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Regulation of ion channels by microRNAs and the implication for epilepsy

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Abstract

Purpose of review: The goal of this focused review is to describe recent studies supporting a critical role of microRNAs in the regulation of ion channels and discuss the resulting implications for the modulation of neuronal excitability in epilepsy.

Recent findings: MicroRNA-induced silencing of ion channels has been shown in several different studies in recent years, and some of these reports suggest a prominent role in epilepsy. The ion channels regulated by microRNAs include ligand- and voltage-gated channels and are not only limited to the central nervous system but have also been found in the peripheral nervous system.

Summary: Ion channel-targeting microRNAs can regulate the intrinsic excitability of neurons and thus influence entire networks in the brain. Their dysregulation in epilepsy may contribute to the disease phenotype. More research is needed to better understand the molecular mechanisms of how microRNAs regulate ion channels to control neuronal excitability, and how these processes are altered in epilepsy.

Keywords

microRNA; ion channels; epilepsy; neuronal excitability; microRNA therapeutics; dendritic translation

Introduction

Epilepsy is characterized by the occurrence of spontaneous seizures, which are episodes of abnormally synchronous neuronal hyperactivity. Seizures can induce structural remodeling in the mammalian brain leading to aberrant cell proliferation, neurogenesis and subsequent tissue destruction. If seizures are not well-controlled, this process, called epileptogenesis,

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Conflict of Interest

Durgesh Tiwari declares no conflict of interest.

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This article does not contain any studies with human or animal subjects performed by any of the authors.

will worsen epilepsy over time [1]. Despite decades of human and animal research, our understanding of the basic pathological mechanisms and pathways underlying neuronal hyperactivity and leading to seizures and cellular abnormalities in an epileptic brain remains incomplete. Even with recent advances in identifying genetic causes and developing more potent and specific drugs, approximately one third of individuals with epilepsy do not respond to treatment [2]. Therefore, epilepsy remains a big challenge to researchers and clinicians.

Understanding the intrinsic cellular mechanisms controlling neuronal excitability can help reveal how seizures and epilepsy develop and may lead to the discovery of new therapeutic targets to prevent or delay disease progression. On a molecular level, neuronal excitability and activity are determined by intra- and extracellular ion concentrations and the ion permeability of neuronal membranes, which are regulated by voltage- or ligand-gated ion channels and ion transporters/exchangers. Unsurprisingly, defects in ion channel function, either caused by mutations or acquired, have been associated with epilepsy [3]. Many current epilepsy drugs are therefore directed at manipulating the ion permeability of these channels to alter neuronal excitability. Yet, increasing evidence points to another level of ion channel dysregulation in epilepsy, namely changes in their expression levels [4, 5]. If the intracellular mechanisms regulating ion channel production, stability and, thus, levels were better understood, those could lead to the identification of novel treatment targets in epilepsy.

A rather novel cellular mechanism regulating post-transcriptional expression of virtually all classes of protein-encoding genes is microRNA-induced silencing [6]. In the last decade, microRNAs have emerged as a novel research area in epilepsy with the potential to not only reveal pathways and mechanisms underlying neuronal excitability but also serve as novel therapeutic targets.

MicroRNAs are master regulators of cellular function

MicroRNAs are a class of small non-coding RNAs (~22 nucleotide), which bind to partially complementary sequences mostly within the 3' untranslated regions of their target messenger RNAs (mRNAs). Through the association with microRNAs, these mRNAs are recruited to the RNA-induced silencing complex (RISC) where they are translationally repressed or degraded [6]. The newest version of miRBase, a repository of miRNA sequences and associated mRNA targets [7], released in March 2018, reports 2,654 mature miRNAs in the human genome. According to target prediction algorithms a single microRNA can repress over 100 mRNAs, and it has been estimated that up to 60% of human mRNAs are regulated by microRNA-induced silencing [8]. Although the number of robust high-confidence microRNAs and their truly functional mRNA targets has recently been challenged and might be somewhat lower than what is reported in miRBase [9, 10], this still reflects the wide potential of microRNAs to influence biological function. It therefore comes to no surprise that many studies suggest an important role for microRNA-induced silencing in epilepsy development [11].

MicroRNA-induced silencing is dysregulated in epilepsy

First associated with epilepsy in 2010 [12], microRNAs have been shown to be differentially expressed across all phases of epileptogenesis, acutely after seizure, during the latent phase before spontaneous recurrent seizures occur, and in chronic epilepsy, in animal models as well as in humans [13–16]. Moreover, various microRNAs have been shown to regulate the development of both acute seizures and epilepsy [17]. A recently developed epilepsy-specific microRNA database provides comprehensive information about these studies, including microRNA expression changes, confirmed target mRNAs as well as links to functional studies [18].

MicroRNA function can be regulated *in vitro* and *in vivo* using antagomirs (antisense oligonucleotides) to block a microRNA, or agomirs (microRNA mimics) to increase microRNA levels. These tools are indispensable for preclinical animal research but are also currently evaluated by various pharmaceutical companies as therapeutic agents in humans. Promising results in phase I or II clinical trials for, for example, Hepatitis C [19] suggest a potential future use in epilepsy [20]. However, there are still challenges impeding our understanding of the role of microRNAs in epilepsy. First, we are far away from a clear picture of which microRNAs are dysregulated and could thus play major roles. Two recent meta-analysis studies, which attempted to identify a consensus of differentially expressed miRNA associated with human and/or experimental epilepsy illustrate the minimal overlap between different studies [21, 22]. Second, the identity of the mRNA targets that mediate the effects of specific microRNAs in epilepsy is largely unknown. It is conceivable that microRNAs directly controlling the intrinsic excitability of neurons are among the most promising candidates as key players in epilepsy. Several studies have recently shown that microRNAs target ion channels directly. These findings provide an exciting novel perspective for microRNAs in epilepsy, given the important role of ion channels in regulating neuronal excitability and the fact that ion channel dysfunction has frequently been identified as an underlying cause of epilepsy [23, 4]. While these studies are still in their infancy, they might pave the way for ion channel-targeted microRNA-based therapies in epilepsy in the future.

This focused review provides an update about recent advancements in our understanding of how microRNAs regulate neuronal excitability through directly targeting neuronal ion channels, describes how dysregulation of these processes may contribute to epilepsy, and discusses what is needed to move the field further. We will emphasize studies in the brain but will also include recent discoveries in the peripheral nervous system that may have implications for epilepsy research. The discussed studies are summarized in Table 1.

MicroRNAs regulate voltage-gated ion channels in the brain

Voltage-gated ion channels change their ion conductance in response to the membrane potential, and alterations in their activity have been associated with epilepsy [4, 24]. It thus seems conceivable that regulation of their expression, for example through microRNAs, is a mechanism underlying changes in excitability under physiological and pathological circumstances. Indeed, in recent years, several reports of epilepsy-associated voltage-gated

potassium and sodium channels that are directly regulated by microRNAs in the brain have been published.

Kv1.1 is regulated by miR-129-5p and mTOR in epilepsy – a potential role for subcellular control of neuronal excitability

In one of the first studies reporting a specific microRNA that regulates a voltage-gated potassium channel in the brain, Raab-Graham and colleagues showed that miR-129-5p suppresses translation of the *Shaker*-like potassium channel Kv1.1 in an mTORC1-dependent manner [•25]. Using a photoconvertible fluorescent translational reporter the authors showed that miR-129-5p suppresses local Kv1.1 translation in dendrites when mTORC1 kinase is active. A follow-up study two years later provided first clues that this mechanism is important for the development of epilepsy by showing that miR-129-5p-mediated translational suppression of Kv1.1 is enhanced three weeks after *status epilepticus* in rats [•26]. miR-129-5p has recently been shown to regulate homeostatic synaptic downscaling, which further corroborates its role in controlling neuronal excitability [27]. In this study, the authors report that miR-129-5p mediates this function through targeting the RNA-binding protein RBfox1 but failed to show a role for Kv1.1 in this process. As the authors only tested general cellular protein synthesis, but not dendritic translation, this suggests the intriguing possibility that miR-129-5p's control of Kv1.1 is restricted to dendrites or synapses. Subcellular location-specific regulation of ion channel expression and function by microRNAs adds additional levels of control of neuronal excitability through the RISC in epilepsy, which will be interesting to further assess.

