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An update for epilepsy research and antiepileptic drug development: Toward precise circuit therapy

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ABSTRACT

Epilepsy involves neuronal dysfunction at molecular, cellular, and circuit levels. The understanding of the mechanism of the epilepsies has advanced greatly in the last three decades, especially in terms of their cellular and molecular basis. However, despite the availability of ~30 anti-epileptic drugs (AEDs) with diverse molecular targets, there are still many challenges (e.g. drug resistance, side effects) in pharmacological treatment of epilepsies today. Because molecular mechanisms are integrated at the level of neuronal circuits, we suggest a shift in epilepsy treatment and research strategies from the "molecular" level to the "circuit" level. Recent technological advances have facilitated circuit mechanistic discovery at each level and have paved the way for many opportunities of novel therapeutic strategies and AED development toward precise circuit therapy.

Contents

1. Introduction

Epilepsy is a common neurological disorder and affects all ages with a prevalence of ~1% (Devinsky et al., 2018; Thijs, Surges, O'Brien, & Sander, 2019). Although the epilepsies are diverse with varying etiologies ranging from genetic to acquired, they are commonly characterized by repeated, spontaneous epileptic seizures caused by excessive or hypersynchronous neuronal activity in the brain. Anti-epileptic drugs (AEDs) are the first-choice treatment for epilepsy, making about 60% patients with epilepsy have seizure controlled effectively. Based on the canonical view that epilepsy is caused by an imbalance of "excitation-inhibition", currently there are ~30 AEDs with diverse molecular targets to block excitatory mechanisms or enhance inhibitory ones.

Abbreviations: AED, Anti-Epileptic Drug; BDNF, Brain Derived Neurotrophic Factor; DBS, Deep Brain Stimulation; DREADDs, Designer Receptors Exclusively Activated by Designer Drugs; GABA-T, GABA Transaminase; GAD, Glutamate decarboxylase; GAERS, Genetic Absence Epilepsy Rat from Strasbourg; HMGB1, High Mobility Group Box 1; IL-1β, Interleukin-1β; KCC2, K^+–Cl^- cotransporter 2; LFS, Low-frequency Electrical Stimulation; MES, Maximal Electroshock Seizure; mTOR, Mammalian Target of Rapamycin; NKCC1, Na^+–K^+–$2Cl^-$ cotransporters 1; PV, Parvalbumin; SST, Somatostatin; SV2A, Synaptic Vesicle Glycoprotein 2A; TLE, Temporal Lobe Epilepsy; TLR4, Toll-like Receptor 4; TrkB, Tropomyosin receptor kinase B; VIP, Vasoactive Intestinal Peptide.

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However, approximately one-third of patients fail to achieve seizure control, becoming drug-resistant for epilepsy, and epilepsies in some patients are even aggravated by AED treatment (Loscher, Klitgaard, Twyman, & Schmidt, 2013). Pharmacological therapeutics has been this way for decades (Chen, Brodie, Liew, & Kwan, 2018), which may be due to an insufficient understanding of the precise mechanism of epilepsy. Long-term poor control of seizures and serious seizure-related injuries and complications are a heavy burden for patients and society (Keezer, Sisodiya, & Sander, 2016). Thus, there is a pressing need to have deeper or alternative insights into the precise mechanism of epilepsy, and thus develop more promising therapeutic approaches or drug targets to control drug-resistant epilepsy.

We have achieved a great progress in understanding of the mechanism of the different types of epilepsies in the last three decades, especially in terms of their molecular and cellular basis. Although we have long known that epilepsy pathogenesis involves neuronal dysfunction at molecular, cellular, and circuit levels, we still have limited information about the neural circuit mechanism of epilepsy. In the present review, we discuss pharmacological targets of current AEDs, current questions about these AEDs, and propose the way forward for AED development, including insights into new targets, new models, and new mechanisms. Particularly, we suggest a shift in epilepsy treatment and research strategies on a neural circuitry basis and emphasize that future AED design and development should be based on abnormal circuit therapies.

2. Pharmacological treatment with AED: Targets and current challenges

2.1. Pharmacological targets of current AEDs

Epileptic seizures have long been considered to be caused by hyperexcitatory or hypersynchronous neuronal activity during which the normal balance between excitation and inhibition is lost. Based on this canonical concept, current AEDs either dampen excitatory mechanisms or boost inhibitory ones (Bialer & White, 2010; Rogawski & Loscher, 2004; Vossler, Weingarten, Gidal, & American Epilepsy Society Treatments, 2018).

Generally, neural excitability is closely related to two factors: one is the intrinsic firing ability of a neuron, which is mainly determined by a variety of voltage-gated ion channels expressed in different compartments of the cell membrane. Voltage-gated ion channels gate the subthreshold electrical firing of the neuron and contribute to the generation of seizure discharges. Gene mutations related to these voltage-gated ion channels, including Na^+, Ca^{2+}, and K^+ channels, are central to the pathophysiology of epileptic seizures (Oyrer et al., 2018; Thomas & Berkovic, 2014a). The other factor is extrinsic neural signal input, which is mainly determined by excitatory or inhibitory synaptic transmission. Presynaptic neural firing contributes to neurotransmitter release and plays a critical role in postsynaptic neural firing behavior, leading to propagation of the epileptic discharge to nearby and distant regions. Based on this principle, we are able to categorize actions of currently available AEDs according to one of the three following aspects (Fig. 1):

2.1.1. Modulation of voltage-gated ion channels

Voltage-gated sodium channels play an essential role in the initiation and propagation of action potentials in neurons (Kohling, 2002; Mantegazza, Curia, Biagini, Ragsdale, & Avoli, 2010). "Sodium channel blockers" are the most frequently used AEDs in the treatment of epileptic seizures. AEDs such as phenytoin, carbamazepine, and valproate are thought to bind to the active state of the channel and reduce high frequency firing during a seizure; some of the newer AEDs such as oxcarbazepine, lamotrigine, and zonisamide act by facilitating the fast inactivation of sodium channels. In addition, lacosamide is thought to enhance slow inactivation of voltage-gated sodium channels (Meldrum & Rogawski, 2007; Vossler, et al., 2018).

Voltage-gated potassium channels, the most diverse group of ion channel, serve to limit excitability in neural cells. Opening of potassium

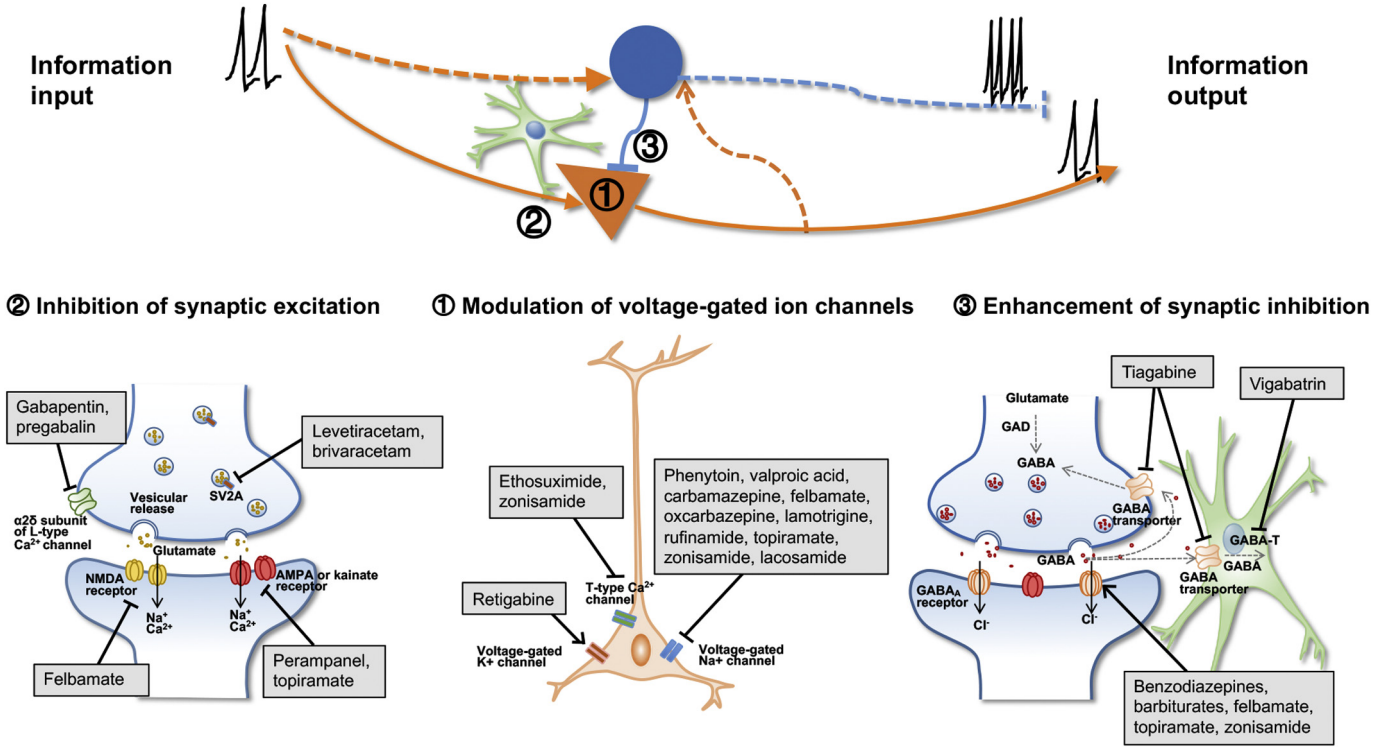

Fig. 1. Molecular targets of current AEDs. Top panel, neural excitability closely related to two factors: intrinsic firing ability of a neuron ①, and extrinsic neural signal input, which is mainly determined by excitatory ② or inhibitory ③ synaptic transmission. Blue, inhibitory neuron; Orange, excitatory neuron. Bottom panel, based on this principle, it is convenient to categorize actions of currently available AEDs according to three aspects: ① Modulation of voltage-gated ion channels; ② Inhibition of synaptic excitation; ③ Enhancement of synaptic inhibition.

channels drives the membrane potential toward hyperpolarization, which causes a generalized reduction in excitability. In particular, M-currents have been reported to be closely involved in epilepsy (Cooper, 2012; Kohling & Wolfart, 2016). Retigabine is the first AED to be identified with an opener of KCNQ channels that selectively acts on neuronal Kv7 potassium channels (M-current) (Main et al., 2000; Schenzer et al., 2005), but now it is not commercial available due to its side effects.

