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REVIEW

Decoding Epileptic Seizures: Exploring In Vitro Approaches to Unravel Pathophysiology and Propel Future Therapeutic Breakthroughs

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Abstract

Epilepsy is a chronic neurological disorder associated with various symptoms, contingent upon the specific brain region involved. Unpredictable seizures characterize epilepsy, significantly influencing the quality of the patient's life. Globally, epilepsy affects 1% of the population, with 30% of individuals developing drug resistant epilepsy despite anti-epileptic pharmacological treatment. While several anticonvulsant drugs alleviate epilepsy symptoms, there is currently no effective medication to cure this neurological disorder. Therefore, overcoming the challenges of predicting and controlling drug-resistant seizures requires further knowledge of the pathophysiology of epilepsy at the molecular and cellular levels. In this review, we delve into in vitro experiments that prove valuable in elucidating the mechanisms of drug-resistant epilepsy, as well as in the development and testing of novel therapeutic approaches prior to extensive animal-based trials. Specifically, our focus is on the utility of multi-electrode array (MEA) recording as an in vitro technique for evaluating aberrant electrical activity within neural networks. Real-time MEA recording from neuronal cultures facilitates monitoring of neurotoxicity, dose response, and the efficacy of newly-designed drugs. Additionally, when coupled with emerging techniques such as optogenetics, MEA enables the creation of closed-loop systems for seizure prediction and modulation. These integrated systems contribute to both prospective therapy and the study of intracellular pathways in drug-resistant seizures, shedding light on their impact on neuronal network activity.

Keywords Epilepsy · Drug-resistant epilepsy · In vitro studies · Multi electrode array · Optogenetics · Closed-loop systems · Prospective therapy · Seizure prediction

Introduction to Epilepsy

Epilepsy is a chronic brain disorder characterized by an unpredictable recurrence of seizures. Seizures are categorized as either focal or generalized [1]. Several different cognitive and emotional dysfunctions can manifest depending on the area of the brain that is affected by epilepsy [2]. The names of the anatomical areas implicated in epilepsy are used to categorize the disorder, such as parietal, temporal, frontal, and occipital-lobe epilepsy [3]. The prevalence of epilepsy is remarkable as it includes 1% of the world population, which highlights the significance of research in this area [2]. Epileptogenesis refers to the causative cascades that resulted in the onset of spontaneous seizures or epilepsy [3]. Epileptogenesis can be influenced by both acquired and genetic factors [4]. Examples of acquired causes of epilepsy include brain trauma, brain tumors, central nervous system infection, and neuroinflammation, while genetic causes include genetic mutations affecting brain development and/or neuronal membrane properties [5]. To study epileptogenesis, the priority should be to understand the main reason of the emergence of seizures as the signaling pathways that initiate epileptic seizures in genetic causes might differ from acquiring or epigenetic causes [4]. One of the most important mechanisms is inflammation. Inflammation is characterized as a dynamic cascade of responses to external threats

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in response to trauma, ischemia, and neurodegenerative diseases in the central nervous system (CNS). Inflammation has a crucial role in increasing the excitability of neurons and eventually seizures [5]. The severity and recurrence of seizures have been linked to inflammatory cytokines such as $IL-1\beta$ [5]. Inflammation acts as a positive feedback loop, driving the development of additional inflammation by making neurons more hyperexcitable, leading to seizures, and subsequent development of epilepsy [5]. Other studies showed that sprouting and new synaptic formation after CNS injury may contribute to epileptogenesis [6]. For instance, mossy-fiber sprouting (MFS) following damage reorganizes the connections between dentate granule cells and CA3 pyramidal cells[1], leading to epileptiform activity [6]. Additionally, recent investigations have shown that similar pathways may contribute to epileptogenesis and neurodegenerative conditions such as Alzheimer's disease [3, 7]. However, further investigation is required to determine the genetic and epigenetic factors underlying these conditions [3]. Genetic mutations causing epilepsy often impact on voltage-gated and ligand-gated ion channel activity [6].

Armed with this knowledge, researchers can use neuronal primary cultures from brain regions of interest to look for therapeutic solutions [3, 8]. Currently, no drugs are available that can both cure and prevent epilepsy; however anti-epileptic drugs (AEDs) can prevent seizures [3]. Around 70% of people will no longer experience seizures after using just one AED [3]. The first AED used for managing seizures was potassium bromide, but it had ineffective results and unfavorable side effects [2]. Benzodiazepines (such as diazepam, clonazepam, etc.) and sodium channel blockers (such as carbamazepine and lamotrigine) are the most frequently used AEDs, with lamotrigine showing the best seizure control in adult epileptic patients [9]. However, we must take into account the fact that roughly 30% of epileptic patients still exhibit drug resistance to all AEDs [2]. Alternative therapies used in drug-resistant epilepsy patients include surgery, deep brain stimulation, and transcranial electrical/magnetic stimulation [10–12]. Non-pharmacological therapies come with barriers that limit their use in some patients. This highlights the need for further research on epilepsy and its associated complications.