There are several indications that Kv1.1 dysfunction can cause epilepsy in humans. Mutations in the gene coding for Kv1.1, *KCNA1*, lead to episodic ataxia type 1 [28], and are, in some individuals, associated with partial onset epilepsy [29], supporting the importance of this channel in limiting neuronal excitability. Mouse models with deletions in *Kcna1* have been suggested as models for sudden unexpected death in epilepsy (SUDEP), which may be associated with Kv1.1's role in regulating parasympathetic control of cardiac function [30, 31]. The hypothesis that miR-129-5p-mediated control of Kv1.1 may likewise affect cardiac function and contribute to SUDEP is intriguing but remains to be tested.

MiR-324–5p suppresses Kv4.2 mRNA translation to control seizure onset

Recently, we discovered a direct role of microRNA-mediated silencing of the *Shal*-related voltage-gated potassium channel, Kv4.2 (encoded by *Kcnd2*) in regulating seizure onset [•32]. Inhibition of the Kv4.2-targeting microRNA miR-324–5p with antagomirs in mice reduced seizure severity in response to kainic acid [•32]. Notably, inhibition of miR-324–5p delayed kainic acid-induced seizure onset in wild type mice, but not in *Kcnd2* knockout mice. These results suggest that miR-324–5p-dependent suppression of Kv4.2 is essential for seizure development following a kainic acid challenge. This is a rather surprising finding, given the many mRNAs shown and suggested to be targeted by miR-324–5p. By contrast, overall EEG power after kainic acid was reduced with miR-324–5p inhibition in both wild type and *Kcnd2* knockout mice, indicating that other targets of miR-324–5p, apart from Kv4.2 mediated this effect. To further delineate miR-324–5p's role in neuronal excitability, it will be important to identify these other mRNA targets that mediate the

neuronal activity-suppressing effect of miR-324–5p antagomirs. Recent meta-analyses of microRNA profiling studies in epilepsy found miR-324–5p to be downregulated in the latent or chronic phase of epilepsy [•21, •22], suggesting an (insufficient) compensatory mechanism that deserves further assessment. Two other microRNAs, miR-223–3p and miR-301a, target Kv4.2 in the heart [33, 34]. The role for miR-301a in the brain is unknown, but miR-223–3p was shown to regulate neuronal excitability in the brain, possibly in the context of epilepsy (further discussed below). A few mutations in *KCND2* have been reported in humans [35, 36], and Kv4.2 function is downregulated in animal models of epilepsy [4, 37], making it a potentially attractive target for microRNA-mediated manipulations as an epilepsy treatment.

MicroRNA-mediated regulation of Nav1.1 may be altered in epilepsy

The voltage-gated sodium channel Nav1.1 plays a prominent role in controlling neuronal excitability. Mutations in *SCN1A*, encoding Nav1.1, are frequently associated with epilepsy, and can lead to epilepsy-associated syndromes, such as Genetic epilepsy with febrile seizures plus (GEFS+), or Dravet's Syndrome, a severe intractable form of epilepsy [38]. Surprisingly little is known about how microRNAs regulate Nav1.1. MiR-155 was shown to target Nav1.1, and may play a role in the seizure-suppressing action of valproic acid [39]. Recent discoveries that epilepsy-associated genetic variants in the 3'UTR of *SCN1A* might affect microRNA-induced silencing [40] corroborate the hypothesis that microRNA-mediated silencing of Nav1.1 is an important regulator of neuronal excitability in epilepsy, and warrant future studies.

MicroRNAs regulating ligand-gated ion channels in the brain

Epilepsy is a disorder of altered neuronal excitability, which may be caused by an imbalance of inhibitory and excitatory signal transduction. Defects in inhibitory or excitatory synapses, GABAergic and glutamatergic synapses, respectively, have often been associated with epilepsy. Loss of inhibitory neurons has been observed in epilepsy [41], and targeted removal of subsets of inhibitory GABAergic neurons leads to seizures, but not epilepsy, in mice [42–44]. Glutamatergic and GABAergic signaling have thus been treatment targets in epilepsy since many years [45]. Transplantation of inhibitory interneurons to normalize the inhibitory/excitatory balance in the brain has shown to be seizure-suppressive [46], and AMPA receptor inhibitors have shown some success in animal studies and in clinic [47]. Although direct examinations of microRNAs targeting glutamate or GABA receptors in the context of epilepsy are scarce, several studies in other disease models show the potential of microRNAs to modulate these ligand-gated ion channels, providing mechanistic insight and potential novel therapeutic strategies for epilepsy.

MicroRNAs targeting ionotropic glutamate receptors regulate synaptic activity

Ionotropic glutamate receptors mediate excitatory postsynaptic currents and are therefore directly involved in excitatory transmission and neuronal excitability. There are three major subgroups of glutamate receptors, AMPA receptors, NMDA receptors and kainate receptors, and all but kainate receptors have been shown to be directly regulated by microRNAs.

MicroRNA-induced silencing of GluA1 impairs AMPA receptor-mediated

synaptic plasticity—AMPA receptors are tetramers of combinations of four subunits, GluA1–4. Rapid insertion or removal of AMPA receptors at the postsynaptic membrane is important for many different forms of synaptic plasticity. It is therefore not surprising that AMPA receptor subunits are under strict regulation at transcriptional, post-transcriptional and subcellular localization levels [48]. The AMPA receptor subunit GluA1 is downregulated after a variety of synaptic or neuronal stimuli, making GluA1's activity-dependent suppression by microRNA-induced silencing an attractive mechanism to study. Indeed, the three microRNAs with a direct role in GluA1 regulation that are discussed here are involved in activity-dependent downregulation of GluA1.

One of the first studies analyzing microRNAs and GluA1 function showed a role for the GluA1-targeting microRNA miR-92a in homeostatic synaptic scaling [••49], which is a mechanism neurons utilize to respond to changes in synaptic activity in order to maintain a stable network [50]. AMPA receptors and especially their GluA1 subunits are critical for homeostatic synaptic scaling, and prolonged inhibition of synaptic activity leads to an upregulation of AMPA receptor function [51]. MiR-92a impairs the incorporation of new GluA1-containing AMPA receptors in response to blockade of synaptic activity, thereby preventing homeostatic scaling [••49]. Homeostatic neuronal scaling has been suggested to be altered in epilepsy [52], but, except of some profiling studies indicating differential expression [53, 54] miR-92a has not been tested in the context of seizures or epilepsy yet.

MiR-137, another GluA1-targeting microRNA [•55, 56], was shown to be important for metabotropic glutamate receptor (mGluR)-mediated long-term depression (LTD) by blocking AMPAR-mediated synaptic transmission and silencing active synapses. MiR-137 has been most prominently associated with schizophrenia [57], but is also differently expressed in several mouse and rat models of epilepsy [58–61]. The findings in these profiling studies were very variable, and no functional studies of miR-137 in epilepsy have been published to date, leaving the role of miR-137-mediated regulation of GluA1 in seizure development obscure.