Voltage-gated calcium channels can be grouped into high voltage-activated and low voltage-activated families (Cain & Snutch, 2012; Rajakulendran & Hanna, 2016). Notably, the latter, including T-type Ca^{2+} channel, is believed to regulate excitability in the brain by participating in neuronal bursting firing and intrinsic oscillations, which is closely involved in epileptic discharge, especially in absence seizures. Ethosuximide is commonly used for the treatment of absence seizures via inhibition of T-type calcium channels in thalamic neurons (Coulter, Huguenard, & Prince, 1989). Another anti-absence seizure AED, zonisamide, also inhibit T-type Ca^{2+} channel (Kito, Maehara, & Watanabe, 1996).

2.1.2. Inhibition of synaptic excitation

Action potentials, spreading from soma to axon, are crucial elements in neurotransmitter release, which is required for synaptic excitatory transmission. Thus, inhibition of synaptic excitation is one of the mechanisms of AED action. High voltage-activated calcium channels are believed to regulate excitability in the brain via regulating the gating of calcium entry and neurotransmitter release from presynaptic nerve terminals, which requires strong presynaptic neuronal membrane depolarization. Gabapentin and pregabalin bind to the α2δ subunit of these voltage-gated calcium channels, which is thought to be associated with a decrease in neurotransmitter release (Hendrich et al., 2008; Stahl et al., 2013). Levetiracetam and its newly structure-modified compound brivaracetam are the only available drugs that bind to synaptic vesicle glycoprotein 2A (SV2A), which might have a role in neurotransmitter release of presynaptic excitatory neurons (Kaminski, Gillard, & Klitgaard, 2010).

The excitatory neurotransmitter glutamate released from presynaptic membrane can bind with inotropic or metabotropic glutamate receptors to induce excitatory neurotransmission in the brain. In particular, ionotropic glutamate receptors (cationic permeable) located in the postsynaptic membrane mediate the bulk of fast excitatory neurotransmission, which is also one of the mechanisms of AED action. Perampanel is a selective noncompetitive antagonist of AMPA glutamate receptor approved for epilepsy treatment (French et al., 2012; Hanada et al., 2011). Topiramate produces anti-seizure effect partly via inhibition of excitatory synaptic transmission by acting on AMPA and kainate receptors (Gryder & Rogawski, 2003; Shank, Gardocki, Streeter, & Maryanoff, 2000). AED felbamate is able to limit excitatory synaptic transmission via inhibition of postsynaptic membrane NMDA receptors (Rho, Donevan, & Rogawski, 1994).

2.1.3. Enhancement of synaptic inhibition

Enhancement of inhibitory neurotransmission mediated by GABA, one of the most important inhibitory neurotransmitters in the brain, is a key mechanism of AED action. GABA is synthesized from glutamate by glutamate decarboxylase (GAD) (Roberts & Frankel, 1950) and then released to reduces neuronal excitability by acting on ionotropic $GABA_A$ receptors and metabotropic G-protein-coupled $GABA_B$ receptors at both pre- and post-synaptic locations (Buzsaki, Kaila, & Raichle, 2007). Then, the GABA can be transported by high-affinity GABA transporters (Conti, Minelli, & Melone, 2004), and subsequently metabolized by GABA transaminase (GABA-T) (Sherif & Ahmed, 1995), which lead to termination of GABAergic transmission. Deficits in any step of the above GABAergic cycle have been reported to closely related with generation of epileptic seizure (During, Ryder, & Spencer, 1995; Loup, Wieser, Yonekawa, Aguzzi, & Fritschy, 2000; Sepkuty et al., 2002).

Many currently available AEDs boost fast inhibitory neurotransmission $GABA_A$ receptor-mediated inhibition by directly allosterically binding with $GABA_A$ receptors. Although the precise binding sites with the $GABA_A$ receptor complex can be diverse, AEDs, like benzodiazepines and barbiturates, have been reported to enhance $GABA_A$ receptor-mediated Cl^- currents by increasing the opening time or frequency of channels (Bai et al., 2001; Rudolph et al., 1999; Walker & Kullmann, 2012). AEDs also alter the cycle of GABA dynamics by modifying GABA transporters and the activity of enzymes so as to produce anti-seizure effect. Tiagabine has been found to inhibit the GABA transporter GAT1 and thus in turn increase GABA concentration in the synaptic cleft (Suzdak & Jansen, 1995); Vigabatrin has been found to inhibit the GABA transaminase (decrease the metabolism of GABA), and thus in turn increase the GABA concentration (Debiase, Barra, Bossa, Pucci, & John, 1991).

2.2. Current challenges in AED treatment

From the early 1900s when only a single bromide was available for treatment to the present day with nearly 30 effective AEDs, we have witnessed the development of pharmacological treatments that have achieved great success in seizure control in many different types of epilepsies. AEDs have also improved by becoming safer and causing fewer side effects. However, there are still several important unmet therapeutic needs and challenges today.

The first and the most important challenge is drug resistance, or pharmacoresistance. Patients who do not achieve adequate seizure control after trials of at least two appropriate AEDs can meet the criteria for pharmacoresistance. This situation has been in place for decades, showing a relatively unchanged rate of seizure-freedom over time (Fig. 2). It is a long-term concern that the efficacy of pharmacological treatment of epilepsy has not greatly improved, although new AEDs are introduced yearly, providing hope for drug-resistant patients (Shorvon, 2009a, 2009b). Most recently, Chen, Brodie, et al. (2018) have checked the AED treatment outcomes in epilepsy patients in a longitudinal observational cohort study and found that 63.7% (1144 out of 1795) patients have been seizure-free for at least 1 year at the end of the 30-year study period (Z. Chen, et al., 2018). As a comparison, the previous result from a two decade longitudinal study with 470 epilepsy patients in 2000 is 64.0% (Kwan & Brodie, 2000). The findings confirm that many patients continue to have drug-resistant seizures, and the urgent need for new highly effective drugs or therapeutic approaches.

Another major concern is side effects of AED. Most, if not all, of the currently available AEDs are associated with one or more side effects, including ataxia, dizziness, sedation, cognitive impairment, and depression (Perucca & Gilliam, 2012; Stephen, Wishart, & Brodie, 2017). These side effects can contribute to the termination of pharmacological treatment in a large proportion (up to 40%) of patients and have a severe impact on quality of their life (Schmidt, 2009). These side effects can result from a drug-disease interaction, drug accumulation, or can emerge after chronic exposure. Notably, many molecules and proteins targeted by AEDs have a natural physiological function. This is the reason for side effects of AED as doses are increased. Encouragingly, the third

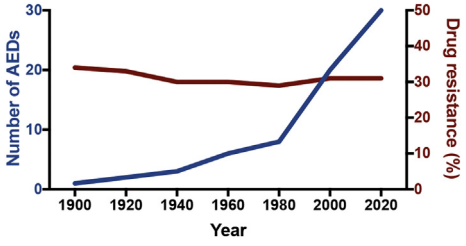

Fig. 2. AED development and drug-resistant rate. Despite the increase of AEDs with diverse molecular targets, the drug-resistant rate in epilepsy has always remained at approximately one-third.

generation AEDs introduced during the past decades have shown little side-effect and better tolerance (Marson, et al., 2007a, 2007b). Other questions remain: current AEDs seem to act purely to symptomatically suppress seizures, no AED has been shown to prevent epileptogenesis (the process from a normal brain to an epileptic one) in patients prior to the first seizure (Kobow et al., 2012). Further, apart from seizure itself, the burden of comorbidities, including depression, anxiety, migraine, and heart disease, is extremely high in epilepsy (Keezer et al., 2016). At present, no single AED seems to treat comorbidities.

These current dilemmas in pharmacological treatment remind us that new concepts and thinking is urgently needed to make a deeper understanding of the precise mechanism of epilepsy or epileptogenesis and thus to produce more effective and precise AED.

3. The way forward for AED development: Insights into new models, new mechanisms, and new targets

Our understanding of the molecular level in the pathophysiology of many different types of epilepsy in the past has helped us to discover many new AEDs with different targets, and we believe that deeper insights into the mechanism of epilepsy will continue to achieve success in the treatment of epilepsy in the future (Leach, 2018; Staley, 2015a). The goal of pharmacological treatment in epilepsy with a new AED is: greater efficacy for those epilepsy patients with pharmacoresistance, or fewer side effects. To achieve this goal, we need new thinking and novel approaches to change the way of AED development, in the following three aspect: new model, new mechanism model, and new targets.

3.1. AED development with new models

Our understanding the pathophysiology of epilepsy and drug discovery is largely dependent on animal models (Table 1). Most of current AEDs have been identified by screening in simple acute-seizure models. The maximal electroshock seizure (MES) test or pentylenetetrazol seizure test are long-term "gold standards" for screening new AEDs (Bialer & White, 2010; Grone & Baraban, 2015). These acute seizure models are technically simple and make it easy to screen large numbers of compounds, but they produce seizure in a normal brain and obviously lack sufficient predictability of developing AEDs with higher efficacy for pharmacoresistant seizures. For example, MES and pentylenetetrazol tests were unable to identify the anticonvulsant activity of levetiracetam. Further, investigational drugs are tested in these acute seizure models after acute administration, while pharmacological treatment of epilepsy patients in the clinic is typically by chronic administration, which may change drug efficacy. Thus, in addition to an acute anticonvulsant screening model, there is increasing recognition of the need for drugs screened in chronic epilepsy models.

Chronic epilepsy models include models of acquired (symptomatic) epilepsy and models of genetic (idiopathic) epilepsy. For acquired epilepsy, epilepsy are induced by electrical or chemical methods in previously healthy (non-epileptic) animals, including the kindling model and post-status epileptic model (Morimoto, Fahnestock, & Racine, 2004). The kindling model involves progressive development of complex partial seizures with secondary generalization induced by repeated electrical stimulation, which is the only chronic model that is currently used by some AED discovery programs (Stables et al., 2002). The kindling model has allowed for identification of the anticonvulsant activity of levetiracetam, which is not done in acute MES or pentylenetetrazol model. Based on the kindling model, several in vivo pharmacoresistant epilepsy models have been developed, including the phenytoin-resistant kindled rat, the lamotrigine-resistant kindled rat, and the 6-Hz psychomotor seizure model of partial epilepsy (Loscher, 2011). However, building these models is costly and laborious, and notably, they do not mimic true epilepsy in human patients, that is, spontaneous seizure occurrence.