This deeper knowledge in the intricate cellular and molecular signaling pathways implicated in epilepsy pathogenesis is pivotal for directing targeted treatments using advanced methods like optogenetics, aiming to correct neural network abnormalities and address specific issues in epilepsy treatment [13]. In accordance with this notion, recent studies have employed optical techniques to selectively modulate specific mechanisms by using optogenetics tools and light stimulation [14]. The use of optogenetics opens up a world of opportunities for studying or modifying the pathophysiological pathways involved in epilepsy through light stimulation. On the other side, recent technological improvements have also led to the development of advanced biocompatible implantable systems known as closed-loop system [15]. These systems use real-time neuronal network electrical activity data to detect early-stage seizures and provide electrical stimulation as feedback. This stimulation aims to regulate neuronal network activity and suppress seizures effectively [15]. Closed-loop systems have shown promising results in the laboratory and are currently in the clinical stage, being used as individual treatments to suppress seizures in patients with drug-resistant epilepsy [16]. One drawback of these systems lies in their use of electrical stimulation, as it affects not only the target region and the neuronal population of interest but also the surrounding neuronal network [17]. New closed-loop systems based on optogenetics and light stimulation to suppress seizures address these drawbacks by improving the specificity and spatial resolution of the mitigation action [18]. In addition, these technologies help our understanding of the root causes of the disease, particularly in in vitro applications, which pave the way towards a personalized therapy for neurological disorders.

In this review, we evaluate the validity of in vitro research in terms of how it affects our knowledge of the pathophysiology of epilepsy and the development of novel treatments. Additionally, we illustrate a novel in vitro setup developed in our laboratory that combines optogenetics, multielectrode array (MEA), and integrated photonic circuits to control network hyperactivity in neuronal cultures [19].

In Vitro Approaches for Investigating Epilepsy

In vitro approaches allow to mechanistically address seizure pathogenesis at its source, enabling the development of targeted treatments. This necessitates the multidisciplinary application of cutting-edge methodology and technology as well as the interaction of experts from multiple fields of study. The relationship between inflammation and drug-resistant epilepsy, for instance, was clarified by in vitro studies that identified Interleukin 1 (IL-1) as a crucial component in this process [20]. The control of inflammatory reactions is also regulated by miR-146a, therefore IL-1 factor is not the only strategy to target inflammation [20]. The pro-inflammatory cytokine IL-1 stimulates the immune response following seizures, while the small RNA molecule miR146a regulates inflammation by specifically targeting several elements of the signaling pathways which suppress the production of pro inflammatory cytokines, including IL1

[1] The class of CA3 neurons, which are present in the hippocampus, includes pyramidal neurons with excitatory properties.

itself [21, 22]. Additional investigations have unveiled that inflammation affects the production of multidrug transporters within the blood–brain barrier (BBB), consequently limiting the entry of AEDs into the brain [23, 24]. Again, specific microRNAs have been shown to control the expression of efflux transporters, which affects the entry of therapeutic drugs into the brain leading to drug resistance in epilepsy [25]. So far, four AEDs have been identified as substrates of multidrug transporters: phenytoin, phenobarbital, lamotrigine, and oxcarbazepine [26].

We previously mentioned that mutations in ion channel genes significantly contribute to the onset of epilepsy. The KCNQ2 (Kv7.2) gene, coding for a voltage-gated potassium channel, is one of such examples [27]. Numerous KCNQ2 gene variants have been linked to a variety of neurological conditions, including drug-resistant epilepsy and self-limited familial neonatal epilepsy [27]. In vitro studies contributed to unravel the pathophysiology of this channel and to identify new drugs able to raise seizure thresholds and effectively reduce neuronal hyperexcitability in KCNQ2-related epilepsy [27, 28].

Other in vitro studies contributed to identify the crucial role of astrocytes in the onset of seizures [29]. Glutamine produced by astrocytes plays a significant role in controlling the activity of GABAergic inhibitory neurons [29]. Specifically, a deficiency in glutamine synthetase is a significant pathogenic mechanism for the onset of seizures [29]. GABAergic inhibitory neurons actively contribute to the dynamics of focal seizures by changing the balance of excitatory to inhibitory neuron transmission as well as by physiologically synchronizing pathological epileptiform activity [30]. Indeed, administration of bicuculline, a GABAA receptor antagonist, leads to rhythmic synchronous activity in hippocampal neurons [31]. Recent in vitro studies also contributed to challenge the conventional belief that seizure onset hub cells are the sole contributors to seizure onset [32], showing that network changes among large populations of neurons induced by pathological processes play a more critical role in driving seizure transitions [32]. Further exploring this field might uncover essential aspects of the pre-ictal stage, leading to improved seizure-prediction strategies and enhancing the effectiveness of surgical interventions [32]. In vitro research also allowed to test the potentially beneficial effect of molecules with anti-convulsant efficacy, such as Cannabis sativa [33] and drugs acting on non-neuronal voltage gated potassium channels [34].

Additional advantages of in vitro studies include the use of induced pluripotent stem cells (iPSCs) as a cell source for epilepsy research. This enables the development of epilepsy-specific neuronal models in a controlled laboratory environment [35]. These methods avoid the need for animal use and offer a scientifically valid substitute for searching the causative mechanisms of epilepsy and potential treatments [35, 36]. Reprogramming adult cells into a pluripotent state allows to differentiate into a range of cell types, including neurons [36]. Adult cells, such as skin or blood cells, can be used for this process [36]. iPSC-derived neurons have a number of advantages. Neurons derived from iPSCs enable personalized studies on the underlying causes of epilepsy by enabling the controlled and repeatable assessment of network activity and seizure-like behavior [37, 38]. They also provide a direct representation of the patient's genetic history, granting studies and investigations into the complex cellular and molecular mechanisms underlying epilepsy [37, 38]. Additionally, just like primary cultures, iPSC-derived neurons can be used as a platform for drug screening. However, the most interesting benefit of iPSC is the development of personalized therapies [37–39]. So, for instance, one can evaluate the efficacy and safety of novel anti-epileptic medications using patient-specific iPSC derived neurons, leading to the identification of more precise and efficient treatment alternatives.