The most recently published work about microRNA-mediated regulation of GluA1 suggests that microRNA-mediated translational suppression of GluA1 is, in addition to mGluR-LTD, also involved in NMDA receptor-dependent LTD. Here, Hu et al. showed that NMDA receptor activation leads to upregulation of miR-501–3p, which reduces GluA1 expression, and is important for NMDA-mediated synaptic spine remodeling [••62]. GluA1 mRNA is localized to dendrites *in vitro* and *in vivo* [63, 64], and there is strong support for its local translation at synapses [65, 64, 66]. Using a rat slice preparation, in which neuronal cell bodies in the CA1 region were mechanically detached from the neuropil, the authors provided evidence that miR-501–3p may regulate local translation of GluA1 in synapses. Again, no functional studies assessing miR-501–3p-regulation of GluA1 in epilepsy has been published so far. Given the prominent role of GluA1 in regulating neuronal excitability, candidate approaches targeting miR-92a, miR-137 or miR-501–3p in animal models are needed to assess their role in epilepsy.

MicroRNA-induced silencing of GluA2 reduces synaptic activity—GluA2 plays a distinct role in regulating neuronal activity because, in contrast to the other AMPA receptor subunits, it is calcium-impermeable and therefore has a strong influence on receptor function [67]. Most AMPA receptors in the adult nervous system contain GluA2 subunits, but rapid membrane insertion of receptors lacking GluA2 provides a mechanism for short-term plasticity, illustrating the need for tight regulation. One of the first studies demonstrating microRNA-mediated regulation of GluA2 showed that miR-181a targets GluA2 in neurons and reduces the frequency of miniature excitatory postsynaptic potentials (mEPSPs) when overexpressed in neurons [68]. Removing GluA2 subunits from AMPA receptors at the synapse is expected to increase calcium-permeability and, thus, synaptic activity. However, this is only true if functional AMPA receptors lacking GluA2 replace the GluA2-containing receptors, which, the authors argue, does not occur in experiments. Instead, the reduction in mEPSP frequency by GluA2 removal may be due to a loss of functional postsynaptic units and/or the generation of silent synapses that lack functional AMPA receptors. While this hypothesis remains to be tested, this study corroborates a role of microRNA-induced silencing of GluA2 in regulating neuronal excitability. In a somewhat controversial finding, a recent publication suggested that inhibition of miR-181a is neuroprotective in a rat model of epilepsy by decreasing the pro-apoptotic factor Caspase-3 and Caspase-9 [69]. However, the effect on the seizure phenotype was not tested, and more studies are needed to assess if miR-181a affects seizure susceptibility. Of note, the earlier study by Schratt and co-authors focused on the nucleus accumbens, whereas Ren et al. analyzed hippocampal tissue, suggesting brain region-specific mechanisms. Notably, a similar effect on spontaneous synaptic activity as for miR-181a was observed with another GluA2-targeting microRNA, miR-223–3p [•70]. Using a knockout approach, the authors showed that lack of miR-223–3p increases miniature excitatory postsynaptic currents (mEPSCs) and is neuroprotective, which is in line with Schratt and colleagues' observations after overexpression of miR-181a. It is noteworthy that miR-223–3p does not only target mGluA2, but also the NMDA receptor subunit GluN2B [•70], and Kv4.2 in the heart [33]. It is not known if both glutamate receptors and Kv4.2 contribute equally to the observed effect on neuronal activity; however, this potentially concerted regulation of several ion channels by a single microRNA makes miR-223–3p a particularly intriguing candidate for future epilepsy studies. MiR-223–3p was recently shown to be decreased in individuals with hippocampal sclerosis and focal cortical dysplasia [71], corroborating a potential role in epilepsy.

Another microRNA, miR-124 was suggested to regulate GluA2 and GluA3 during demyelination in multiple sclerosis [72]. Notably, two recent studies showed a role of miR-124 in seizure regulation [••73, 74]. Although these studies did not test effects on GluA2 and were somewhat contradictory, this adds an additional microRNA to the pool of potential regulators of GluA2. It will be interesting to directly compare how these different GluA2-targeting microRNAs affect neuronal excitability and epilepsy. In our experience, the physiological effect of microRNA inhibition on seizure frequency and severity in epilepsy models can strongly vary for different microRNAs, even though these microRNAs target the same ion channel (*unpublished*). We speculate that brain region- and subcellular expression levels as well as other mRNA targets of these microRNAs may influence if and how they change the epileptic phenotype.

NMDA receptor subunits are targeted by microRNAs—Among the three classes of glutamate receptors, NMDA receptors have a distinct role in synaptic plasticity because of their ability to integrate internal and external cues. They are activated by binding of a ligand, like AMPA receptors, but additionally have an intracellular Mg^{2+} block that is only removed if the cell is in a depolarized state. This puts NMDA receptors in the ideal position to mediate “Hebbian plasticity”, which postulates that the correlated activity of two neurons strengthens their synaptic connection and is believed to underlie learning and memory [75, 76]. Several microRNAs were shown to directly target NMDA receptor subunits and play an important role in synaptic plasticity [77], but evidence for their importance in epilepsy is less prominent. NMDA receptors are heterotetramers consisting of two GluN1 subunits and two GluN2A-D or GluN3A/B subunits. Notably, most, if not all microRNAs, directly regulating NMDA receptors have been shown to target GluN2 subunits. Two microRNAs, miR-19a and miR-539, were suggested to be involved in the developmental switch of GluN2B- to GluN2A-containing receptors, with miR-19a suppressing GluN2A during early development, and miR-539 suppressing GluN2B at later stages [78]. However, this study only assessed the effect of miR-19a or miR-539 manipulation in cultured neurons *in vitro* and provided correlative evidence *in vivo*. It remains to be seen if *in vivo* modulation of miR-19a or miR-539 alters GluN2B:GluN2A ratios and neuronal excitability. The ratio of GluN2B:GluN2A was shown to increase in epileptic rats, which may underlie the enhanced long-term potentiation (LTP) in hippocampal slices from these rats [79]. LTP is believed to be the synaptic correlate of learning and memory [80], suggesting that the change in GluN2B:GluN2A ratio in epilepsy may contribute to cognitive deficits often associated with epilepsy [81]. It will be interesting to study if miR-19a or miR-539 are involved in these processes and contribute to cognitive impairment in epilepsy.

A few NMDA receptor-targeting microRNAs have been associated with altered neuronal excitability. MiR-125b, for example directly targets NR2A and regulates its expression in cooperation with the Fragile X Mental Retardation Protein (FMRP) [82]. Loss of FMRP leads to Fragile X Syndrome (FXS), an intellectual disability associated with hypersensitivity to auditory and visual stimuli as well as increased risk for childhood epilepsy [83]. MiR-125b overexpression reduced mEPSCs in wild type mice; however, it is unclear if dysregulated miR-125b-mediated silencing of NR2A contributes to the neuronal hyperexcitability in FXS. A more direct link between microRNA-mediated regulation of NR2A and epilepsy was recently provided by Alsharafi et al., who showed that miR-139–5p targets NR2A and is down-regulated in both human temporal lobe epilepsy and rat epilepsy models [84]. Increasing miR-139–5p using an agomir prevents *status epilepticus*-induced upregulation of NR2A in the rat model, but it is unknown if this manipulation affects seizure severity or the development of epilepsy. NMDA dysfunction has been reported in epilepsy, including mutations in the genes coding for NMDA receptor subunits [85], but also anti-NMDA-antibody positive encephalitis associated with seizures [86]. Notably, NMDA receptor mutations associated with epilepsy and intellectual disability can either activate or impair NMDA function [85], making microRNAs a potentially valuable therapeutic tool to either enhance (by blocking microRNAs with antagomirs) or decrease (by supplementation with agomirs) NMDA expression and thus function. Such strategies should be tested in animal models in the future.