Another important category of chronic epilepsy models is post-status epileptic models, in which recurrent, spontaneous seizures can be induced by a chemical convulsant (pilocarpine or kainic acid) or through sustained electrical stimulation (Morimoto et al., 2004). In these models, status epilepticus is induced first and maintained for more than an hour. Animals then appear to have an overtly normal latency period (usually several weeks) and subsequently the animals begin to exhibit spontaneous seizures frequently, which usually persist for the remainder of their life. The post-status epileptic models have been reported to have hippocampal pathology resembling typical pathological patterns of hippocampal sclerosis evident in patients with temporal lobe epilepsy (TLE) (Coulter, McIntyre, & Loscher, 2002). The post-status epileptic models are now widely used to study the pathophysiology of epileptogenesis. However, the post-status epileptic models are currently not widely used for drug discovery because they are expensive and time-consuming. As these models also allow selection of AED responders and non-responders (Loscher, 1997), we believe that they are suited for discovering novel drugs with higher efficacy against not only epileptogenesis but also chronic drug-resistant epilepsy.

Table 1
Seizure and epilepsy models.

Animal model	Epilepsy type	Pathological change	AED screen	References
Maximal electroshock seizure model	Generalized tonic-clonic seizure	No	The most widely used	(Bialer & White, 2010; Loscher, 2017)
Pantyleneterrazol seizure model	Generalized myoclonic seizure	No	The most widely used	(Bialer & White, 2010; Loscher, 2017)
6-Hz psychomotor seizure model	Partial seizure	No	"As needed" use	(Barton, Klein, Wolf, & White, 2001; Metcalf et al., 2017)
Kindling epilepsy model (stimulation at amygdala or hippocampal et al. regions)	Focal seizure with secondarily generalized seizure	No significant brain damage; Functional change, including: changed synaptic plasticity, neurotransmitter release, receptors expression, gliosis et al	"As needed" use	(Morimoto et al., 2004; Sato, Racine, & McIntyre, 1990)
Post-status epilepsy model (kainite or pilocarpine)	Temporal lobe epilepsy with hippocampal sclerosis	Hippocampal circuit rearrangement and functional change, including neuronal loss, mossy fiber sprouting, abnormal neurogenesis et al.	Not regularly used	(Bouilleret et al., 1999; Curia, Longo, Biagini, Jones, & Avoli, 2008; Morimoto et al., 2004)
GAERS or WAG/Rij rat	Absence epilepsy	Functional change, including increased T-type Ca^{2+} channel current, abnormal thalamus-cortex circuit et al.	"As needed" use	(Depaulis, David, & Charpier, 2016; van van Luijtelaar & Zobeiri, 2014; Vergnes et al., 1982)
Other transgenetic epilepsy model (mutation of Na^{+} or K^{+} channel)	Genetic (idiopathic) epilepsy	Recapitulate many of the features of human genetic epilepsy resulting from a specific defect in a particular receptor or voltage-gated ion channel.	Not regularly used	(Bialer & White, 2010; Grone & Baraban, 2015; Thomas & Berkovic, 2014b)

In addition, numerous transgenic animal models have been developed to mimic the phenotypic of human genetic (idiopathic) epilepsy, including mice with mutation in $Na_{V1.1}$ (*SCN1A*), and $K_{7.3}$ (*KCNQ3*), and rats with spontaneous absence seizure (the Genetic Absence Epilepsy Rat from Strasbourg, GAERS) (Bialer & White, 2010). Recently, transgenetic zebrafish models of epilepsy, including mutation in *UBE3A* (mimic Angelman's syndrome), and *SCN1A* (mimic Dravet syndrome), have gradually been used to screen drugs in acutely evoked seizure events with advantages of rapid and relatively simple genetic modifications for modeling genetic epilepsies (Grone & Baraban, 2015). Drug screening is not widely conducted in these genetic mutant models, apart from the GAERS model that is now used for testing drugs for anti-absence seizure properties. Such models may ultimately represent a valuable platform in the routine evaluation of investigational AED for precise epilepsy treatment.

Epilepsy research and drug screening programs are still hampered by a lack of adequate experimental models of "real" chronic drug-resistant epilepsy that mimic clinical patients. The current screening models may be biased toward a restricted set of anti-seizure targets and mechanisms in "molecular" level. The epilepsies are diverse with varying etiologies ranging from genetic to acquired, but a single animal epilepsy model used to predict the therapeutic potential of an investigational AED for drug-resistant epilepsy is obviously not enough. Thus, the inclusion of these chronic epilepsy models may potentially help us to identify more new and promising AEDs for the treatment of patients with drug-resistant epilepsy. A "real" epileptic model with an abnormal pathological basis would be an ideal option for a drug screening model.

3.2. AED development with new molecular mechanisms

New antiepileptic compounds that are under investigation can be grouped into two categories: (1) AEDs with new structure but a mechanism of action similar to current AEDs. In many cases, these compounds are structurally modified from existing ones. (2) AEDs with new structure and novel (or unknown) mechanism that are different from current AEDs. We provide a detailed summary in Table 2.

3.2.1. Structural modifications to existing AEDs

The purpose of designing structural modifications to existing AEDs (second-generation to existing AEDs) is to improve the efficacy and/or tolerability of existing AEDs. For example, brivaracetam is a (4*R*)-propyl derivative of levetiracetam, and is identified in a high-throughput screen of 12,000 compounds. Brivaracetam has a 10-fold higher affinity for SV2A than levetiracetam (Kenda et al., 2004), leading to more potent in antiepileptic efficacy in animal epilepsy models (Bialer et al., 2009). Another example is valrocemide (valproyl glycinamide), which is the most promising second-generation drug to valproic acid (Bialer et al., 2009). The use of valproic acid, one of the most widely prescribed AEDs, is limited by two major side effects, teratogenicity and hepatotoxicity (Huber-Mollema, Lindhout, Oort, Rodenburg, & Dev, 2018). Valrocemide is designed to a broad-spectrum AED with fewer side effects. Other AEDs, including carbamazepine, lamotrigine, and gabapentin, have their second-generation AEDs under investigation (Bialer & White, 2010).

Table 2
New antiepileptic compounds under investigation.

Type	Drug	Potential action of mechanism	Reference
Mechanism similar to current AED	XEN901	Selective Nav1.6 inhibitor	(Bialer et al., 2018)
	Brivaracetam	Selective ligand for synaptic vesicle protein 2A	(Bialer et al., 2017)
	Ganaxolone	GABA receptor modulator	(Pieribone et al., 2007)
	Allopregnanolone (SAGE-547)	$GABA_A$ receptor modulator	(Belelli, Peden, Rosahl, Wafford, & Lambert, 2005)
	Valnoctamide	$GABA_A$ receptor agonist	(Barel et al., 1997)
	OV329	Inactivation of GABA-T	(Bialer et al., 2018)
	YKP3089 (cenobamate)	Selective blocker for the inactivated state of the sodium channel, facilitates presynaptic GABA release	(Bialer et al., 2015)
	ICA-105665	Selective opener of neuronal Kv7 potassium channels	(Kasteleijn-Nolst Trenite et al., 2013)
	1OP-2198	Selective opener of neuronal Kv7 potassium channels	(Bialer et al., 2017)
	XEN1101	Enhances activation of neuronal Kv7.2-7.5 potassium channels	(Bialer et al., 2018)
	FV-137	Inhibition of P/Q-type Ca^{2+} channels	(Bialer et al., 2018)
	Selurampanel (BGG492)	Competitive antagonist for AMPA and kainate receptors	(Faught, 2014)
	JNJ-55511118	Selective negative modulator of AMPA receptor	(Bialer et al., 2018)
Novel mechanism	Everolimus	Selective inhibitor of mTOR pathway	(French et al., 2016)
	Anakinra	IL-1β receptor antagonist	(DeSena et al., 2018)
	BUM5	Selective inhibitor of NKCC1	(Bialer et al., 2017)
	Cannabidiol (Epidiolex)	Selective activation of Cannabidiol Signaling	(Bialer et al., 2017)
	MRSMRS4204	Adenosine kinase inhibitor	(Bialer et al., 2018)
	JNJ-40411813	Allosteric modulator site independent of the agonist binding site on the mGlu2 receptor.	(Bialer et al., 2018)
	Huperzine A (BIS-001)	Acetylcholinesterase inhibitor	(Bialer et al., 2018)
	2-Deoxy-D-glucose	A glucose analogue and a reversible inhibitor of glycolysis.	(Bialer et al., 2018)
	Pitolisant	Histamine 3 receptor antagonist	(Kasteleijn-Nolst Trenite et al., 2013)
	Minocycline	Anti-inflammatory effects, including inhibiting mitochondrial cytochrome-c release, minocycline blocks death receptor pathways and activation of microglia.	(Bialer et al., 2017)
	Fenfluramine (ZX008)	Serotonin reuptake inhibitor	(Ceulemans, Schoonjans, Marchau, Paelinck, & Lagae, 2017)
	Nalutozan	Nonazapirone 5-HT1A partial agonist	(Merlet et al., 2004)
	NAX 810-2	Galanin receptor agonist	
	Quinidine	Partial antagonist of KCNT1	(Bearden et al., 2014)
	Verapamil	Inhibitor of P-glycoprotein	(Borlot et al., 2016)
	TAK-935	Competitive inhibitor of cholesterol 24-hydroxylase	(Bialer et al., 2018)
Unknown mechanisms	JNJ-26489112	Unknown	(Di Prospero et al., 2014)
	FV-082	Unknown	(Bialer et al., 2018)

The existing second-generation AEDs can be designed to improve the efficacy, and/or tolerability of existing AEDs to some extent. However, as the antiepileptic action of these new AEDs is still similar to the old ones, it remains to be determined whether drug-resistant patients will benefit from those new second-generation AEDs. There are similar worries for many other novel AEDs with novel structures but mechanisms of action similar to old AEDs (Table 1).

3.2.2. AEDs with new structure and novel mechanism

Recent progress in our understanding the new "molecular level" mechanism of epilepsy has helped us to identify other promising and novel targets (using an old drug for new indications in some cases) for clinical trials. In the below part, we describe particularly interesting novel mechanisms involved in seizure and epilepsy and some potential druggable targets which may be benefit for refractory epilepsy.