In conclusion, in vitro studies enable the analysis of ion channel dysfunctions, synaptic connectivity, neuroinflammation, and other factors contributing to epileptogenesis, and provide valuable insights into the pathophysiology of the condition. The insights gained through this method are instrumental in formulating targeted therapies that address the core issue. Even when targeting the primary disease cause presents challenges, understanding alternative pathways allows for innovative treatment strategies [40]. Furthermore, this platform serves as a vital arena for evaluating the potential toxicities of newly developed drugs.

In Vitro Models of Epileptic Seizures

In the field of epilepsy research, neuronal cultures and brain slices have become fundamental models for enhancing our knowledge of seizures. These models effectively replicate the intricate mechanisms of epileptic activity, allowing to explore the cellular molecular, and network-level processes involved in seizures [41]. This section describes several in vitro models used in epilepsy research, along with their advantages and contributions to comprehend this debilitating neurological condition. Two major methods are used to mimic epileptic episodes in primary neuron cultures [41]. A first method includes changing the extracellular ion balance, while a second strategy focuses on increasing the excitability of neuronal cultures by activating excitatory pathways with excitatory agonists or inhibiting inhibitory neurons with antagonist drugs (Fig. 1) [41].

In cultured neurons, the disruption of extracellular ion balance emerges as a potent trigger, capable of precisely generating seizures [41]. The Goldman equation states that the ion gradient controls the excitability of neurons [41]. We therefore predict that by changing the concentrations

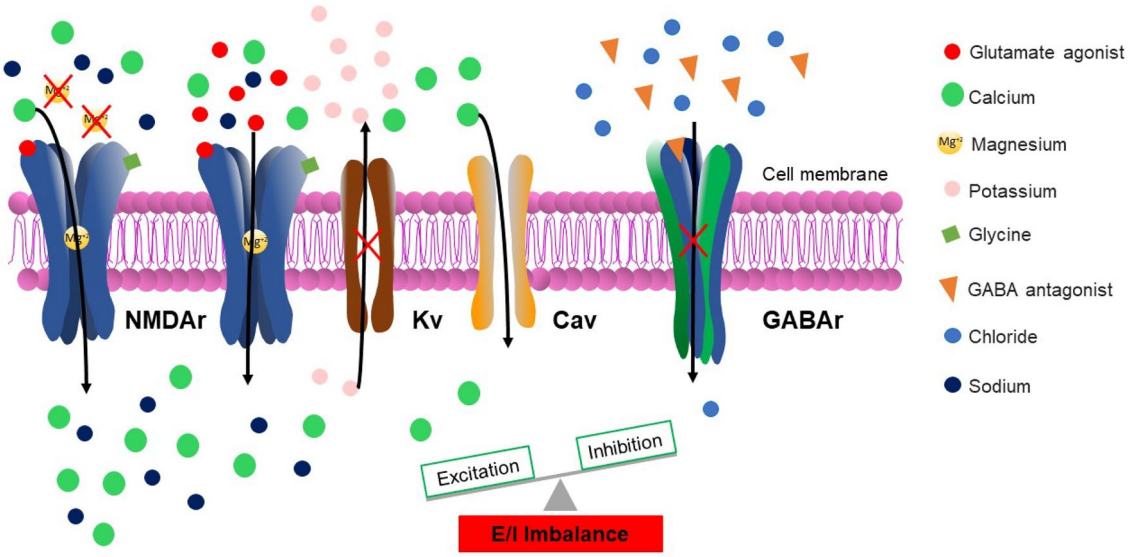

Fig. 1 The figure shows the principal mechanisms inducing hyperexcitability in neurons. Glutamate agonists increase Na + and Ca2 + influx through glutamate NMDA receptors (NMDAr); GABA antagonists block Cl- influx through GABAA receptors; K + channel blockers block K + efflux through K + channels. All these mechanisms shift excitation/inhibition (E/I) balance towards excitation

of these ions, we may increase the excitability of the neurons and induce seizure-like activity in cultures [41, 42]. Several studies demonstrate increasing extracellular potassium content as a potential means of reaching this objective [41]. Also, more recent studies claim that there is a clear association between extracellular potassium concentrations and the onset of seizures through depolarization, increased neuronal firing, and maybe burst firing [41, 42]. Indeed, the sodium–potassium pumps precisely maintain the membrane gradients, resulting in a resting membrane voltage of around − 65 mV in most neurons [43]. In vitro, the voltage may be slightly higher due to the elevated potassium concentration in commercial media [42]. But as extracellular potassium levels rise, the potassium gradient declines, causing a generalized depolarization [42]. The transition from burst firing to tonic spike firing or the complete cessation of neuronal activity can be caused by this change in the external potassium levels [42]. This effect is caused by depolarization-sensitive processes, which also result in the deactivation of voltage-gated channels and neurotransmitter depletion [42]. This makes it more difficult to analyze seizure-like activity in cell cultures employing multi electrode array (MEA) recording.

Recent studies have revealed a compelling association between brain regions affected by epileptic seizures and a decrease in extracellular calcium concentrations when compared to unaffected tissues [44]. This discovery underscores the pivotal role of extracellular calcium, which can be likened to the importance of potassium [44]. These findings shed light on the intricate mechanisms at play during epileptic seizures [44]. Voltage-gated calcium channels and N-methyl-D-aspartate (NMDA) receptors facilitate calcium entry into neurons, resulting in a drop in extracellular calcium concentration [44, 45]. This rise in calcium influx has the potential to trigger synchronized, spontaneous bursts of activity, which are distinct features of epileptiform discharges. These bursts may contribute to the development and manifestation of epileptic seizures [44].

Another ion that has an important effect on seizure-like impulses in neuronal cultures is Mg2 + when its extracellular concentration drops [46]. Increased neuronal excitability and seizure-like activity can result from a drop in extracellular Mg2 + levels in cultured neurons [46]. This phenomenon is linked to the regulation of NMDA receptors, since Mg2 + typically functions as a voltage-dependent blocker for these receptors [46]. A decrease in Mg2 + levels causes NMDA receptors to unblock, leading to an influx of calcium ions [46]. This influx, in turn, promotes hyperexcitability, resulting in the emergence of seizure-like bursts in the neuronal cultures [44–46].