MiR-33 targets GABA_A receptors but the role in epilepsy is unknown

GABA is the predominant inhibitory neurotransmitter in the brain. There are two types of GABA receptors, ionotropic (GABA_A) and metabotropic (GABA_B) receptors. For the purpose of this review, we will focus on GABA_A receptors, which mediate chloride currents, thereby hyperpolarizing the cell in the adult organism. GABA_A receptors have a complex structure, containing α , β , γ , and δ subunits assembled as heteropentamers [87]. So far, there is not much known about microRNAs directly regulating GABA_A receptors. One exception is miR-33, which was recently shown to regulate two subunits of the GABA_A receptor, $\alpha 4$ and $\beta 2$, as well as the potassium chloride symporter KCC2 supporting a major function of miR-33 to control inhibitory signaling in the brain [88]. This study focused on miR-33's role in encoding fear memory but did not assess neuronal excitability. MiR-33 is downregulated in the dentate gyrus of animal models of epilepsy [89, •22], which could either indicate a compensatory mechanism upregulating GABA-mediated inhibition or a general dysregulation of GABAergic signaling in epilepsy. The effect of manipulating miR-33 levels on seizure susceptibility in animal models has yet to be tested but will be essential to reveal underlying mechanisms.

MicroRNA-induced silencing of ion channels in chronic pain – what can be learned?

Chronic pain, like epilepsy, is characterized by neuronal hyperexcitability, and therefore mechanisms identified in chronic pain models may be translatable to epilepsy [90]. Several recent studies have demonstrated a role of ion channel-targeting microRNAs in the control of chronic pain. Targeted channels include voltage-gated potassium and sodium channels. The miR-17–92 microRNA cluster, for example, was shown to regulate neuronal excitability in chronic neuropathic pain through regulation of several members of the A-type voltage-gated potassium channel family [•91]. The miR-17–92 cluster contains three polycistronic genes coding for 15 microRNAs [92]. Overexpression or inhibition of members of this microRNA cluster induced or alleviated allodynia in rats through regulation of A-type currents. As discussed above, A-type currents are downregulated in animal models of epilepsy [4], and miR-324–5p, which targets the A-type potassium channel Kv4.2, regulates seizure susceptibility [•32]. So far, there is not much known about the miR-17–92 microRNA cluster in epilepsy, apart from one expression study suggesting miR-19b to be downregulated in cerebrospinal fluid of epilepsy patients [93]. It will be interesting to assess how manipulating the miR-17–92 cluster affects epilepsy.

Another example is miR-30b, which is downregulated in dorsal root ganglia of a rat nerve injury model. Local injection of a miR-30b agomir in dorsal root ganglia decreases elevated expression of the voltage-gated sodium channel Nav1.7 and alleviates pain [94]. Mutations in the Nav1.7-encoding gene *SCN9A* have been associated with epilepsy [95], but a role of miR-30b in epilepsy has not been reported yet [•21, •22]. MiR-7a, which is also downregulated in the dorsal root ganglion of a spinal cord injury rat model of chronic pain [96], is another example of a voltage-gated sodium channel-targeting microRNA. Local viral overexpression of miR-7a reduced action potentials and alleviated pain-related behavior through direct regulation of the Nav $\beta 2$ subunit (*SCN2B*). Co-expression of miR-7a with

recombinant miR-7a-insensitive Nav β 2 removed the analgesic effect of miR-7a, supporting the importance of this specific miR-7a target for pain regulation. There is currently no strong support for causative *SCN2B* mutations in epilepsy [97, 98], but *Scn2b* knockout mice have a higher susceptibility to seizures [99]. Therefore, altered expression of *SCN2B* due to defective microRNA-induced silencing may contribute to epilepsy. Nav β 2 was shown to be regulated by another microRNA, miR-9 in the brain [100], further supporting this hypothesis.

Conclusions

Ion channels are often mutated in epilepsy disorders and are therefore frequent targets of pharmacological manipulations to reduce or prevent pathologic hypersynchronous activity in the epileptic brain. However, epilepsy-related defects in ion channels do not only occur on the genomic level but can also manifest as changes in expression levels or subcellular localization. Under physiological conditions, changes in expression levels of ion channels can provide powerful cellular tools to alter a neuron's intrinsic excitability but may be detrimental when pathologically altered. MicroRNA-induced silencing is a molecular mechanism that can mediate rapid changes in a protein's expression levels, including ion channels. The here described studies illustrate that, although there is already some published evidence for direct regulation of different ion channels by microRNAs, only little is known about how microRNA-mediated silencing of ion channels is affected in epilepsy. To advance the field, more studies are needed that test if manipulation of these ion channel-targeting microRNAs affects seizures and epilepsy in animal models. Assessing microRNAs and targeted ion channels identified in pain research for their role in epilepsy may be a promising strategy. In addition, it will be essential to gain a better understanding of the mechanisms of microRNA-mediated regulation of ion channels under physiological conditions. For example, more studies are needed to directly test if and how microRNAs modulate the ion currents mediated by their target ion channels. Moreover, the underlying mechanisms of translational repression need to be elucidated: some studies indicate that RNA-binding proteins cooperate with microRNAs to regulate ion channels, but it is unknown if this is a common mechanism. A few studies suggest that microRNAs are involved in the local translation of ion channels at the synapse and in dendrites, which adds an additional layer of complexity and deserves further assessment in the context of epilepsy. With more studies elucidating the exact molecular mechanisms of microRNA-induced silencing of ion channels in neurons, a better understanding of how these processes may be dysregulated in epilepsy will be achieved. Ultimately, this will help to reveal how this mechanism could be used as a tool to alter neuronal excitability and reduce or prevent seizures in epilepsy.

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References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Dudek FE , Staley KJ . The Time Course and Circuit Mechanisms of Acquired Epileptogenesis In: Noebels JL , Avoli M , Rogawski MA , Olsen RW , Delgado-Escueta AV , editors. Jasper's Basic Mechanisms of the Epilepsies. Bethesda (MD): National Center for Biotechnology Information (US); 2012.
2. Golyala A , Kwan P . Drug development for refractory epilepsy: The past 25 years and beyond. *Seizure*. 2017;44:147–56. doi:10.1016/j.seizure.2016.11.022. [PubMed: 28017578]
3. Lerche H , Shah M , Beck H , Noebels J , Johnston D , Vincent A . Ion channels in genetic and acquired forms of epilepsy. *J Physiol*. 2013;591(4):753–64. doi:10.1113/jphysiol.2012.240606. [PubMed: 23090947]
4. Bernard C , Anderson A , Becker A , Poolos NP , Beck H , Johnston D . Acquired dendritic channelopathy in temporal lobe epilepsy. *Science*. 2004;305(5683):532–5. doi:10.1126/science.1097065. [PubMed: 15273397]
5. Poolos NP , Johnston D . Dendritic ion channelopathy in acquired epilepsy. *Epilepsia*. 2012;53:32–40. doi:10.1111/epi.12033. [PubMed: 23216577]
6. Bartel DP . Metazoan MicroRNAs. *Cell*. 2018;173(1):20–51. doi:10.1016/j.cell.2018.03.006. [PubMed: 29570994]
7. Griffiths-Jones S The microRNA Registry. *Nucleic acids research*. 2004;32(suppl_1):D109–D11. doi:10.1093/nar/gkh023. [PubMed: 14681370]
8. Friedman RC , Farh KK , Burge CB , Bartel DP . Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*. 2009;19 Doi: 10.1101/gr.082701.108.
9. Fromm B , Billipp T , Peck LE , Johansen M , Tarver JE , King BL et al. A Uniform System for the Annotation of Vertebrate microRNA Genes and the Evolution of the Human microRNAome. *Annual review of genetics*. 2015;49:213–42. doi:10.1146/annurev-genet-120213-092023.
10. Agarwal V , Bell GW , Nam JW , Bartel DP . Predicting effective microRNA target sites in mammalian mRNAs. *eLife*. 2015;4. doi:10.7554/eLife.05005.
11. Henshall DC , Hamer HM , Pasterkamp RJ , Goldstein DB , Kjems J , Prehn JHM et al. MicroRNAs in epilepsy: pathophysiology and clinical utility. *The Lancet Neurology*. 2016;15(13):1368–76. doi:10.1016/S1474-4422(16)30246-0. [PubMed: 27839653]
12. Aronica E , Fluiter K , Iyer A , Zurolo E , Vreijling J , van Vliet EA et al. Expression pattern of miR-146a, an inflammation-associated microRNA, in experimental and human temporal lobe epilepsy. *Eur J Neurosci*. 2010;31(6):1100–7. doi:10.1111/j.1460-9568.2010.07122.x. [PubMed: 20214679]
13. Jimenez-Mateos EM , Bray I , Sanz-Rodriguez A , Engel T , McKiernan RC , Mouri G et al. miRNA Expression profile after status epilepticus and hippocampal neuroprotection by targeting miR-132. *Am J Pathol*. 2011;179(5):2519–32. doi:10.1016/j.ajpath.2011.07.036. [PubMed: 21945804]
14. Kaalund SS , Venø MT , Bak M , Møller RS , Laursen H , Madsen F et al. Aberrant expression of miR-218 and miR-204 in human mesial temporal lobe epilepsy and hippocampal sclerosis—Convergence on axonal guidance. *Epilepsia*. 2014;55(12):2017–27. doi:10.1111/epi.12839. [PubMed: 25410734]
15. Li MM , Jiang T , Sun Z , Zhang Q , Tan CC , Yu JT et al. Genome-wide microRNA expression profiles in hippocampus of rats with chronic temporal lobe epilepsy. *Scientific reports*. 2014;4:4734. doi:10.1038/srep04734. [PubMed: 24751812]
16. Lee JY , Park AK , Lee ES , Park WY , Park SH , Choi JW et al. miRNA expression analysis in cortical dysplasia: regulation of mTOR and LIS1 pathway. *Epilepsy Res*. 2014;108(3):433–41. doi:10.1016/j.epilepsyres.2014.01.005. [PubMed: 24560344]
17. Tiwari D , Peariso K , Gross C . MicroRNA-induced silencing in epilepsy: Opportunities and challenges for clinical application. *Developmental dynamics : an official publication of the American Association of Anatomists*. 2018;247(1):94–110. doi:10.1002/dvdy.24582. [PubMed: 28850760]