Inflammatory pathways. A large amount of evidence suggests that inflammation is involved in the pathophysiology of epilepsy (Barker-Haliski, Loscher, White, & Galanopoulou, 2017; Vezzani, French, Bartfai, & Baram, 2011; Vezzani, Lang, & Aronica, 2015). Cytokine interleukin-1β (IL-1β), one of the important players in inflammation, is mainly produced by glia cells and contributes to the hyperexcitability of neurons in epilepsy. Activation of IL-1β-converting enzyme (also known as caspase-1) and downstream IL-1β – IL-1 receptor 1 signaling occur in human and experimental epilepsy (Vezzani & Baram, 2007). We also found that transient increase of IL-1β after prolonged febrile seizures enhance seizure susceptibility in adults (B. Chen et al., 2016; Feng & Chen, 2016; Feng et al., 2016). In addition, IL-1β is a key regulatory factor for postictal suppression (Tao et al., 2015) and diazepam-resistant status epilepticus (Xu et al., 2016). From the perspective of therapy, VX-765, a selective caspase-1 inhibitor (that inhibits IL-1β biosynthesis), reduced both acute and chronic epileptic activity in animal models (Maroso et al., 2011; Ravizza et al., 2008). Meanwhile, anakinra, a human recombinant IL-1β receptor antagonist used to the treatment of rheumatoid arthritis, have showed therapeutic effects in animal epilepsy models (Librizzi, Noe, Vezzani, de Curtis, & Ravizza, 2012; Noe et al., 2013), and showed some potential benefits in clinical patients (DeSena, Do, & Schulert, 2018). Another potentially interesting target is the high mobility group box 1 (HMGB1), which is closely related to the pathology of epilepsy (Maroso et al., 2010; Zurolo et al., 2011). HMGB1 can be activated by epileptogenic injuries in the brain. It exerts powerful proconvulsant effects via the toll-like receptor 4 (TLR4), and downstream NMDA receptor-related signaling network (Balosso, Liu, Bianchi, & Vezzani, 2014; Park et al., 2004). Thus, inhibition of HMGB1 - TLR4 signaling may represent a novel antiepileptic treatment strategy. We demonstrated the antiepileptic therapy potential of an anti-HMGB1 monoclonal antibody in multiple animal models and human epilepsy tissue (Zhao et al., 2017). Antagonists or knockout of TLR4 decrease acute and chronic seizure recurrence in an animal model (Iori et al., 2013). Notably, TLRs use signaling molecules that partially overlap with IL-1R1 (Vezzani, Maroso, Balosso, Sanchez, & Bartfai, 2011). Therapeutic efficacy would be better if the target is common overlapping molecular signaling.

mTOR pathway. The mammalian target of rapamycin (mTOR) signaling pathway modulates cell growth, differentiation, and synaptic plasticity by regulating metabolism and protein synthesis in the brain (Crino, 2016; Lipton & Sahin, 2014). Recent studies indicate mTOR dysregulation in the pathogenesis of different types of epilepsy, such as tuberous sclerosis complex patients with epilepsy, focal cortical dysplasia, and TLE (Crino, 2015; Wong, 2013). Zeng, Xu, Gutmann, and Wong (2008) first reported that the mTOR inhibitor rapamycin alleviates epilepsy in a mouse epilepsy model of tuberous sclerosis complex (Zeng et al., 2008). More recently, mTOR inhibitors have been reported to have consistent protective effects in various epilepsy models, including tuberous sclerosis complex, absence epilepsy, and post-status epilepticus, suggesting rapamycin as a potential antiepileptic target. Furthermore, the effectiveness of mTOR inhibitors has been verified in clinical epilepsy patients with tuberous sclerosis complex and cortical dysplasia (Citraro, Leo, Constanti, Russo, & De Sarro, 2016). Rapamycin, however, has many severe side effects, which makes therapeutic intervention by targeting the mTOR pathway unsatisfactory, unless tolerability is improved. At present, the rapamycin analogue everolimus, which is developed initially as an antitumor agent, is being assessed for its new indication in epilepsy patients, and has shown some promising preliminary results (Krueger et al., 2010; Krueger et al., 2013). In future, the efficacy of mTOR inhibitors as epilepsy treatments is likely to be assessed in other mTOR-associated epilepsy types.

BDNF pathway. Brain derived neurotrophic factor (BDNF) has important effects on neuronal activity in the brain via rapidly modulation of both excitatory and inhibitory synaptic transmissions (Huang & Reichardt, 2001). Emerging evidence suggests that activation of BDNF and its tropomyosin receptor kinase B (TrkB) exists in both animal models and humans with epilepsy (Binder, Croll, Gall, & Scharfman, 2001; McNamara & Scharfman, 2010). Augmentation of BDNF - TrkB signaling, by infusion of BDNF or transgenic overexpression of BDNF/TrkB enhances seizure susceptibility (Heinrich et al., 2011; Koyama et al., 2004; Scharfman, Goodman, & Sollas, 1999), while conditional knockout or chemical-genetic inhibition of TrkB protects against epileptogenesis (He et al., 2004; Liu et al., 2013). These findings suggest that inhibiting BDNF-TrkB signaling could represent a promising antiepileptic strategy. Recently, Gu et al. (2015) designed a novel peptide (pY816) that uncouples TrkB from its phospholipase Cγ1, preventing TLE following status epilepticus (Gu et al., 2015). It remains to be determined how this novel peptide targeting could be translated into clinical treatment. Further analysis of the cellular/molecular mechanisms that influence excitability in the brain by BDNF-TrkB signaling could provide other novel targets for antiepileptic therapy.

Cation chloride co-transporters. Cation-chloride cotransporters have critical roles in shaping GABAergic signaling and neuronal connectivity (Blaesse, Airaksinen, Rivera, & Kaila, 2009; Doyon, Vinay, Prescott, & De Koninck, 2016). The expression of these cation-chloride cotransporters are sensitive to neuronal activity, and thus neuronal Cl^- regulation is affected in multiple pathophysiological conditions, including epilepsy (Kaila, Price, Payne, Puskarjov, & Voipio, 2014; Miles, Blaesse, Huberfeld, Wittner, & Kaila, 2012). A common feature of chronic epilepsy is the dysregulation of chloride homeostasis-regulating genes and proteins, in particular with down-regulation of K^+–Cl^- cotransporter 2 (KCC2) and up-regulation of Na^+–K^+–$2Cl^-$ cotransporters 1 (NKCC1), which leads to a positive shift of reversal potential of $GABA_A$ receptors and hyper-excitability network activity (Wang, Wang, & Chen, 2018). At present, there is still a lack of specific KCC2-agonist drugs (Moore, Kelley, Brandon, Deeb, & Moss, 2017), and further development of such drugs are vitally important for the treatment of epilepsy. The NKCC1 blocker bumetanide, which is now used as a clinical diuretic, has received considerable interest for treating epilepsies that are often refractory to standard therapies. Bumetanide suppresses seizure activity and enhances anticonvulsant efficacy of some AED acting on $GABA_A$ receptor in animal epilepsy models (Loscher, Puskarjov, & Kaila, 2013). Encouragingly, bumetanide has been reported to alleviate the severity of seizure in a small number of TLE patients (Eftekhari et al., 2013). However, bumetanide is not easily to pass through the blood-brain barrier, which limits its clinical antiepileptic applications. Therefore, much attention is now paid to designing bumetanide prodrugs that can penetrate the blood-brain barrier more easily, like BUM5 (Erker et al., 2016; Tollner et al., 2014), and thus produce more effective antiepileptic effects.

Cannabinoid Receptors. Endocannabinoid signaling is an important regulator of synaptic neurotransmission and network patterns in the brain (Lu & Mackie, 2016). Cannabinoid receptor antagonists produce enhanced brain excitability. Epilepsy has been treated with cannabidiol for a long time, but the anticonvulsant mechanism of action of cannabidiol is still not fully understood (may be independent of cannabinoid receptors) (Billakota, Devinsky, & Marsh, 2019; Brodie & Ben-

Menachem, 2018). The NIH funded Epilepsy drug screening program have provided the most recent evidence of anti-convulsant efficacy for cannabidiol in different acute seizure models (Kaplan, Stella, Catterall, & Westenbroek, 2017; Klein et al., 2017). Cannabidiol (Epidiolex) is presently in phase III development for Dravet syndrome, Lennox-Gastaut syndrome, tuberous sclerosis complex, and infantile spasms (Devinsky et al., 2016; Devinsky et al., 2017; Devinsky et al., 2018; Thiele et al., 2018). Encouragingly, current data indicates that cannabidiol might have a promising antiepileptic effect with adequate safety profile in children and young adults with highly drug-resistant epilepsy. Cannabidiol could result in a greater reduction in seizure frequency among patients with Dravet syndrome, but is associated with higher rates of adverse events. Nevertheless, cannabinoid-targeting drugs may have very different effects on hyperexcitability and seizures when used for acute vs. long-term treatment (Armstrong, Morgan, & Soltesz, 2009; Soltesz et al., 2015), which may complicate the therapeutic effects within the central nervous system. Thus, more multi-center randomized controlled trials are warranted to characterize true antiepileptic efficacy and the safety profile of this compound.

Others. There are other important mechanisms or hypotheses involved in refractory epilepsy have been proposed. (1) Transporter hypothesis. Increased expression or function of efflux transporter proteins in the blood-brain barrier, including P-glycoprotein, multidrug resistance-associated proteins, breast cancer-resistance protein, lead to the decrease of effective concentration of AEDs at their target (Feldmann & Koepp, 2016; Kwan & Brodie, 2005; Schmidt & Loscher, 2005). (2) Target hypothesis. Altered properties of drug target results in decreased drug sensitivity and lower anti-seizure effect (Loscher, 2005; Remy & Beck, 2006). (3) Circuit hypothesis. Formation of abnormal neural circuit, including synaptic reorganization, axonal sprouting, disrupted neurogenesis and gliosis, not only decreases the endogenous inhibitory effect of anti-seizure system, but also changes functional output of AED targets (see detail in below section) (Fang, Xi, Wu, & Wang, 2011; Tang, Hartz, & Bauer, 2017). Currently, it is still unclear which hypothesis is the most predominant mechanism underlying refractory epilepsy. Drug resistance in epilepsy can be a multifactorial phenomenon, which is involved in either genetic or acquired manner. Notably, there is no promising drug was developed based on above hypothesis, apart from the use of a non-specific P-glycoprotein inhibitor verapamil as a test drug in epilepsy treatment (Narayanan et al., 2016; Pirker & Baumgartner, 2011; Summers, Moore, & McAuley, 2004).

Searching for novel target strategies in AED development (old drugs for new indications in some cases) may offer a compelling direction for basic research and even industry investment in order to provide new promising treatment options for epilepsy. It remains to be determined how these above-mentioned novel targets could be translated into clinical treatment for epilepsy that is refractory to standard therapies.

3.3. AED development with neural circuit mechanism

The ~30 AEDs available and other potential novel targets under investigation all focus on diverse molecular targets. Molecular mechanisms are integrated at the level of neuronal circuits and thus achieve their whole functions. Thus, an alternative more promising way to control a particular type of epilepsy (especially those refractory epilepsy) may focus on targeting specific neural circuits, instead of targeting a particular molecular mechanism (Krook-Magnuson & Soltesz, 2015). One important answer to the dilemma in pharmacological treatment today may be that the action of AED design takes neuronal circuits into account (Fig. 3).