4-Aminopyridine (4-AP, fampridine, or dalfampridine) is also used to induce seizures in vitro [47]. By acting as a blocker of voltage-gated potassium channels, 4-AP prolongs action potentials, leading to increased neurotransmitter release [47]. As a consequence, synaptic transmission strength is enhanced, contributing to the observed convulsive effects in the neuronal cultures [48]. Indeed, rat hippocampus neurons grown on MEA plates increase their activity when exposed to 4-AP [48].

Apart from manipulating extracellular ion concentration, a second method to induce seizure-like behavior in neuronal cultures involves increasing excitation by targeting excitatory or inhibitory pathways [41]. Certain medications that act as GABA antagonists, specifically blocking inhibitory circuits, are capable of causing convulsive behavior in both induced human pluripotent stem cell (ihPSC) cultures and primary cultures of rodent neurons [48, 49]. Several potential candidates for this purpose exist, with some of the more frequently used, e.g., pentylenetetrazole, isoniazid, bicuculline, gabazine, and isoniadine [41, 48, 49]. These compounds have been widely employed in research settings to study epileptic activity and its underlying mechanisms in controlled laboratory environments [41, 48–50]. To induce electrical behavior similar to epileptic seizures in different types of neuronal cultures, drugs are used to stimulate excitatory circuits [41]. A strong candidate for this purpose is the glutamate agonist kainic acid, which targets glutamatergic receptors inducing long-lasting seizures in rodents. However, it is worth noting that kainic acid application to rat and hPSC-derived cultures resulted in a decrease in electrical activity, as measured by MEA [49]. Treatment of neuronal cultures with glutamate instead leads to long-term changes in neuronal excitability, lasting up to 8 days [51].

An important issue in this field is the lack of a reliable or established method for modeling seizures in neuronal cultures. This issue may arise due to the wide range of used neuronal cultures, varying in their phenotypes and origins, each displaying distinct behaviors when exposed to candidate drugs based on their unique identities. Additionally, the lack of consistency in dosage and monitoring of neuronal behavior, coupled with the absence of comprehensive studies comparing the effects of different drug dosages on various types of neuronal cultures, prevents the development of a solid knowledge in this field. Nevertheless, it is important to remember that the main aim of in vitro epileptic seizure assessment is to comprehend the cellular and molecular mechanisms behind seizures, discover possible therapeutic targets, and evaluate the efficacy of medication to prevent seizures. These models offer a safe and convenient setting for researching epileptic activity and may help with the development of novelapproaches to treat epilepsy.

In Vitro Epileptic Seizure Assessment

Uncovering the underlying causes of seizures involves closely monitoring the neural activity while manipulating various factors in a controlled in vitro environment [52]. Patch-clamp recordings are one possibility since they let measuring electrical currents directly across particular neurons, giving precise information about the synaptic activity and firing patterns of these neurons (Fig. 2A) [51, 53]. If we instead need to monitor neuronal activity at the network scale, MEAs represent an important method for electrophysiological assessment of neuronal function, since they offer crucial insights into the alterations of network dynamics (Fig. 2B) [54]. Furthermore, MEAs enable the simultaneous monitoring of electrical activity originating from multiple neurons within neural cultures, thus enhancing our comprehensive understanding of their collective activity [8, 54]. This makes it possible to analyze network-wide synchronization and find seizures across a population of neurons [54]. Calcium imaging is a useful method for evaluating seizures in vitro, too. According to this technique, calcium ions, which are important for neural signaling, are bound by fluorescent dyes (Fig. 2C) [8, 41]. Researchers can indirectly monitor neuronal activity in culture by tracking changes in calcium levels [8, 41]. Calcium imaging, which provides a spatial picture of neuronal dynamics, allows to pinpoint specific network regions or particular neurons that contribute to seizure initiation and propagation [8, 55]. Researchers are able to simultaneously do electrophysiological recordings and calcium imaging to gain a better understanding of the electrical and chemical activity in neuronal cultures during epileptic convulsions [8, 55]. In order to discover new therapeutic approaches for epilepsy, this method allows a rapid and controlled screening of a wide range of drugs. The following sections will provide more details about these monitoring techniques.

Patch Clamp Recording

Patch clamp recording provides direct and accurate measurements of membrane potential and current flow through cell membranes [56, 57]. In the patch clamping technique, voltage clamp (V-clamp) and current clamp (I-clamp) are used to address certain research questions [57]. In a nutshell, the V-clamp approach allows to precisely quantify the ionic currents that flow through the cell by clamping the membrane potential at a predefined level [57]. In contrast, the I-clamp approach allows to monitor changes in membrane potential by clamping the current rather than the voltage [57]. I-clamp is used to record resting membrane potential and synaptic potentials while V-clamp is employed to record firing activity [56]. Consequently, patch clamp recording techniques enable to get substantial knowledge of the cellular and network dynamics involved in seizures [56–58]. Seizures are commonly identified by atypical and synchronous firing of neurons; therefore, during a seizure, patch clamp recording can capture the action potentials of specific neurons, providing data on their firing rates, amplitudes, and altered patterns [57]. This knowledge helps the comprehension of the processes involved in the genesis and spread of seizures [58]. Patch clamp recording has however some limitations that require attention. One such concern relates to the invasive nature