18. Mooney C , Becker BA , Raoof R , Henshall DC . EpimiRBase: a comprehensive database of microRNA-epilepsy associations. *Bioinformatics*. 2016;32(9):1436–8. doi:10.1093/bioinformatics/btw008. [PubMed: 26748106] •• This database provides an useful tool for researchers to easily obtain information about published studies related to microRNAs in epilepsy.
19. Chakraborty C , Sharma AR , Sharma G , Doss CGP , Lee S-S . Therapeutic miRNA and siRNA: Moving from Bench to Clinic as Next Generation Medicine. *Molecular Therapy - Nucleic Acids*. 2017;8:132–43. doi:10.1016/j.omtn.2017.06.005. [PubMed: 28918016]
20. Henshall DC . Antagomirs and microRNA in status epilepticus. *Epilepsia*. 2013;54 Suppl 6:17–9. doi:10.1111/epi.12267. [PubMed: 24001063]
21. Korotkov A , Mills JD , Gorter JA , van Vliet EA , Aronica E . Systematic review and meta-analysis of differentially expressed miRNAs in experimental and human temporal lobe epilepsy. *Scientific reports*. 2017;7(1):11592. doi:10.1038/s41598-017-11510-8. [PubMed: 28912503] • This study provides useful databases of microRNAs shown to be differentially expressed in temporal lobe epilepsy in animal models and humans.
22. Srivastava PK , Roncon P , Lukasiuk K , Gorter JA , Aronica E , Pitkänen A et al. Meta-Analysis of MicroRNAs Dysregulated in the Hippocampal Dentate Gyrus of Animal Models of Epilepsy. *eNeuro*. 2017;4(6):ENEURO.0152-17.2017. doi:10.1523/ENEURO.0152-17.2017. • This is the first study that performed a meta analysis of studies reporting differentially expressed microRNAs in the dentate gyrus of epilepsy mouse models.
23. D'Adamo MC , Catacuzzeno L , Di Giovanni G , Franciolini F , Pessia M . K(+) channelopathy: progress in the neurobiology of potassium channels and epilepsy. *Frontiers in cellular neuroscience*. 2013;7:134. doi:10.3389/fncel.2013.00134. [PubMed: 24062639]
24. Brenner R , Wilcox KS . Potassium Channelopathies of Epilepsy In: th, Noebels JL , Avoli M , Rogawski MA , Olsen RW , Delgado-Escueta AV , editors. *Jasper's Basic Mechanisms of the Epilepsies*. Bethesda (MD): National Center for Biotechnology Information (US); 2012.
25. Sosanya NM , Huang PP , Cacheaux LP , Chen CJ , Nguyen K , Perrone-Bizzozero NI et al. Degradation of high affinity HuD targets releases Kv1.1 mRNA from miR-129 repression by mTORC1. *J Cell Biol*. 2013;202(1):53–69. doi:10.1083/jcb.201212089. [PubMed: 23836929] •• This is the first study showing that a microRNA is involved in the local dendritic translation of an ion channel.
26. Sosanya NM , Brager DH , Wolfe S , Niere F , Raab-Graham KF . Rapamycin reveals an mTOR-independent repression of Kv1.1 expression during epileptogenesis. *Neurobiol Dis*. 2015;73:96–105. doi:10.1016/j.nbd.2014.09.011. [PubMed: 25270294] •• Together with [25] this study suggests that microRNA-mediated regulation of dendritic translation of Kv1.1 is involved in epilepsy.
27. Rajman M , Metge F , Fiore R , Khudayberdiev S , Aksoy-Aksel A , Bicker S et al. A microRNA-129-5p/Rbfox crosstalk coordinates homeostatic downscaling of excitatory synapses. *The EMBO Journal*. 2017;36(12):1770–87. doi:10.15252/embj.201695748. [PubMed: 28487411]
28. Browne DL , Gancher ST , Nutt JG , Brunt ER , Smith EA , Kramer P et al. Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1. *Nat Genet*. 1994;8(2):136–40. doi:10.1038/ng1094-136. [PubMed: 7842011]
29. Zuberi SM , Eunson LH , Spauschus A , De Silva R , Tolmie J , Wood NW et al. A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. *Brain*. 1999;122 (Pt 5):817–25. [PubMed: 10355668]
30. Glasscock E , Yoo JW , Chen TT , Klassen TL , Noebels JL . Kv1.1 potassium channel deficiency reveals brain-driven cardiac dysfunction as a candidate mechanism for sudden unexplained death in epilepsy. *J Neurosci*. 2010;30(15):5167–75. doi:10.1523/jneurosci.5591-09.2010. [PubMed: 20392939]
31. Moore BM , Jerry Jou C , Tatalovic M , Kaufman ES , Kline DD , Kunze DL . The Kv1.1 null mouse, a model of sudden unexpected death in epilepsy (SUDEP). *Epilepsia*. 2014;55(11):1808–16. doi:10.1111/epi.12793. [PubMed: 25377007]
32. Gross C , Yao X , Engel T , Tiwari D , Xing L , Rowley S et al. MicroRNA-Mediated Downregulation of the Potassium Channel Kv4.2 Contributes to Seizure Onset. *Cell reports*. 2016;17(1):37–45. doi:10.1016/j.celrep.2016.08.074. [PubMed: 27681419] • This is the first study

showing that regulation through a specific microRNA-mRNA pair is essential to control seizure onset.