As for pharmacoresistance, “sodium channel blockers” inhibit action potentials of all type of neurons and their downstream neural transmission; GABA release may also be inhibited rather than only glutamate release. Parvalbumin (PV) positive GABAergic neurons, fire at high frequencies, which is a key component in feed-forward microcircuits (Hu, Gan, & Jonas, 2014) and may be susceptible to reduced firing by these AEDs. Meanwhile, glutamatergic transmission may form a feedback circuit with local GABAergic interneurons (Buckmaster, Zhang, & Yamawaki, 2002; Dengler & Coulter, 2016) and inhibition of glutamatergic transmission may also impair local inhibitory function. These situations mean that AEDs could potentially worsen seizures.

GABAergic synaptic transmission in seizures is double-edged. In particular, $GABA_A$ receptor-mediated fast inhibitory transmission can exert both seizure-suppressing and seizure-promoting actions, depending on the “activity-dependent” or “pathology-dependent” changes in Cl^- plasticity (Kaila & Miles, 2010; Wang, Wang, & Chen, 2018). Further, GABAergic synapses can have diverse integration at the level of neuronal circuits or microcircuits, such as feed-back circuit, feed-forward circuit, disinhibition circuit, and counter-inhibition circuits (Paz & Huguenard, 2015a). Thus, those “GABAergic potentiation” AEDs may not always be antiepileptic, and in many situations can even aggravate epileptic seizures. These examples suggest that we need to design drugs in a cell type-specific manner and take into account the action of AEDs at the microcircuit and circuit level.

In terms of side effects, one single molecule is involved in many different brain circuits with diverse signals, and it may exist in different type of cells or circuits with different functional outcome. Although epilepsy may be caused by a change in the function of a specific protein in a specific type of cell, the same protein in other cell types or circuit may be functioning normally and even perform other important physiological function. Similarly, firing of a single neuron can easily spread to its linked neuronal networks. Block of seizure-related firing, rather than disturbing normal firing, can be used to reduce side effects. This situation would challenge traditional pharmacological treatment involving the systemic delivery of drugs acting on a particular molecule but that does not distinguish among precise neural circuit elements.

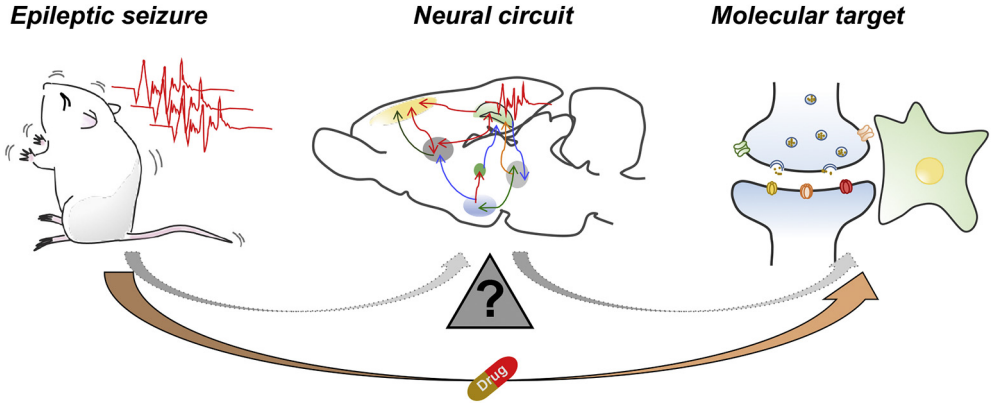

Fig. 3. Schematic illustrating macro-to-micro insights into the mechanism of epileptic seizure. Pathogenesis of epilepsy involves neural dysfunction at the molecular, cellular, and circuit levels, but we still have very limited information for the neural circuit mechanism. Pharmacological treatment and AED design need to take neuronal circuits into account.

In summary, in the last three decades, researchers have identified changes at the molecular level in the pathology of epilepsy, in neurotransmitters, receptors, and enzymes; and in individual circuit components, which ultimately cause a hyperexcitable epileptic brain (Avoli, Louvel, Pumain, & Kohling, 2005; McNamara, 1994; Staley, 2015b). Notably, these changes do not exist alone, but are integrated at the level of larger neuronal circuits; the fine balance between these changes dictates the functional outcome of large-scale networks (Fig. 3). Thus, the interpretation of the implications of these "molecular level" changes is often limited, if we do not place these observed changes appropriately in "circuit level" of the brain. Understanding circuit-level interactions of epileptic seizures may guide us not only to better understanding of the pathophysiology of epilepsy and but also to identify more effective pharmacologic treatment for drug-resistant epilepsy by targeting precise neural circuit.

4. Epilepsy research on neural circuits

4.1. Network research development in epilepsy

Much of epilepsy research leads to epilepsy being gradually accepted as a circuit-level syndrome pathologically characterized by hypersynchronous seizure activity with enhanced neuronal excitability within neural circuits (Goldberg & Coulter, 2013; Paz & Huguenard, 2015b). The search item of "(epilepsy and neural network) or (epilepsy and neural circuit)" in the US National Library of Medicine "PubMed" database yielded ~1600 papers. Notably, we can see from Fig. 4, there are two peak periods for network research in epilepsy.

After 1995, the wide application of multiple-channel EEG recordings and neuroimaging techniques promoted network research in epilepsy. At present, EEG recordings serve as a "gold standard" to diagnose epilepsy and are now used to predict seizures (Kuhlmann, Lehnertz, Richardson, Schelter, & Zaveri, 2018; Perucca, Dubeau, & Gotman, 2014). Neuroimaging techniques like PET scanning, structural or functional MRI, and DTI are used to delineate the epileptic network and greatly contribute to improved outcomes in epilepsy surgery (Duncan, 1997; Duncan, Winston, Koepp, & Ourselin, 2016). In addition, lesional or pharmacological intervention of neural circuits has provided some insight into how activity in some key brain regions is involved in the particular process of epileptic seizures. Advances in the above techniques have largely helped us identify many functional and structural networks underlying epilepsy. However, EEG and neuroimaging techniques have notable limitations in terms of cell- or circuit-type specificity because they are an average measurement of the activity of all kinds of cells.

Another peak period came after the year 2010, with more modern advances including optogenetics, trans-synaptic viral tracing, single-unit recordings, and two-photon microscopy providing precise descriptions of individual cells, microcircuits, and circuit function in both humans and animal models of epilepsy. These technologies for cell- and circuit-specific modulation have gradually made a great progress

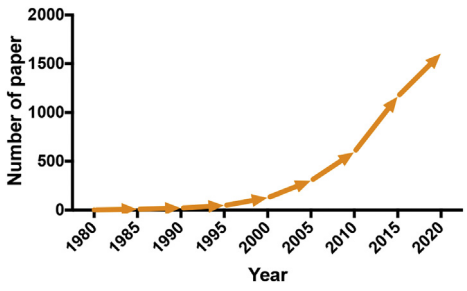

Fig. 4. Cumulative citations for neural circuit studies in epilepsy over time. Data were retrieved using the US National Library of Medicine "PubMed" database trend tool. The search terms were "(epilepsy and neural network) or (epilepsy and neural circuit)".

in our understanding of the circuit mechanisms of epilepsy. For example, large-scale single-unit recordings in epilepsy patients have shown that, epileptic seizures are not synchronized events as traditionally viewed (Truccolo et al., 2011). In particular, by using a powerful approach called optogenetics (Fenno, Yizhar, & Deisseroth, 2011; Truccolo et al., 2011), it is possible to elucidate the causal role of circuits in epilepsy by control of spatial cell-specific neural activity with millisecond precision. The precise definition of optogenetics is: the combination of genetic and optical methods to achieve gain or loss of function of well-defined events in specific cells of living tissue, which was first proposed and pioneered by the Deisseroth group (Yizhar, Fenno, Davidson, Mogri, & Deisseroth, 2011). Optogenetics have advanced the field of neuronal circuit analysis in neuroscience research (Paz & Huguenard, 2015c; Tye & Deisseroth, 2012). For example, optogenetic experiments have shown clear evidence that activation of inhibitory GABAergic circuits is unexpectedly sufficient to induce epileptic seizure (Shiri, Manseau, Levesque, Williams, & Avoli, 2015, 2016). The emergence of these novel approaches is beginning to provide new insights into how neural circuits organize and contribute to epileptic seizure, challenge the traditional theory of epilepsy.

4.2. Research advances on neural circuits in temporal lobe epilepsy

Here we take TLE as an example to show how recent studies involved investigations of epilepsy from the perspective of neural circuits. TLE is highly drug-resistant at rates up to 75% (Kwan, Schachter, & Brodie, 2011). Moreover, surgical resection of epileptic foci within the temporal lobe might still fail to control seizures (Engel Jr., 2018). The main factor in these challenges is probably that a complex epileptogenic network has formed in the brain of TLE patients. Structural and metabolic imaging from both clinical and experimental studies demonstrate that abnormal pathological changes in TLE were associated with not only the neighboring epileptogenic structures but also with remote brain regions, extending from local seizure foci to subcortical limbic structures and other remote structures (Englot et al., 2015). Thus, identification of the neuronal circuitry involved in TLE is urgently needed for developing precise and safe interventions to control TLE.

The electrographic feature of a seizure is its evolution: a seizure usually has a beginning, a middle, and an end, which is usually linked with seizure initiation, spread and termination, respectively (Bertram, 2013; Rao & Lowenstein, 2015). Different networks can be involved in the initiation, spread, or termination of seizures (Fig. 5A).

4.2.1. Seizure initiation circuit

The seizure focus for epilepsy is the site where the seizure starts or generates. The initiating circuit is a group of linked regions that are sufficient to support the focus to generate a seizure. As the hippocampus is strongly linked with the clinical entity of mesial TLE and has an obvious low threshold for seizure onset (Lothman, 1994), the hippocampus connected circuit and microcircuit have long been studied in the basic neurobiology of TLE.