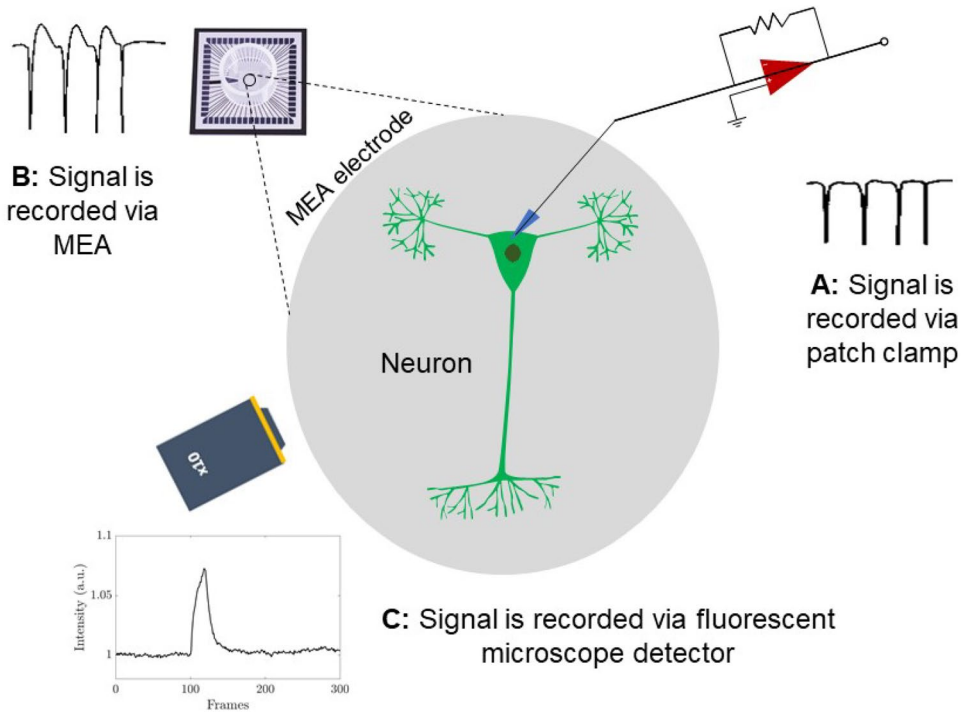

Fig. 2 Experimental approaches to measure neuronal activity. **A** Patch clamp allows to record the flow of ions through the attachment of a tiny glass pipette (electrode) to the cell membrane, enabling to investigate the electrical activity at the resolution of single channels (extracellular recordings or single cell recordings without patch-clamping do not allow to record single channel activity). **B** Multi-electrode arrays (MEA) works by arranging several microelectrodes on a substrate to allow for simultaneous extracellular recording of electrical activity from multiple neurons. **C** Calcium imaging for neuronal activity involves the use of fluorescent dyes to follow changes in intracellular calcium levels, which increase when neurons are active. This method allows for a real-time view of neural responses and network dynamics

of inserting a glass pipette into the cell membrane for the purpose of tight seals formed during recording [57]. This invasive approach to monitor cellular activity raises legitimate concerns, as studies have indicated that even the composition of the pipette itself may result in variations in recorded data [57]. Moreover, patch clamp recordings are often carried out in vitro, so they might not accurately reflect the complicated in vivo environment [56, 58]. However, employing acute slices, patch clamp recording has been shown to be a successful method for investigating epilepsy in vitro [27]. By directly monitoring the electrical activity of individual neurons, patch clamp recording enables the characterization of abnormal firing patterns and alterations in membrane potentials related to seizures [57]. Patch clamp recordings also allow studying how pharmacological agents alter neuronal excitability, advancing the search for new anti-seizure treatments [56, 59]. Additionally, one can evaluate and set up the appropriate dosage of novel drugs using the concentration–response curve [60, 61]. In conclusion, patch clamp recording offers a powerful tool for deciphering the cellular mechanisms developing epilepsy, enhancing our understanding of the condition and promote the development of novel therapeutic strategies.

Multi Electrode Array Recording

A MEA is designed to record extracellular electrical activity of all excitable or electrogenic cells and tissues [54, 62]. MEAs typically have insulated microelectrodes covered in conductive materials like indium-tin oxide, palladium, or gold on a photoetched glass chip [62]. Depending on the purpose of the study, the number, arrangement, and size of the MEA chips can vary. For example, arrays have a number of electrodes that vary from few tenths up to many thousands (high density MEA or HD-MEA). Each electrode has a size from 10 to 30 µm [62]. The ability to record local field potentials (LFPs) or high-frequency single spikes through MEAs depends on both the cell culture density and the proximity of cells to the electrodes. In cases of high cell density or recording from acute slices, the resulting electrical activity could lead to either constructive or destructive interference, influencing the expected outcome of LFP recordings [62, 63].

EA recordings provides valuable data regarding action potentials (spikes) and local field potentials (LFPs), which reflect the collective activity of small groups of neurons. These data give insights into the spike rate of action potentials or into the burst rate. The burst represents groupings of action potentials, which are rapid and synchronized sequences of spikes. In addition, the overall spike and burst rates within the network can be recorded [62]. Furthermore, by conducting more advanced analysis, valuable information can be obtained, including autocorrelograms, cross-correlograms, and advanced statistical models of network connectivity [62, 64]. This information can be used in toxicology research to examine how drug affect network function [62, 63]. In epilepsy research, MEAs enable to investigate the intricate dynamics and fundamental factors contributing to abnormal electrical activity associated with seizure generation and occurrence. Experimental observations, data analysis, and simulation obtained through MEA recording can all be used to shed light on the pathology of epilepsy and suggest prospective treatments. To identify seizures in a culture using MEA, one can analyze various parameters based on the specific objectives of the study and the characteristics of the cell types being investigated [65, 66]. Spike rate, minimal spike amplitude, interburst interval, burst duration, synchronized time windows, synchronized burst number are some key factors to consider for studying epileptic seizures in neuronal cultures [66]. MEA is a non-invasive technique for the long-term analysis of electrical activity of neuronal network. By using MEAs, accurate predictions can be achieved in the early stages of drug production, enabling a better assessment of the seizure-inducing potential of newly designed drugs [65, 67].