33. Liu X , Zhang Y , Du W , Liang H , He H , Zhang L et al. MiR-223-3p as a Novel MicroRNA Regulator of Expression of Voltage-Gated K⁺ Channel Kv4.2 in Acute Myocardial Infarction. *Cell Physiol Biochem*. 2016;39(1):102–14. doi:10.1159/000445609. [PubMed: 27322747]
34. Panguluri SK , Tur J , Chapalamadugu KC , Katnik C , Cuevas J , Tipparaju SM . MicroRNA-301a mediated regulation of Kv4.2 in diabetes: identification of key modulators. *PLoS One*. 2013;8(4):e60545. doi:10.1371/journal.pone.0060545. [PubMed: 23573265]
35. Singh B , Ogiwara I , Kaneda M , Tokonami N , Mazaki E , Baba K et al. A Kv4.2 truncation mutation in a patient with temporal lobe epilepsy. *Neurobiol Dis*. 2006;24(2):245–53. doi:10.1016/j.nbd.2006.07.001. [PubMed: 16934482]
36. Lee H , Lin MC , Kornblum HI , Papazian DM , Nelson SF . Exome sequencing identifies de novo gain of function missense mutation in KCND2 in identical twins with autism and seizures that slows potassium channel inactivation. *Hum Mol Genet*. 2014;23(13):3481–9. doi:10.1093/hmg/ddu056. [PubMed: 24501278]
37. Lugo JN , Smith GD , Arbuckle EP , White J , Holley AJ , Floruta CM et al. Deletion of PTEN produces autism-like behavioral deficits and alterations in synaptic proteins. *Frontiers in molecular neuroscience*. 2014;7. doi:10.3389/fnmol.2014.00027.
38. Catterall WA , Kalume F , Oakley JC . Nav1.1 channels and epilepsy. *J Physiol*. 2010;588(Pt 11): 1849–59. doi:10.1113/jphysiol.2010.187484. [PubMed: 20194124]
39. Zhang Z , Wang Z , Zhang B , Liu Y . Downregulation of microRNA155 by preoperative administration of valproic acid prevents postoperative seizures by upregulating SCN1A. *Molecular medicine reports*. 2018;17(1):1375–81. doi:10.3892/mmr.2017.8004. [PubMed: 29115566]
40. Li T , Kuang Y , Li B . The genetic variants in 3' untranslated region of voltage-gated sodium channel alpha 1 subunit gene affect the mRNA-microRNA interactions and associate with epilepsy. *BMC genetics*. 2016;17(1):111. doi:10.1186/s12863-016-0417-y. [PubMed: 27473590]
41. Ribak C , Harris A , Vaughn J , Roberts E . Inhibitory, GABAergic nerve terminals decrease at sites of focal epilepsy. *Science*. 1979;205(4402):211–4. doi:10.1126/science.109922. [PubMed: 109922]
42. Spanpanato J , Dudek FE . Targeted Interneuron Ablation in the Mouse Hippocampus Can Cause Spontaneous Recurrent Seizures. *eNeuro*. 2017;4(4):ENEURO.0130-17.2017. doi:10.1523/ENEURO.0130-17.2017.
43. Drexel M , Romanov RA , Wood J , Weger S , Heilbronn R , Wulff P et al. Selective Silencing of Hippocampal Parvalbumin Interneurons Induces Development of Recurrent Spontaneous Limbic Seizures in Mice. *J Neurosci*. 2017;37(34):8166–79. doi:10.1523/jneurosci.3456-16.2017. [PubMed: 28733354]
44. Gross C Lost Inhibition - Brain Activity Temporarily Out of Control. *Epilepsy Curr*. 2018;18(1): 53–5. doi:10.5698/1535-7597.18.1.53. [PubMed: 29844768]
45. Rowley NM , Madsen KK , Schousboe A , Steve White H . Glutamate and GABA synthesis, release, transport and metabolism as targets for seizure control. *Neurochemistry International*. 2012;61(4):546–58. doi:10.1016/j.neuint.2012.02.013. [PubMed: 22365921]
46. Hunt RF , Baraban SC . Interneuron Transplantation as a Treatment for Epilepsy. *Cold Spring Harbor Perspectives in Medicine*. 2015;5(12). doi:10.1101/cshperspect.a022376.
47. Rogawski MA . AMPA Receptors as a Molecular Target in Epilepsy Therapy. *Acta neurologica Scandinavica Supplementum*. 2013(197):9–18. doi:10.1111/ane.12099. [PubMed: 23480151]
48. Hanley JG . Subunit-specific trafficking mechanisms regulating the synaptic expression of Ca²⁺-permeable AMPA receptors. *Seminars in Cell & Developmental Biology*. 2014;27:14–22. doi: 10.1016/j.semcdb.2013.12.002. [PubMed: 24342448]
49. Letellier M , Elramah S , Mondin M , Soula A , Penn A , Choquet D et al. miR-92a regulates expression of synaptic GluA1-containing AMPA receptors during homeostatic scaling. *Nat Neurosci*. 2014;17(8):1040–2. doi:10.1038/nn.3762. [PubMed: 25017011] •• This study shows that microRNA-mediated regulation of GluA1 regulates homeostatic scaling, one of the first studies demonstrating a direct role of microRNA-induced silencing of an ion channel in synaptic plasticity.

50. Turrigiano G Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function. *Cold Spring Harb Perspect Biol.* 2012;4(1):a005736. doi:10.1101/cshperspect.a005736. [PubMed: 22086977]
51. Turrigiano GG , Leslie KR , Desai NS , Rutherford LC , Nelson SB . Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature.* 1998;391:892. doi:10.1038/36103. [PubMed: 9495341]
52. Trasande CA , Ramirez JM . Activity deprivation leads to seizures in hippocampal slice cultures: is epilepsy the consequence of homeostatic plasticity? *Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society.* 2007;24(2):154–64. doi:10.1097/WNP.0b013e318033787f. [PubMed: 17414971]
53. McKiernan RC , Jimenez-Mateos EM , Bray I , Engel T , Brennan GP , Sano T et al. Reduced Mature MicroRNA Levels in Association with Dicer Loss in Human Temporal Lobe Epilepsy with Hippocampal Sclerosis. *PLOS ONE.* 2012;7(5):e35921. doi:10.1371/journal.pone.0035921. [PubMed: 22615744]
54. Roncon P , Soukupová M , Binaschi A , Falcicchia C , Zucchini S , Ferracin M et al. MicroRNA profiles in hippocampal granule cells and plasma of rats with pilocarpine-induced epilepsy – comparison with human epileptic samples. *Scientific reports.* 2015;5:14143. doi:10.1038/srep14143. [PubMed: 26382856]
55. Olde Loohuis NF , Ba W , Stoerchel PH , Kos A , Jager A , Schrott G et al. MicroRNA-137 Controls AMPA-Receptor-Mediated Transmission and mGluR-Dependent LTD. *Cell reports.* 2015;11(12):1876–84. doi:10.1016/j.celrep.2015.05.040. [PubMed: 26095359] • This study is the first to report a role for a microRNA in mGluR-LTD.
56. Thomas KT , Anderson BR , Shah N , Zimmer SE , Hawkins D , Valdez AN et al. Inhibition of the Schizophrenia-Associated MicroRNA miR-137 Disrupts Nrg1 α Neurodevelopmental Signal Transduction. *Cell reports.* 2017;20(1):1–12. doi:10.1016/j.celrep.2017.06.038. [PubMed: 28683304]
57. Sakamoto K , Crowley JJ . A comprehensive review of the genetic and biological evidence supports a role for MicroRNA-137 in the etiology of schizophrenia. *Am J Med Genet B Neuropsychiatr Genet.* 2018;177(2):242–56. doi:10.1002/ajmg.b.32554. [PubMed: 29442441]
58. Gorter JA , Iyer A , White I , Colzi A , van Vliet EA , Sisodiya S et al. Hippocampal subregion-specific microRNA expression during epileptogenesis in experimental temporal lobe epilepsy. *Neurobiology of Disease.* 2014;62(0):508–20. doi: 10.1016/j.nbd.2013.10.026. [PubMed: 24184920]
59. Song YJ , Tian XB , Zhang S , Zhang YX , Li X , Li D et al. Temporal lobe epilepsy induces differential expression of hippocampal miRNAs including let-7e and miR-23a/b. *Brain Res.* 2011;1387:134–40. doi:10.1016/j.brainres.2011.02.073. [PubMed: 21376023]
60. Bot AM , Debski KJ , Lukasiuk K . Alterations in miRNA levels in the dentate gyrus in epileptic rats. *PLoS One.* 2013;8(10):e76051. doi:10.1371/journal.pone.0076051. [PubMed: 24146813]
61. Schouten M , Fratantoni SA , Hubens CJ , Piersma SR , Pham TV , Bielefeld P et al. MicroRNA-124 and -137 cooperativity controls caspase-3 activity through BCL2L13 in hippocampal neural stem cells. *Scientific reports.* 2015;5:12448. doi:10.1038/srep12448. [PubMed: 26207921]
62. Hu Z , Zhao J , Hu T , Luo Y , Zhu J , Li Z . miR-501–3p mediates the activity-dependent regulation of the expression of AMPA receptor subunit GluA1. *J Cell Biol.* 2015;208(7):949–59. doi:10.1083/jcb.201404092. [PubMed: 25800054] •• This study provides in vivo evidence that the local dendritic translation of GluA1 is regulated by a microRNA.
63. Grooms SY , Noh KM , Regis R , Bassell GJ , Bryan MK , Carroll RC et al. Activity bidirectionally regulates AMPA receptor mRNA abundance in dendrites of hippocampal neurons. *J Neurosci.* 2006;26(32):8339–51. [PubMed: 16899729]
64. Muddashetty RS , Kelic S , Gross C , Xu M , Bassell GJ . Dysregulated metabotropic glutamate receptor-dependent translation of AMPA receptor and postsynaptic density-95 mRNAs at synapses in a mouse model of fragile X syndrome. *J Neurosci.* 2007;27(20):5338–48. doi:10.1523/jneurosci.0937-07.2007. [PubMed: 17507556]