As authoritative theory states that epileptic seizure originates from imbalances between excitation and inhibition; many current studies of network epilepsy focus on modulation of specific cell types (excitatory glutamate neuron or inhibitory GABAergic neuron) by optogenetics and aim to test their role in seizure initiation. Tonnesen, Sorensen, Deisseroth, Lundberg, and Kokaia (2009) first used the optogenetic method to selectively inhibit principal cells of the hippocampus (Tonnesen et al., 2009). Optogenetic hyperpolarization of these cells prevented generation of action potentials and epileptiform discharge in an in vitro seizure model. Later, studies from different groups verified that optogenetic inhibition of excitatory principal cells in the hippocampus significantly delayed the initiation of status epilepticus in a pilocarpine-induced seizure model (Sukhotinsky et al., 2013) and greatly control seizure activities in an in vivo mouse model of TLE (Berglind et al., 2014; Sukhotinsky et al., 2013). In addition, Osawa

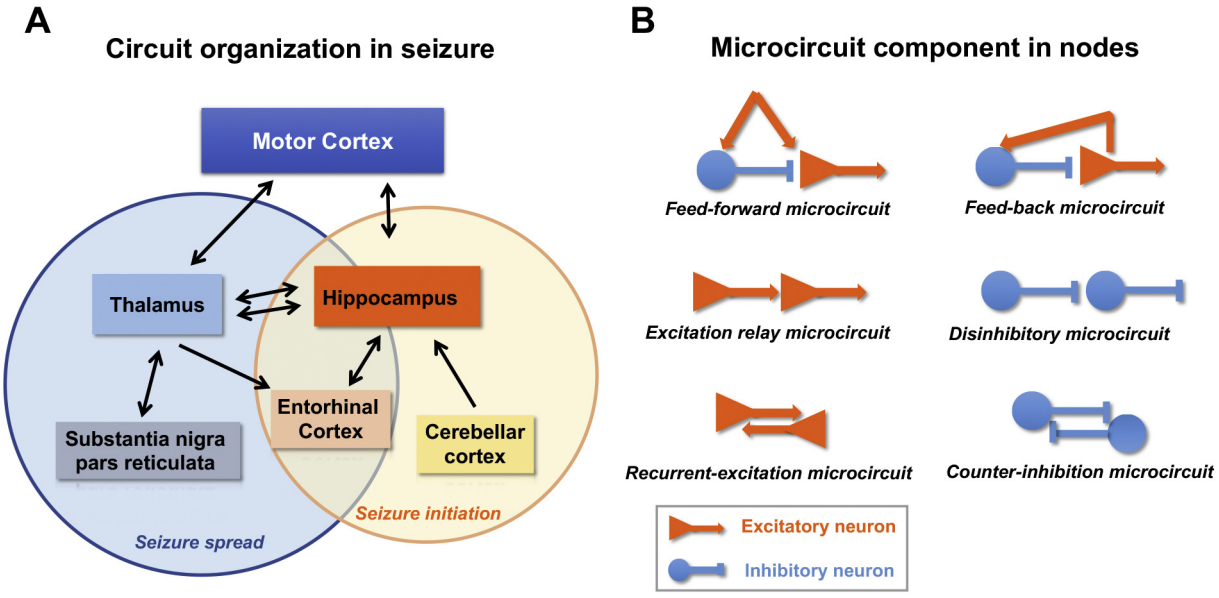

Fig. 5. Schematic illustrating insights into seizure circuits in TLE. (A) Some possible networks can be involved in the different stage of seizures (seizure initiation, spread, or termination) in TLE. (B) Different microcircuit organization can be involved in critical nodes of seizure circuits.

et al. (2013) found that optogenetic activation of hippocampal excitatory neurons directly induced focal seizure-like afterdischarges in rat hippocampus (Osawa et al., 2013), providing additional evidence of the potential role of hippocampal excitatory neurons in seizure genesis. Interestingly, enhanced excitatory function does not always produce pro-epileptic effects. Recently, Bui et al. (2018), using a combination of optogenetic, electrophysiological, and behavioral approaches, provided elegant evidence that optogenetic activation of mossy cells in hippocampal dentate gyrus (a type of glutamatergic neuron) controlled the activity of spontaneous seizure (Bui et al., 2018). This effect can be attributed to its downstream circuit function. Mossy cells have widespread projections to nearby granule cells and GABAergic interneurons (Scharfman, 2016), and thus block runaway excitation via modulating the activities of downstream distributed microcircuits.

In addition to hippocampal excitatory neurons, this is also true for hippocampal GABAergic neurons, which have diverse roles in seizure initiation. The traditional view is that epileptic seizure can be triggered by impaired inhibitory function, thus optogenetic activation of hippocampal GABAergic neurons was used to control seizures in both in vitro and in vivo epileptic models (Krook-Magnuson, Armstrong, Oijala, & Soltesz, 2013; Ladas, Chiang, Gonzalez-Reyes, Nowak, & Durand, 2015; Ledri, Madsen, Nikitidou, Kirik, & Kokaia, 2014). Surprisingly, in some situations, optogenetic activation of the inhibitory GABAergic circuit failed to block seizure generation (Sessolo et al., 2015), and even promoted or directly induced seizure generation (Shiri et al., 2015, 2016). The reason for the above inconsistency may be due to multiple factors. One important factor is that activation of the inhibitory GABAergic circuit may induce postinhibitory rebound spiking in postsynaptic pyramidal neurons through positive shifts in the GABAergic reversal potential, thus enhancing neuronal synchrony and promoting seizure genesis (Alfonsa et al., 2015). Another important factor is that GABAergic neurons are very diverse. For example, somatostatin (SST)-positive GABAergic neurons, targeting dendritic domains, and PV-positive GABAergic neurons, targeting perisomatic compartments, have different functional feature of controlling the excitability in principal cells (Freund & Buzsaki, 1996; Paul et al., 2017). Different subtypes of GABAergic neurons may form different microcircuits in the hippocampus, such as feed-back circuit, feed-forward circuit, disinhibition circuit, and counter-inhibition circuits (Fig. 5B), and thus produce different outcomes in seizure generation.

Apart from the seizure focus itself, many remote brain regions sending long-range projections to the seizure initiation circuits would affect the level of excitability of seizure focus and seizure initiation. These modulatory inputs can also be part of a seizure initiation circuit. For TLE, the entorhinal cortex and cerebellar cortex, were reported to have a potential role the onset of limbic seizures. Krook-Magnuson, Szabo, Armstrong, Oijala, and Soltesz (2014) found that optogenetic excitation of the midline cerebellum results in a reduction in seizure frequency (Krook-Magnuson et al., 2014). These findings demonstrate that the cerebellum is a powerful modulator of seizure initiation in TLE. A recent study demonstrated that cerebellar output circuits are also potent modulators of pathological thalamocortical network activity during absence seizures (Kros et al., 2015), suggesting its potential therapeutic benefit for controlling different types of epilepsy.

In the entorhinal cortex, optogenetic activation of inhibitory GABAergic circuits can directly induce seizure generation (Shiri et al., 2015); either entorhinal PV or SST interneurons is able to trigger ictal discharges (Yekhlef, Breschi, Lagostena, Russo, & Taverna, 2015). Interestingly, optogenetic activation of excitatory principal cells also induces a hypersynchronous seizure onset pattern that is different from that induced by optogenetic activation of an inhibitory GABAergic circuit (Shiri et al., 2016). These findings indicate that a diverse neural circuit mechanism may be involved in different types of seizure onset in TLE, providing insight in the treatment of TLE depending on seizure onset patterns. However, in our previous study, we found that low-frequency optogenetic activation of entorhinal glutamatergic neurons retarded hippocampal seizure development; this anti-seizure effect is mediated by activating the "entorhinal glutamatergic neuron – hippocampal GABAergic neurons" circuit (Xu et al., 2016). Similarly, two later studies found that low-frequency optogenetic activation of either excitatory or inhibitory neural networks in the EC is effective in controlling seizure generation, while stimulation targeting at excitatory cells produced a more prolonged inhibition of ictogenesis (Shiri et al., 2017; Yekhlef, Breschi, & Taverna, 2017). These complicated results can be attributed to the possibility that glutamatergic or GABAergic neurons trigger seizure-like activity or interrupt ongoing seizures in different circuit-based functional outputs. The functional status of aberrant excitatory and inhibitory synaptic connections at the level of the neural circuit determines the final epileptic state.

4.2.2. Seizure spread circuit

For a seizure to spread, a larger network including other regions outside of initiating circuit, is needed to sustain the seizure activity (Fig. 5A). Understanding the circuit of seizure spread would help to identify potential key nodes that are remote from the focus of the initial dysfunction. This area is now gradually receiving attention, because inhibition of seizures spread would minimize damage for a person's life.

Ellender et al. (2014) found that specific GABAergic circuit dynamics in the seizure focus underlie later seizure spread phase of the epileptiform activity in in vitro hippocampal slice models. Later seizure spread (clonic afterdischarge) requires excitatory (depolarized) GABAergic synaptic transmission due to a transient collapse in the chloride reversal potential. Optogenetic activation of PV positive GABAergic neurons during the clonic phase was sufficient to elicit synchronous afterdischarge activity across the network via a mechanism of depolarized GABAergic responses in pyramidal neurons. However, this situation can differ from modulation by PV neurons in distant regions. Optogenetic activation of PV neurons distant from the seizure focus delayed seizure propagation and shortened seizure duration in a similar in vitro seizure model (Sessolo et al., 2015). The functional diversity of PV positive GABAergic neurons here described provide new insights into our understanding of how GABAergic inhibitory circuits gate generation and spread of epileptic seizure.

Recently, we demonstrated that the subiculum, the gate of hippocampal information output, is crucially involved in spreading and generalizing hippocampal seizures in in vivo epileptic models (Wang et al., 2017). We demonstrated that depolarized GABAergic signaling caused by the change of chloride transporters in subicular microcircuit mediates secondary generalized seizures in TLE. Specifically, we found that optogenetic activation of subicular GABAergic neurons retarded the spread of generalized seizures by inhibiting the firing of pyramidal neurons. Once a generalized seizure had been stably acquired, optogenetic activation of these GABAergic neurons aggravated the expression of generalized seizures via depolarized GABAergic signaling. More interestingly, subicular PV neurons were more easily depolarized than those of SST subtype GABAergic neurons, as optogenetic activation of subicular SST neurons produced an anti-epileptic effect on both acquisition and expression of generalized seizures. These data suggested that even in one region, a different subtype of GABAergic neurons might have totally diverse roles in seizure control.

Similar conclusion was also verified in a neocortical epileptic role. Khoshkhoo, Vogt, and Sohal (2017) found that seizure activity rapidly recruits PV, SST, and vasoactive intestinal peptide (VIP)-positive GABAergic neurons, and then recruits excitatory neurons within several seconds later. Optogenetic inhibition of VIP neurons consistently increased seizure threshold (inhibiting seizure generation) and reduced seizure duration (preventing seizure spread). Inhibiting PV and SST interneurons consistently reduced seizure duration (Khoshkhoo et al., 2017).

Apart from the brain region in the temporal lobe, the midline/intralaminar thalamus is a crucial relay for seizure spread from common seizure focus in TLE. Forcelli's group found that selective silencing of the midline thalamus by using chemogenetics suppressed seizure activities evoked from a distal site (i.e., the amygdala) (Wicker & Forcelli, 2016). Recently, we found that a long-range disinhibitory PV-expressing GABAergic circuit, from the substantia nigra pars reticulata to the intralaminar thalamus parafascicular nucleus, is responsible for regulating seizure propagation in TLE, and inactivation of this circuit can alleviate the severity of epileptic seizures (unpublished data). These results indicated that GABAergic neurons have diverse roles not only in seizure initiation, but also in seizure spread, depending on subtype, location, and timing involved in seizure.