Calcium Sensing Indicators

Fluorescent dyes called calcium sensing indicators (CSIs) change in fluorescence intensity or wavelength when they bind to calcium ions [68]. CSIs can be administered to neuronal cultures, where they permeate cell membranes and bind to calcium ions exclusively [68, 69]. Examples of such CSIs are indo-1, Fluo-4, and Fura-2. CSIs go through a conformational shift upon calcium binding, causing an obvious shift in emission/excitation wavelength [68, 69]. This shift can be observed and quantified using spectroscopy or fluorescence microscopy, which makes them valuable tools for tracking changes in intracellular calcium levels to evaluate epilepsy in neuronal cultures [68, 69]. This is because calcium ions play a significant role in the abnormal and excessive neuronal activity that defines seizures [8,44]. CSIs provide plenty of advantages for evaluating epilepsy in neuronal cultures. These benefits include extraordinary spatial precision, continuous live tracking, and easy integration with additional (e.g., electrophysiological) techniques. However, the use of CSIs in epilepsy investigation necessitates invasive loading processes, precise calibration, and comprehensive signal processing. The potential phototoxic effects caused by the excitation light used for CSI visualization also represents an important drawback.

Innovative, Powerful Tools for Epilepsy Research

Within the field of epilepsy research, innovative and robust methodologies possess the capacity to enhance our understanding of this complex neurological disorder. These approaches offer the opportunity to unveil the mechanisms underlying epileptic activity, thereby paving the way for more efficient diagnostic protocols, alternative therapeutic options, and novel treatment strategies. In this section will focus mainly on optogenetics and genetic modification approaches.

Genetic analysis has the potential to reveal critical mutations within genes linked to both ion channels and non-ion channels, thereby contributing to our understanding of idiopathic epilepsy in patients [4, 70]. As an example, recent studies on specific gene mutations, notably those related to non-ion channel gates, have shed light on the genetic basis of rare familial epilepsy types [4]. However, it is crucial to understand the equally significant role that epigenetics plays in epilepsy development [4]. Epigenetic processes, which include heritable changes in gene expression without changes in the underlying DNA sequence, have been shown to have an important effect on the pathophysiology of epilepsy. Environmental exposures, lifestyle decisions, and early life experiences are just a few examples of the external factors that might have an impact on these epigenetic alterations and potentially cause or aggravate epilepsy [4, 70]. Scientists can now closely investigate the role of genetics and epigenetics in epilepsy using novel technologies of DNA sequencing and gene manipulation [4, 71]. The difficulty of predicting seizure events, combined with the concerning reality that a notable percentage of individuals with epilepsy exhibits resistance to conventional drug therapies, has led to venture into sophisticated strategies of gene modulation [1, 4]. Within this landscape, optogenetics emerges as an exceptionally promising path, offering a potential solution to effectively address this pressing concern. Optogenetics, a cutting-edge approach in neuroscience, allows to genetically modify targeted neurons in order to render them responsive to light stimulation [1]. Opsins are light-sensitive proteins that allow for precise control and manipulation of the activity of targeted neurons [70]. By employing this powerful tool, we can acquire fresh insights into the neural circuits responsible for epilepsy, which could provide a unique opportunity to explore

the functional aspects of neuronal activity during seizures, while genetic and epigenetic insights reveal specific mutations and regulatory processes contributing to epilepsy [1, 70, 71]. The convergence of these powerful methodologies, combined with advancements in computer science, has given rise to smart closed-loop systems [72]. These systems have been shown to effectively modulate seizure-like behaviours in vivo. In a noteworthy study, not only did light stimulation of medial septal GABAergic neurons suppress seizures, but it also allowed for anatomical exploration of brain regions to map brain connectivity [72]. These studies provide valuable insights into brain anatomy at this scale [72]. However, it is essential to emphasize the significance of in vitro studies, which offer crucial information at the molecular and cellular levels. In vitro experiments enable the examination of, for instance, the role of astrocytes in epilepsy onset and help address unanswered questions surrounding the root causes of epilepsy [73]. It's crucial to acknowledge that in vitro studies do come with limitations. Cultures cannot entirely replicate the intricate complexity of circuits and diverse cell types. Additionally, as mentioned earlier in model induction, the expected outcomes in vitro don't consistently mirror those observed in vivo [74]. Consequently, this multiple strategy combining genetic, epigenetic, and optogenetic studies has a significant potential for more successful therapeutic interventions for epilepsy.