65. Sutton MA , Ito HT , Cressy P , Kempf C , Woo JC , Schuman EM . Miniature Neurotransmission Stabilizes Synaptic Function via Tonic Suppression of Local Dendritic Protein Synthesis. *Cell*. 2006;125(4):785–99. doi:10.1016/j.cell.2006.03.040. [PubMed: 16713568]
66. Smith WB , Starck SR , Roberts RW , Schuman EM . Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. *Neuron*. 2005;45(5):765–79. doi:10.1016/j.neuron.2005.01.015. [PubMed: 15748851]
67. Isaac JTR , Ashby MC , McBain CJ . The Role of the GluR2 Subunit in AMPA Receptor Function and Synaptic Plasticity. *Neuron*. 2007;54(6):859–71. doi:10.1016/j.neuron.2007.06.001. [PubMed: 17582328]
68. Saba R , Storchel PH , Aksoy-Aksel A , Kepura F , Lippi G , Plant TD et al. Dopamine-regulated microRNA MiR-181a controls GluA2 surface expression in hippocampal neurons. *Mol Cell Biol*. 2012;32(3):619–32. doi:10.1128/mcb.05896-11. [PubMed: 22144581]
69. Ren L , Zhu R , Li X . Silencing miR-181a produces neuroprotection against hippocampus neuron cell apoptosis post-status epilepticus in a rat model and in children with temporal lobe epilepsy. *Genetics and molecular research : GMR*. 2016;15(1). doi:10.4238/gmr.15017798.
70. Harraz MM , Eacker SM , Wang X , Dawson TM , Dawson VL . MicroRNA-223 is neuroprotective by targeting glutamate receptors. *Proc Natl Acad Sci U S A*. 2012;109(46):18962–7. doi:10.1073/pnas.1121288109. [PubMed: 23112146] • This study, together with [33] suggests that a microRNA regulates neuronal activity by targeting several different ion channels.
71. Srivastava A , Dixit AB , Paul D , Tripathi M , Sarkar C , Chandra PS et al. Comparative analysis of cytokine/chemokine regulatory networks in patients with hippocampal sclerosis (HS) and focal cortical dysplasia (FCD). *Scientific reports*. 2017;7(1):15904. doi:10.1038/s41598-017-16041-w. [PubMed: 29162878]
72. D Ranjan , CA M , C Ansi , RM V , DS A , DM K et al. Hippocampal demyelination and memory dysfunction are associated with increased levels of the neuronal microRNA miR-124 and reduced AMPA receptors. *Annals of Neurology*. 2013;73(5):637–45. doi:doi:10.1002/ana.23860. [PubMed: 23595422]
73. Brennan GP , Dey D , Chen Y , Patterson KP , Magnetta EJ , Hall AM et al. Dual and opposing roles of microRNA-124 in epilepsy are mediated through inflammatory and NRSF-dependent gene networks. *Cell reports*. 2016;14(10):2402–12. doi:10.1016/j.celrep.2016.02.042. [PubMed: 26947066] •• This study illustrates that the physiological function of microRNAs is the summary of actions on all targets and therefore not always predictable.
74. Wang W , Wang X , Chen L , Zhang Y , Xu Z , Liu J et al. The microRNA miR-124 suppresses seizure activity and regulates CREB1 activity. *Expert Reviews in Molecular Medicine*. 2016;18. doi:10.1017/erm.2016.3.
75. Iacobucci GJ , Popescu GK . NMDA receptors: linking physiological output to biophysical operation. *Nature Reviews Neuroscience*. 2017;18:236. doi:10.1038/nrn.2017.24. [PubMed: 28303017]
76. Lüscher C , Malenka RC . NMDA Receptor-Dependent Long-Term Potentiation and Long-Term Depression (LTP/LTD). *Cold Spring Harbor Perspectives in Biology*. 2012;4(6):a005710. doi:10.1101/cshperspect.a005710. [PubMed: 22510460]
77. Shen H , Li Z . miRNAs in NMDA receptor-dependent synaptic plasticity and psychiatric disorders. *Clinical science (London, England : 1979)*. 2016;130(14):1137–46. doi:10.1042/CS20160046.
78. Corbel C , Hernandez I , Wu B , Kosik KS . Developmental attenuation of N-methyl-D-aspartate receptor subunit expression by microRNAs. *Neural Development*. 2015;10:20. doi:10.1186/s13064-015-0047-5. [PubMed: 26381867]
79. Muller L , Tokay T , Porath K , Kohling R , Kirschstein T . Enhanced NMDA receptor-dependent LTP in the epileptic CA1 area via upregulation of NR2B. *Neurobiol Dis*. 2013;54:183–93. doi:10.1016/j.nbd.2012.12.011. [PubMed: 23313317]
80. Morris RG , Moser EI , Riedel G , Martin SJ , Sandin J , Day M et al. Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*. 2003;358(1432):773–86. doi:10.1098/rstb.2002.1264. [PubMed: 12744273]