4.2.3. Seizure termination circuit

Most seizures are usually self-limited within a few minutes. Failure of seizure termination leads to status epilepticus - a state of continuous seizure activity that can cause severe and permanent brain damage and even directly induce sudden death. Seizure termination could be involved in multi-level factors, ranging from molecules to circuits and many hypothesis have been suggested about how seizures stop (Lado & Moshe, 2008), unfortunately, how exactly seizures stop is still a mystery. Understanding the mechanisms participating in seizure termination would definitely help us to identify novel targets of drug for the treatment of epilepsy.

Truccolo et al. (2011) used multi-channel microelectrode arrays to analyze firing patterns in a large population of neurons during seizures in epilepsy patients (Truccolo et al., 2011). Contrary to the traditional view, epileptic seizure is not a synchronous event in single neuron level. Neuronal spiking activity during seizure initiation and spread was highly heterogeneous, while seizure termination in the later stage of seizure is a nearly synchronous phenomenon that almost complete cessation of spiking across recorded neuronal ensembles. These findings suggested that seizure initiation, propagation, and termination are involved in distinct mechanisms. At the circuit level, seizure termination seems to have an overall increase in synchronization within many different regions. Seizure termination may occur at discrete locations with wide spacing, which might more easily be targeted by therapeutic intervention. However, our understanding of the relationships between seizure termination in neural circuits still remains largely phenomenological and needs further studies.

In summary, as epilepsy has many causes and can arise in many different regions of the brain (Devinsky, Vezzani, et al., 2018; Gavvala & Schuele, 2016), thus, the underlying neural circuit-based pathophysiology in different types of epilepsy will also vary considerably and need to be investigated. At present, we are still in the early stages of research and are far from clear knowledge of precise neural circuit mechanisms of different types of epilepsy. Our current knowledge about neural circuit mechanisms of epilepsy is limited to circuit or microcircuit function of a few important brain regions. However, the emergence of optogenetics, single-unit recording, voltage-sensitive dye, calcium imaging and other state-of-the-art approaches give us many opportunities to have a much deeper insight into how networks are organized and contribute to seizure activity in epilepsy, and challenge the established, yet somewhat simplistic, views.

Researchers are gradually realizing that the traditional theory that epilepsy is caused by an increase in excitation and a decrease in inhibition is clearly an over-simplification. We now know that enhancing inhibitory function does not always produce anti-epileptic effects, while enhancing excitatory function does not always produce pro-epileptic effects. The functional status of aberrant excitatory and inhibitory synaptic connections at the level of the neural circuit determines the final epileptic state, which may be a necessary component of traditional epilepsy the “imbalanced excitation-inhibition” concept. However, there is still a long way to go to fully understand what the abnormal circuit connections are in epilepsy or epileptogenesis. A neural circuit-based mechanism of epilepsy may be a very important reason why the drug-resistance rate is so high in TLE.

5. Circuit-based therapeutic approaches in epilepsy

By using deep brain stimulation, optogenetics, chemogenetics and other novel intervention approaches, we are starting to reveal key choke points in critical neural circuits that likely represent promising targets for highly specific and effective anti-epileptic or anti-epileptogenetic therapies.

5.1. Potential therapeutic applications of deep brain stimulation

Deep brain stimulation (DBS), which consists of electrical pulses administered to certain brain regions to modulate activity, is emerging as a new option for the treatment of medically refractory epilepsy (Fisher & Velasco, 2014; Jobst, Darcey, Thadani, & Roberts, 2010). Although the

mechanisms are still unclear, DBS is thought to have an effect by directly or indirectly influencing the pathological epileptogenic network (Laxpati, Kasoff, & Gross, 2014; Lozano & Lipsman, 2013; McIntyre & Hahn, 2010). The anti-epileptic effect of DBS is determined by several factors: stimulating parameters, stimulating targets, timing patterns, and even epileptic types (Li & Cook, 2018; Theodore & Fisher, 2004). To date, a variety of brain structures, such as the anterior nucleus of the thalamus and the hippocampus, have been studied as targets for brain stimulation in animal models of epilepsy as well as in patients with epilepsy. Recently, a large multicenter double-blind randomized clinical study suggested that high-frequency stimulation of the anterior nucleus of the thalamus decreased the frequency of seizures and about half of all patients experienced a 46%–90% seizure reduction (R. Fisher, et al., 2010). However, the majority of patients still would not become seizure-free.

Stimulating the epileptogenic cortexes or nearby structures to interrupt epileptic seizures appears to be parameter-dependent. High-frequency stimulation of such structures may induce a "kindling effect", thus facilitating seizures. On the other hand, low-frequency electrical stimulation (LFS) achieved promising effect in animal studies (D'Arcangelo, Panuccio, Tancredi, & Avoli, 2005; Goodman, Berger, & Tcheng, 2005; Mohammad-Zadeh et al., 2007) and in clinical studies (Koubeissi, Kahriman, Syed, Miller, & Durand, 2013; Vonck, Boon, Achten, De Reuck, & Caemaert, 2002). We found LFS targeting not only at the seizure focus (Liu et al., 2013; Wang et al., 2014) but also in regions that have intensive connections with seizure focus, such as the piriform cortex (Yang et al., 2006; Zhu-Ge et al., 2007), cerebellar fastigial nucleus (Wang et al., 2008), and hippocampal CA3 (Sun et al., 2010; Zhang et al., 2009), inhibited kindling-induced seizures. The anti-epileptic effect of LFS is polarity-dependent (Z. Xu et al., 2013), suggesting that electrode polarity, especially that for anodal current, is a key factor affecting the anti-epileptic effects of LFS. Notably, we found that LFS achieved its anti-epileptic effect with time-dependent characteristics (Wu et al., 2008; Xu et al., 2010). That is, LFS has a better effect when delivered shortly after seizure induction compared with tens of seconds after seizure induction. Interestingly, the subiculum is a target that does not have the characteristics of time-dependent effect (Zhong et al., 2012), which may be a promising and suitable target for clinical application.

DBS, because of the advantage of reversibility and adjustability thus can be delivered safely and is emerging as an alternative treatment. Though this emerging therapy is not conclusive and has risks of side effects, the overall results are encouraging. Further study is still needed to search for optimal stimulation parameters and brain targets. Meanwhile, we need to improve our knowledge of the neural circuits in different types of epilepsies, which would support the optimal application of DBS in clinical.

5.2. Potential therapeutic applications of optogenetics

Optogenetics in seizure suppression confers cellular specificity and even circuit specificity while maintaining high temporal precision, which is not feasible with DBS. Optogenetic tools include genetically engineered light-responsive opsins, transmembrane proteins that translocate ions across the cell membrane upon exposure to a specific wavelength of light (Deisseroth, 2015; Fenno et al., 2011). Two main groups of opsins are available, allowing optogenetic activation or inhibition.

The tools used for optogenetic activation come from naturally-occurring Channelrhodopsin-2 and other genetically-engineered opsins (Deisseroth & Hegemann, 2017; F. Zhang, Wang, Boyden, & Deisseroth, 2006). Channelrhodopsin-2 opens and allows passive movement of cations following the electrochemical gradient in the presence of blue light (~470 nm), depolarizing cell membranes and generating action potentials. The tools used for optogenetic inhibition are constituted by opsins, like the most frequently used halorhodopsin and archaerhodopsin, which can hyperpolarize the cell membrane and inhibit the generation of action potentials. Halorhodopsin (Gradinaru et al., 2010), can cause active pumping of chloride ions into the cell in the presence of orange light (~570 nm), thereby hyperpolarizing the membrane potential and inhibiting neural excitability. Archaerhodopsin (Chow et al., 2010), an outward proton pump driven by orange light, also hyperpolarizes the membrane potential and inhibits action potential generation.

Current optogenetic approaches for controlling seizure focus on either block of excitation or augment of inhibition. Seizure control in epilepsy with optogenetics is first demonstrated in hippocampal slice cultures, in which halorhodopsin is expressed in excitatory neurons (Tonnesen et al., 2009). Shortly after, it is shown that optogenetic inhibition of excitatory cells with halorhodopsin could alleviate epileptiform activity in behaving animals (Berglind et al., 2014; Krook-Magnuson et al., 2013). However, the use of halorhodopsin can significantly increase spiking probability after prolonged illumination, which most probably comes from a collapse in the Cl^- transmembrane gradient (Alfonsa et al., 2015; Chang et al., 2018). Seizure control could be also obtained by targeting and activating inhibitory GABAergic neurons with ChR2. Optogenetic activation of hippocampal GABAergic neurons is used to control seizure in both in vitro and in vivo epileptic models (Krook-Magnuson et al., 2013; Ladas et al., 2015; Ledri et al., 2014). In particular, Soltesz et al. demonstrated that optogenetic activation of <5% of PV positive GABAergic neurons could effectively reduce seizures in a model of spontaneous epilepsy with a real-time seizure detection system (Armstrong, Krook-Magnuson, Oijala, & Soltesz, 2013; Krook-Magnuson et al., 2013). This online closed-loop feed-back system has very high sensitivity and specificity, which can also be used to react to diverse types of epilepsy combined with optogenetic and other interventions (Berenyi, Belluscio, Mao, & Buzsaki, 2012; Paz et al., 2013). Given the diversity of inhibitory microcircuits organized in large-scale network (Fig. 5B), modulation one of single elements could have disparate and even opposite consequences for seizure control.

Although the impact of these recent investigations with optogenetics in the field of seizure control has already been substantial, there remains much work to do. Optogenetic systems still require a light-delivery technique and need to express ectogenic viruses in specific type of neurons. In addition, the application of optogenetics to non-human primates is still in its infancy, and many types of epilepsy remain unexplored. These questions have to be addressed before the system has a chance of reaching the clinic. The development of red-shifted variants of opsins (Chuong et al., 2014; Lin, Knutsen, Muller, Kleinfeld, & Tsien, 2013) or near-infrared up-conversion nanoparticle-mediated optogenetics (Chen et al., 2018; He et al., 2015), makes optogenetic intervention a noninvasive approach, which may be an attractive way for further clinical application.