Genetic and Epigenetics Modulation

Genetic alterations have shown to play a significant role in the development and manifestation of epileptic disorders [71, 75–77]. Epigenetic mechanisms, which include heritable changes in gene expression without altering the fundamental DNA sequence, are also crucially involved in epilepsy pathogenesis [4]. Epigenetic changes include DNA methylation, posttranslational histone modifications, and noncoding RNA, each of which has significant effects on gene activity and physiological processes [4]. As an example, studies performed in the last 10 years clearly show a crucial role of micro RNAs (miRNAs) in epilepsy [78, 79]. Levels of certain miRNAs are changed in both human and experimental epilepsy models, consequently impacting on the expression of several targets including transcription factors, neurotransmitter signalling components, and modulators of neuroinflammation. Novel approaches to manipulate miRNAs have been tested, including injection of miRNA agonists/antagonists to modulate brain levels of miRNAs [78, 79]. Most importantly, studies on DNA methylation indicated a clear epigenetic regulation of miRNA expression [4, 78, 79]. Current techniques also allow to extensively modify gene function both in vitro and in vivo [80, 81]. As an example, investigation showed how particular genes affect neuronal excitability and contribute to the emergence of epileptic activity by changing, deleting, downregulating or upregulating the expression of genes of interest. Parallel to this, in vitro research allows to investigate how epigenetic modification contributes to epilepsy, by monitoring changes in gene expression patterns and find putative epigenetic modulators affecting DNA methylation, histone acetylation, or other epigenetic markers [4, 80, 81]. Lentiviruses, adenoviruses, and adeno-associated viruses (AAVs) are also examples of genetically modified viruses that efficiently carry particular genetic material into target cells to modulate neuronal activity [80, 81]. An insightful study demonstrates the potential of gene therapy in rescuing epilepsy [82]. Using AAV NeuroD1-based gene therapy, a successfully regeneration of GABAergic interneurons from astrocytes in the hippocampus was reported. This method notably suppressed spontaneous seizure activity in a rat model of drug-resistant temporal lobe epilepsy (TLE) [82].

Optogenetics

Optogenetics is a powerful technology that empowers us to precisely modulate neuronal electrical activity, gene expression, and intracellular signalling by ectopically expressing light-sensitive proteins [78, 83]. To deliver optogenetic tools into specific target cells, different types of viral vectors, including adeno-associated viruses, lentiviruses, and herpes simplex viruses (HSV) have been used [75, 84]. Depending on the intended use and the particular targeted neuronal subpopulation, viral vectors can efficiently deliver their transcriptional code to their targeted destinations, ensuring accurate and seamless integration within the appropriate cell population, allowing to achieve precise targeting [75]. In epilepsy research, three widely recognized forms of optogenetic tools—Channelrhodopsin2 (ChR2), Halorhodopsin (NpHR), and Archaerhodopsin (Arch)—are employed to exert precise control over neuronal function [75, 84]. ChR2, originally derived from green algae, opens in response to blue light (470 nm), thus allowing the inflow of sodium and calcium ions into the neuron while simultaneously facilitating the outflow of potassium ions. Consequently, neurons rapidly depolarize, leading to the generation of action potentials with exceptional temporal resolution [75, 84]. Investigations into neural transmission and circuit mapping are considerably facilitated by these capabilities [75, 84]. Additionally, ChR2 can successfully prevent seizures when they are ectopically expressed in inhibitory interneurons [75]. NpHR is a light-gated chloride pump that is generated from halobacteria [85]. Yellow (590 nm) light activates NpHR, prompting chloride ions to enter neurons [85]. This influx of chloride ions hyperpolarizes the neuron, effectively decreasing its excitability and raising the threshold for action potential firing [85]. Thus, NpHR permits to selectively inhibit neuronal activity of target neurons within different brain circuits [75, 85].

This controlled inhibition of action potentials offers valuable opportunities for studying neural processes and their contributions to various functions. Similar to NpHR, Arch functions as an inhibitory opsin. In fact, when subjected to green light at 565 nm, this archaea-derived opsin operates as a proton pump, facilitating the transport of protons into the neuron [85]. The neural action potential is inhibited as a result of this proton pumping by raising the threshold for depolarization. Arch is distinguished from other optogenetic tools by its singular capacity to produce consistent and long-lasting inhibition, which makes it essential for implementing long-term changes in neuronal activity [13]. These optogenetic approaches can help better understanding the complex processes behind epileptic activity. By precisely activating or inhibiting specific neurons, optogenetics can in fact reveal details on how these neurons promote the development and progression of seizures, enabling investigations into the dynamics of neural networks and the relationships between neuronal activity and epileptogenesis. Additionally, optogenetics has a potential for studying and modifying several biochemical processes within neuronal cells, resulting in novel therapeutic treatments different from those offered by traditional pharmacological methods [13, 75, 86]. An in vivo study highlights the effectiveness of this advanced technique. The study showed that light-stimulation of the deep and intermediate layers of the superior colliculus via Chr2 could suppress spontaneous limbic seizures across various experimental epilepsy models [87]. In this way, optogenetic tools emerge as a promising avenue to target specific pathways implicated in epilepsy onset in vivo. Translating this understanding into novel applications holds the potential for developing more effective drugs to suppress drug-resistant epilepsy [84].

Future Directions for Prospective Interventions to Control and Predict Drug Resistant Seizures

In the pursuit of improved seizure control and prediction, a key focus is on in vitro epilepsy models. These models serve as a robust platform for evaluating the safety of newly developed drugs and investigating their potential impact on seizures [79]. A possible path in this direction is the use of optogenetics [10]. The development of highly effective prediction algorithms is paramount to seizure prediction [88]. As an example, predicting seizures in the future could involve conducting a thorough analysis of genetic profiles and signals from in vitro electrophysiological assessments of neurons derived from iPSCs of epileptic patients [89]. These studies, which require data analysis from a variety of algorithms to find patterns and correlations between genetic defects and aberrant electrical behavior of epileptic neurons, may pave the way for the development of personalized medicine, in which medical interventions are customized to a patient's unique genetic profile and neuronal responses [79, 83, 86, 90].

In addition, closed-loop in vitro systems, provide customized responses based on inputs from neural network activity, successfully controlling aberrant neuronal behavior [18, 91]. Closed-loop systems use optical or electrical stimulation in response to signals from the neural network to regulate the electrical activity of neurons [18, 90, 92].

