appropriately addressing age-specific difficulties of patients with epilepsy. The mental health difficulties experienced by patients during their transition also concerned sleep patterns, as indicated by the higher

score on the relative EpiPedsQL sub-domain. The literature regarding developmental trends of sleep during the transition from adolescence to young adulthood has indicated a general decline in sleep duration and efficiency [56]. Sleep problems during these developmental ages are frequent in patients with epilepsy compared to healthy controls [57] and may affect medication compliance and negatively impact autonomy from caregivers [58]. Moreover, research has shown that caregivers of patients with epilepsy exhibit disrupted sleep patterns and sleep deprivation [59], and parental sleep dysfunction is influenced by a child's seizure severity [60]. In our investigation, parents of patients with epilepsy syndrome/rare diseases reported greater sleep difficulties and epilepsy-related impact on quality of life as compared to parents of patients with epilepsy. This result concurs well with the evidence that the impact of sleep can be specific considering epilepsy in general and specific epilepsy syndromes (e.g., Lennox-Gastaut syndrome, West syndrome, Tuberous Sclerosis Complex) [61]. These findings seemed to indicate that parents of patients with epilepsy syndrome/rare diseases might particularly benefit from interventions of parental sleep. Considering that the seizure frequency of patients with epilepsy syndrome/rare diseases was low compared to that reported in the previous literature, it appeared evident that sleep problems persist also when seizure frequency is reduced by the use of ASMs. Increased comprehension of the association between poor sleep and epilepsy, possibly also mediated by other factors, may suggest the need to set strategies to improve the sleep quality of patients with epilepsy and their parents to help also mitigate psychiatric comorbidities and behavioral problems at home and school [62].

The present study clearly had some limitations. First, its cross-sectional nature prevents drawing conclusions about the causality of associations. Additional research is needed to explore the associations found with longitudinal methods. Second, the mere use of questionnaires to collect data may be subject to social desirability and recall bias. Future studies should employ more rigorous methods of evaluation, such as qualitative structured interviews or daily diaries to collect subjective data on all the psychological aspects investigated. Moreover, objective measures such as actigraphy or polysomnography to describe sleep parameters are encouraged in the future. Another limitation pertains to the small sample size of the group of patients and parents included, which affected the conclusions of the study in terms of their generalizability. Moreover, the non-random sampling affected our findings in terms of their external validity. Future studies should employ alternative approaches to analyze preference data, such as cluster analysis [63]. The use of non-parametric methods of estimating group differences may have affected our results. Future studies are required to use more sophisticated methods of analysis. For instance, actor–partner interdependence models (APIMs) [64] could be useful in detecting mutual influences of psychological aspects between parents and youths with epilepsy during transition. Finally, future research should collect data on the life history of patients with epilepsy, in order to assess whether deteriorations in sleep patterns may depend on the environment and life events, as mentioned elsewhere [65].

Despite these weaknesses, this study suggests that epilepsy transition programs need to be implemented to ensure the efficient transition of youngsters with epilepsy from pediatric to adult care. One of the main findings of the present study is the delay in transition documented, which reflects the need for guidelines for the transition from child to adult neurological centers in epilepsy care. Another potential practical implication that could be highlighted is the continuous monitoring of mental health in persons with epilepsy approaching adult healthcare, especially given the documentation of depressive and anxiety symptoms found in our sample. In this regard, depression and anxiety were directly related to higher seizure frequency [66]. Therefore, clinicians should take the time to screen these patients in order to combine seizure treatment with mental health interventions through individualized programs. Moreover, starting the introduction of transition planning early could be crucial for improving transition-related emotional problems. Results on sleep further emphasized the need to include assessments of sleep in the management protocols of youngsters with epilepsy. Practitioners are encouraged to promote knowledge of sleep

among both patients and their caregivers, especially considering the well-known association between deteriorated sleep and seizure severity [62]. In conclusion, the evidence that emerged indicated the need to improve care not only of patients with epileptic syndrome and epilepsy in comorbidity with rare diseases, but also of their parents, who experience disease-related stress for many years and, considering their increasing age, should be assisted by health professionals who can limit their assistance.

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