5.3. Potential therapeutic applications of chemogenetics

Optogenetics is not the only means for selective manipulation of circuit elements. Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)-based chemogenetics (also called pharmacogenetics) is another valuable tool for manipulating neuronal and non-neuronal signal transduction in a specific cell type manner (Roth, 2016; Urban & Roth, 2015). The designer receptors include hM3Dq, hM4Di (genetically engineered from human muscarinic receptors), and the recently developed KORD (genetically engineered from the kappa opioid receptor), which display high affinity for other ectogenic designer drugs (e.g., Clozapine-n-oxide, Compound 21, Salvinorin B). The hM3Dq has been reported to activate neuronal activity via increasing Gq signaling and thus intracellular Ca^{2+} (used as excitatory DREADDs) (Alexander et al., 2009), while hM4Di and KORD have been reported to inhibit neuronal activity via either activation of G-protein inwardly rectifying potassium channels, or inhibition of the presynaptic release of neurotransmitters (used as inhibitory DREADDs) (Stachniak, Ghosh, & Sternson, 2014; Vardy et al., 2015).

As another tool set being used to address circuit-based treatment in epilepsy research, chemogenetics in epilepsy treatment lags behind optogenetics. Katzel, Nicholson, Schorge, Walker, and Kullmann (2014) first tested the potential of chemogenetics as a promise novel approach to treat drug-resistant epilepsy. They genetically expressed hM4Di to the seizure focus and found that this treatment silenced seizure activity in three focal neocortical epilepsy models (Katzel et al., 2014). Similar suppression of seizure activity has been reported following hM4Di-mediated silencing in an in vitro TLE model (Avaliani, Andersson, Runegaard, Woldbye, & Kokaia, 2016) and an in vivo TLE model (Wicker & Forcelli, 2016). Further, we transduce PV neurons in the seizure focus in the hippocampus by using hM3Dq, and test its therapeutic potential in several animal TLE models compared to that of chemogenetic inactivation of pyramidal neurons using hM4Di (Wang et al., 2018). We found that chemogenetic activation of hippocampal PV neurons using hM3Dq greatly alleviate the severity of seizure onset in the TLE models and does not affect the physical cognitive function.

Although chemogenetic inhibition of pyramidal neurons using hM4Di produce a similar anti-ictogenic effect, interestingly, it is sensitive to impair cognitive function. These findings suggested that chemogenetic seizure attenuation through targeting PV neurons rather than pyramidal neurons may be a novel and relatively safe approach for treating refractory TLE. Recently, hM4Di-mediated suppression of hippocampal newborn dentate granule cells is found to significantly reduce epileptic spikes and spontaneous recurrent seizures in a TLE model in an inducible and reversible manner (Zhou et al., 2019). This study reveals a critical role for hippocampal newborn cells in epileptic neural circuits, providing critical insights into hippocampal newborn dentate granule cells as a potential therapeutic target for treating TLE.

Although the temporal specificity of pharmaco-genetics does not match that of optogenetics, chemogenetics has the following advantages: (1) it avoids the need for invasive devices to deliver light to the specific brain area because the designer drugs can be administered systemically; (2) a relatively large area or multiple seizure focus region may be targeted, which is limited by light stimulation with optogentics. These advantages might be more rapidly accepted into clinical practice. People might quickly take the drug when a seizure is predicted, which may be suitable for patients whose seizures are predictable, such as in catamenial epilepsy (Herzog, 2015). Alternatively, an implanted drug-delivery system that is seizure-activated can be designed, which will greatly improve seizure control by chemogenetics treatment in the future.

5.4. Potential therapeutic applications of other minimally-invasive approaches

All of the above-mentioned treatments targeting abnormal circuits of epilepsy are invasive methods. The development of novel minimally-invasive or even noninvasive intervention approaches in this field may be essential for further improvements both in experimental research and in clinical therapeutic efficacy and safety. The following new types of intervention approaches may give us some hints of future directions of epilepsy research and treatment.

Recently, Grossman et al. (2017) reported a form of noninvasive electrical stimulation called “temporal interference”, which can stimulate neurons in the deep brain, like the hippocampus, of free-moving mice without recruiting neurons of the overlying cortex (Grossman et al., 2017). The basic mechanism is that when two tones with similar very high frequencies (e.g., >1 kHz) are simultaneously emitted from different locations of the skull, the envelope of the net low-frequency oscillates equal to the difference of the two tones. It may be an attractive method for future clinical applications. However, an open question is how small a focal volume may be achieved. Meanwhile, noninvasive electrical “temporal interference” is not cell-type specific, but whether and how effectively it can be used in epilepsy treatment is still unknown.

Chen, Romero, Christiansen, Mohr, and Anikeeva (2015) have developed wireless magnetothermal stimulation to remote control neural excitation through the activation of the heat-sensitive capsaicin receptor TRPV1 and to facilitate the study of intact brain circuits (R. Chen et al., 2015). The basic mechanism is that the magnetic nanoparticles dissipate heat in the presence of alternating magnetic fields, and thus trigger and activate firing of $TRPV1^{+}$ neurons. Wireless magnetothermal stimulation of well-defined neuronal populations may also be promising for the treatment of epilepsy. However, this magnetothermal stimulation should be used for two following cautions: (1) it may be easily trigger thermosensitive ion channels endogenously expressed in the brain and bring other side effects; and (2) it may heat the tissue and damage brain function.

Sonogenetics is another non-invasive approach to facilitate the study of intact brain circuits. It is able to achieve gain or loss of function in specific cells by the combination of genetic and ultrasound methods. Ibsen, Tong, Schutt, Esener, and Chalasani (2015) used low-pressure ultrasound to activate specific ultrasonically sensitized neurons in *C. elegans*, resulting in behavioral outputs (Ibsen et al., 2015). Further, Ye et al. (2018) have screened a mutation version of mechanosensitive channel of large conductance, which could be easily activated by low-pressure ultrasound (Ye et al., 2018). They expressed this mechanosensitive channel in hippocampal neurons in primary pulses and succeed in triggering action potential by ultrasound stimulation. In further studies, sonogenetics may be broadly applied to manipulate neuronal functions in vivo with the development of miniature ultrasound device. In addition, sonogenetics may also be developed to inhibit neuronal function, apart from current version of neuronal activation.

6. Perspectives on AEDs toward abnormal circuits in epilepsy

Traditional pharmacological treatment in epilepsy is not based on a comprehensive understanding of the diverse functional and structural network mechanisms and lack regional and cell- or circuit-type specificity. We believe that a better understanding of the complex microcircuit and circuit alterations leading to epilepsy would permit the definition of novel targets for new AEDs, leading to a shift from conventional “molecular” to modern “circuit” in both epilepsy research and AED development. Although there is no available AED targeting a particular pathological node or circuit at present, we believe that the “circuit” may be a promising direction for AED development. We list three following emerging perspectives on AEDs therapeutics toward abnormal circuits in epilepsy:

(1) The simplest way is for circuit-based AED targeting the functionally active or abnormal circuit during epileptic seizure. A neuronal circuit is based on anatomical connections between brain regions, which essentially constitute the structural organizational basis. A functional network is different from the neuroanatomy of a circuit in that the participation of a group of neurons is variable with potentially extensive degrees of change (Faingold, 2004). Many synaptic events in neural connections in physiological conditions are “subthreshold”, while neural activities or synaptic events can exceed threshold or even largely be “amplified” (neuronal firing with high frequency or bursting type) during epileptic seizures. Thus, based on the principle of targeting the enlargement of the functionally active circuit for AED design, we have developed electro-responsive hydrogel nanoparticles, which transported AEDs into the brain and released them under electroencephalograph epileptiform abnormalities (Wang et al., 2016; Ying et al., 2014). The distribution of AED in the brain can be largely increased in the regions that are functionally active during epileptic seizure. This nanoparticle delivery system improved antiepileptic effects in various kinds of seizure and epilepsy models. This concept may change the therapeutic paradigm of current antiepileptic treatment with AED

into a type of on-demand "active circuit" control for epilepsy in the future. Further, apart from high-frequency neuronal firing, other characteristics during seizure (e.g. high K^+ or glutamate release) can also be used as a trigger for on-demand "active circuit" control for epileptic seizure.

(2) Deconstructing current neuropharmacology using cellular or circuit specificity. Shields et al. (2017) have recently developed a more elegant approach that combined the speed and molecular specificity of pharmacology with cell type specificity (Shields et al., 2017). They call it DART (drugs acutely restricted by tethering), and it is a technique with the basic mechanism where the bacterial enzyme HaloTag is used to tether drugs to the surface of defined cells. Drug capture proceeds rapidly within up to few seconds, leading to ~100 enrichment of the drug concentration at the surface of HaloTag-expressing cells, which minimized drug side effect on non-HaloTag-expressing cells. In the future, if HaloTag were located in a specific epileptogenetic circuit, the DART system could inform new translational strategies by providing a road map for the design of novel AEDs based on particular circuits in epilepsy therapeutics.

(3) The final goal of precise circuit-based AED development is to reveal and target a "specific" molecule or protein within a "specific" circuit for a "specific" epilepsy at a "specific" pathological stage. We call this approach "4S AED". New method advances, such as single-cell or projection RNA sequencing (Nectow, Ekstrand, & Friedman, 2015; Saunders et al., 2018; Zeisel et al., 2018), novel viral tracing system (Beier et al., 2017; Ciabatti, Gonzalez-Rueda, Mariotti, Morgese, & Tripodi, 2017), crystal brain (Richardson & Lichtman, 2015), and membrane voltage dynamics (Gong et al., 2015; Marshall et al., 2016) allow precise classification of cell types and provide genetic access to diverse neuronal types in epilepsy. If we could find molecule "A" specifically expressed in one specific seizure initiate circuit of TLE at a specific stage, then we may finally succeed in discovering a precise circuit-based "4S AED" for epilepsy treatment by targeting molecule "A" with high efficacy and low side effects. In addition, single-target "4S AED" treatments may be less effective than multiple-target "4S AED" treatments that act on different molecules and proteins or are commonly involved in the epileptogenetic network. The aim of multiple-target "4S AED" treatments is to develop combinations of existing modified drugs or totally novel drugs that modulate common or diverse mechanisms via different targets within different epileptogenic networks to effectively treat epilepsy, especially for those drug-resistant patients.

7. Conclusion

Epilepsy involves neuronal dysfunction at molecular, cellular, and circuit levels. Our understanding of the pathophysiology of epilepsy, especially in terms of their cellular and molecular basis, has advanced dramatically in the past three decades. The incomplete understanding of circuit-level function has left a gap in our knowledge of how disruption at a molecular or cellular level generates epilepsy in intact organisms, and further has led to many challenges in pharmacological treatment in epilepsy. At present, the deep insight into the hyperexcitable circuit is bridging this gap and is gradually poised to take a center stage in epilepsy research and pharmacological treatment. Technological advances have facilitated circuit mechanistic discovery at each level and paved the way for providing many opportunities of novel therapeutic strategies and AED development toward precise circuit therapy.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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