In this context, our research endeavors focus on the precise control over target neural function through a closed-loop in vitro light stimulation techniques, as illustrated in Fig. 3 [19]. This approach leverages the principles of optogenetics in conjunction with integrated photonics networks. Specifically, an integrated photonic neural network [93] undergoes training to discern deviant behavior within a neuronal culture and subsequently administers a tailored light stimulation to arrest seizure-like activity in a genetically modified neuronal culture. This photonic network interfaces directly with the neural population, enabling the monitoring of neuronal activity and identification of irregular network behavior, and promptly responding to any aberrant network behavior.

Significant progress has already been achieved in advancing along this trajectory. Diverse integrated photonic neural networks have been successfully demonstrated [93, 94], showcasing proficiency in signal processing of temporal sequences. We have developed integrated photonic circuits capable of generating specific light patterns to activate channel rhodopsin [95]. Furthermore, we have devised an artificial intelligence-based model for neuronal cultures, which possesses the capability to infer the connectivity map of the culture from MEA recordings [96]. This model accurately predicts the locations within the culture to elicit a response in a subset of the neuronal population by light stimulation [97].

This method offers the potential to assess the electrophysiological characteristics of neurons sourced from either animals or induced pluripotent stem cells (iPSCs) derived from epileptic patients. It enables the investigation of their network connectivity and pharmacological responses under seizure suppression induced by light. Through thorough analysis of these datasets, coupled with the application of predictive algorithms, we could attain early seizure predictions, and further employ the platform for screening and identifying novel anticonvulsant compounds.

Conclusion

In vitro studies serve as invaluable tools for gaining insights into molecular and cellular complexity, principally facilitated by cutting-edge technologies, such as optogenetics and gene therapy. These methodologies enable researchers to delve deeper into the role of specific cell types, such as astrocytes,

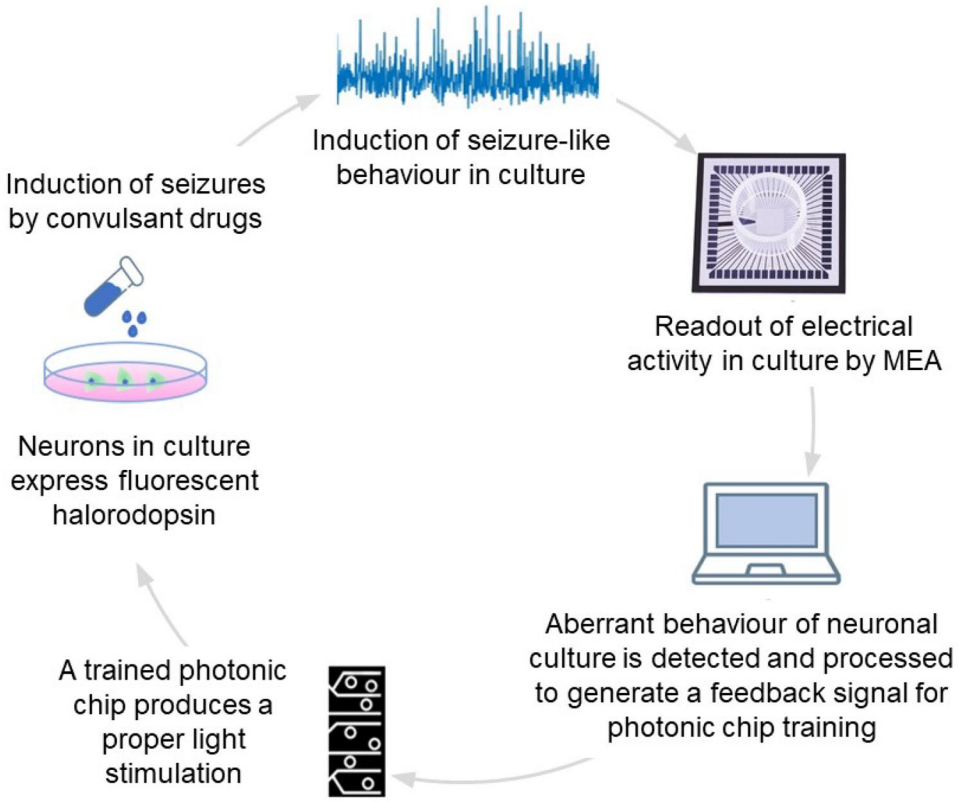

Fig. 3 In a system that combines a photonic chip with a multielectrode array (MEA), seizures can be significantly reduced. This is achieved by training the photonic chip to use the feedback from the electrical activity of neuronal cultures. This technology allows us to observe the electrical activity of neurons in real-time, and we can influence neuronal activity through light stimulation, by the expression of halorhodopsin in neurons. When the system detects unusual electrical patterns signaling a seizure, it automatically administers precisely-timed stimuli, effectively putting an end to the seizure activity. This innovative approach presents a promising approach to develop novel treatment option for epilepsy and related neurological disorders

in the genesis of epilepsy. By unraveling these complex mechanisms, novel therapeutic approaches can be developed. Moreover, in vitro studies provide an essential platform for testing new drugs, serving as a sophisticated ground to assess drug efficacy and potential side effects before clinical trials. This robust testing environment armed with newly developed technologies not only expedites evaluations but also mitigates risks associated with unforeseen outcomes in clinical trials, significantly advancing epilepsy treatment strategies. Among the proposed tools to achieve this aim, there are cutting-edge technologies effectively recording network activity and stimulating neurons accordingly, such as closed-loop systems. These innovations will undoubtedly yield new therapeutic perspectives. By integrating feedback from cross-talk between neuromorphic photonic integrated circuits and living neurons, a deeper understanding of the mechanisms underlying seizure generation and progression will be attained. Ultimately, these newly developed technologies offer hope for more effective treatments, paving the way for targeted therapies to alleviate the burden of epilepsy.

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Data Availability Data sets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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