81. Lenck-Santini PP , Scott RC . Mechanisms Responsible for Cognitive Impairment in Epilepsy. *Cold Spring Harb Perspect Med.* 2015;5(10). doi:10.1101/cshperspect.a022772.
82. Edbauer D , Neilson JR , Foster KA , Wang CF , Seeburg DP , Batterton MN et al. Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. *Neuron.* 2010;65(3):373–84. doi:10.1016/j.neuron.2010.01.005. [PubMed: 20159450]
83. Gross C , Hoffmann A , Bassell GJ , Berry-Kravis EM . Therapeutic Strategies in Fragile X Syndrome: From Bench to Bedside and Back. *Neurotherapeutics.* 2015;12(3):584–608. doi: 10.1007/s13311-015-0355-9. [PubMed: 25986746]
84. Alsharafi WA , Xiao B , Li J . MicroRNA-139–5p negatively regulates NR2A-containing NMDA receptor in the rat pilocarpine model and patients with temporal lobe epilepsy. *Epilepsia.* 2016;57(11):1931–40. doi:10.1111/epi.13568. [PubMed: 27731509] • The first direct link of a microRNA regulating NMDA receptors and epilepsy.
85. Swanger SA , Chen W , Wells G , Burger PB , Tankovic A , Bhattacharya S et al. Mechanistic Insight into NMDA Receptor Dysregulation by Rare Variants in the GluN2A and GluN2B Agonist Binding Domains. *Am J Hum Genet.* 2016;99(6):1261–80. doi:10.1016/j.ajhg.2016.10.002. [PubMed: 27839871]
86. Dalmau J , Lancaster E , Martinez-Hernandez E , Rosenfeld MR , Balice-Gordon R . Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol.* 2011;10(1):63–74. doi:10.1016/s1474-4422(10)70253-2. [PubMed: 21163445]
87. Wu C , Sun D . GABA receptors in brain development, function, and injury. *Metab Brain Dis.* 2015;30(2):367–79. doi:10.1007/s11011-014-9560-1. [PubMed: 24820774]
88. Jovasevic V , Corcoran KA , Leaderbrand K , Yamawaki N , Guede AL , Chen HJ et al. GABAergic mechanisms regulated by miR-33 encode state-dependent fear. *Nature neuroscience.* 2015;18(9):1265–71. doi:10.1038/nn.4084. [PubMed: 26280760]
89. Hu K , Xie YY , Zhang C , Ouyang DS , Long HY , Sun DN et al. MicroRNA expression profile of the hippocampus in a rat model of temporal lobe epilepsy and miR-34a-targeted neuroprotection against hippocampal neurone cell apoptosis post-status epilepticus. *BMC neuroscience.* 2012;13:115. doi:10.1186/1471-2202-13-115. [PubMed: 22998082]
90. Albert S , Stephanie S . Changing channels in pain and epilepsy: Exploiting ion channel gene therapy for disorders of neuronal hyperexcitability. *FEBS Letters.* 2015;589(14):1620–34. doi:doi: 10.1016/j.febslet.2015.05.004. [PubMed: 25979170]
91. Sakai A , Saitow F , Maruyama M , Miyake N , Miyake K , Shimada T et al. MicroRNA cluster miR-17–92 regulates multiple functionally related voltage-gated potassium channels in chronic neuropathic pain. *Nature communications.* 2017;8:16079. doi:10.1038/ncomms16079. • The study shows that microRNAs of the same family/gene cluster can regulate the same physiological outcome, namely A-type potassium currents, providing further insight into microRNA-induced silencing and suggesting similar mechanisms in epilepsy.
92. Concepcion CP , Bonetti C , Ventura A . The microRNA-17–92 family of microRNA clusters in development and disease. *Cancer journal (Sudbury, Mass).* 2012;18(3):262–7. doi:10.1097/PPO.0b013e318258b60a.
93. Raoof R , Jimenez-Mateos EM , Bauer S , Tackenberg B , Rosenow F , Lang J et al. Cerebrospinal fluid microRNAs are potential biomarkers of temporal lobe epilepsy and status epilepticus. *Scientific reports.* 2017;7(1):3328. doi:10.1038/s41598-017-02969-6. [PubMed: 28607431]
94. Shao J , Cao J , Wang J , Ren X , Su S , Li M et al. MicroRNA-30b regulates expression of the sodium channel Nav1.7 in nerve injury-induced neuropathic pain in the rat. *Mol Pain.* 2016;12. doi:10.1177/1744806916671523.
95. Singh NA , Pappas C , Dahle EJ , Claes LR , Pruess TH , De Jonghe P et al. A role of SCN9A in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. *PLoS Genet.* 2009;5(9):e1000649. doi:10.1371/journal.pgen.1000649. [PubMed: 19763161]
96. Sakai A , Saitow F , Miyake N , Miyake K , Shimada T , Suzuki H . miR-7a alleviates the maintenance of neuropathic pain through regulation of neuronal excitability. *Brain.* 2013;136(Pt 9):2738–50. doi:10.1093/brain/awt191. [PubMed: 23861446]
97. Baum L , Haerian BS , Ng HK , Wong VC , Ng PW , Lui CH et al. Case-control association study of polymorphisms in the voltage-gated sodium channel genes SCN1A, SCN2A, SCN3A, SCN1B,

- and SCN2B and epilepsy. *Hum Genet.* 2014;133(5):651–9. doi:10.1007/s00439-013-1405-1. [PubMed: 24337656]
98. Lu Y , Yu W , Xi Z , Xiao Z , Kou X , Wang XF . Mutational analysis of SCN2B, SCN3B and SCN4B in a large Chinese Han family with generalized tonic-clonic seizure. *Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology.* 2010;31(5):675–7. doi:10.1007/s10072-010-0390-6.
99. Chen C , Bharucha V , Chen Y , Westenbroek RE , Brown A , Malhotra JD et al. Reduced sodium channel density, altered voltage dependence of inactivation, and increased susceptibility to seizures in mice lacking sodium channel beta 2-subunits. *Proc Natl Acad Sci U S A.* 2002;99(26):17072–7. doi:10.1073/pnas.212638099. [PubMed: 12481039]
100. Sun LH , Yan ML , Hu XL , Peng LW , Che H , Bao YN et al. MicroRNA-9 induces defective trafficking of Nav1.1 and Nav1.2 by targeting Navbeta2 protein coding region in rat with chronic brain hypoperfusion. *Mol Neurodegener.* 2015;10:36. doi:10.1186/s13024-015-0032-9. [PubMed: 26259688]

Table 1:
Summary of ion channels directly regulated by microRNAs in the brain and in the peripheral nervous system and their implication in epilepsy.

Highlighted in bold are microRNAs that were shown to be consistently down-regulated in epilepsy models in a recent meta-analysis [•22].

Channel	MicroRNA	Reference	Role in epilepsy shown?
Kv1.1	miR-129-5p	[••26,••25]	yes, enhanced 3 weeks after seizure
Kv4.2	miR-324-5p	[•32]	yes, antagomir delays seizure onset
	miR-223-3p	[33]	no (<i>heart</i>)
	miR-301a	[34]	no (<i>heart</i>)
Multiple A-type channels	miR-17-92	[•91]	no, regulate A-type channels in chronic pain (<i>dorsal root ganglia</i>)
Nav1.1	miR-155	[39]	yes, downregulated by valproic acid
	multiple	[40]	yes, epilepsy-associated genetic variants in microRNA-binding sites
Nav1.7	miR-30b	[94]	no, involved in pain (<i>dorsal root ganglia</i>)
Navβ2	miR-9	[100]	no, involved in chronic cerebral ischemia
	miR-7a	[96]	no, plays a role in chronic pain (<i>dorsal root ganglia</i>) (downregulated in epilepsy models)
GluA1	miR-92a	[••49]	no, involved in homeostatic scaling
	miR-137	[•55, 56]	no, regulates mGluR-dependent LTD
	miR-501-3p	[••62]	no, regulates NMDA-dependent LTD
GluA2	miR-181a	[69, 68]	yes, overexpression reduces mEPSPs, but antagomir shown to reduce caspase activation in epilepsy model
	miR-223-3p	[•70, 71]	yes, deletion increases mEPSCs, downregulated in focal cortical dysplasia
	miR-124	[72–74]	yes, indirect: agomir suppresses seizure-induced gene expression, but upregulates neuroinflammation (no effect on seizure [••73]); another study showed seizure suppression with agomir [74] (no connection to GluA2 shown in the epilepsy studies)
GluN2A	miR-19	[78]	no, potential role in developmental GluN2A expression
	miR-125b	[82]	no, potentially involved in FXS
	miR-139-5p	[•84]	yes, overexpression prevents seizure-induced increase in GluN2A
GluN2B	miR-223-3p	[•70, 71]	yes, deletion increases mEPSCs, downregulated in focal cortical dysplasia
	miR-539	[78]	no, potential role in developmental GluN2B expression
GABA_A	miR-33	[88]	no, regulates fear memory (downregulated in epilepsy models)