Brain Stimulation 12 (2019) 1390–1401

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Electrical stimulation of the ventral hippocampal commissure delays experimental epilepsy and is associated with altered microRNA expression

Lara S. Costard [a, b, *], Valentin Neubert [a, b, 1], Morten T. Venø [c], Junyi Su [c], Jørgen Kjems [c], Niamh M.C. Connolly [d], Jochen H.M. Prehn [d], Gerhard Schratt [e], David C. Henshall [d, f], Felix Rosenow [a, b], Sebastian Bauer [a, b]

[a] Epilepsy Center Frankfurt Rhine-Main, Department of Neurology, University Hospital Frankfurt and Center for Personalized Translational Epilepsy Research (CePTER), Goethe University, Frankfurt, Germany
[b] Department of Neurology, Philipps University, Marburg, Germany
[c] Department of Molecular Biology and Genetics and Interdisciplinary Nanoscience Center, Aarhus University, Aarhus, Denmark
[d] Department of Physiology & Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland
[e] Lab of Systems Neuroscience, Institute for Neuroscience, Department of Health Science and Technology, ETH Zurich, Zurich, Switzerland
[f] FutureNeuro Research Centre, Royal College of Surgeons in Ireland, Dublin, D02, Ireland

ARTICLE INFO

Article history:
Received 18 January 2019
Received in revised form
18 April 2019
Accepted 5 June 2019
Available online 7 June 2019

Keywords:
microRNA
Mesial temporal lobe epilepsy
Hippocampal sclerosis
Deep brain stimulation
Ventral hippocampal commissure

ABSTRACT

Background: Up to 80% of mesial temporal lobe epilepsy patients with hippocampal sclerosis (mTLE-HS) are resistant to pharmacological treatment, often necessitating surgical resection. Deep brain stimulation (DBS) has emerged as an alternative treatment for patients who do not qualify for resective brain surgery. Brain stimulation may also exert disease-modifying effects, and noncoding microRNAs have recently been proposed to shape the gene expression landscape in epilepsy.
Objective: We compared the effect of DBS of 4 different hippocampal target regions on epileptogenesis and manifest epilepsy in a rat model of mTLE-HS. To explore mechanisms, we profiled the effect of the most effective DBS paradigm on hippocampal microRNA levels.
Methods: MTLE-HS was induced by electrical stimulation of the perforant pathway (PP) in rats. This paradigm leads to spontaneous seizures within 4 weeks. We investigated DBS of 4 targets: PP, fimbria fornix (FF) formation, dentate gyrus (DG) and ventral hippocampal commissure (VHC). We applied both high- (130 Hz) and low-frequency (5 Hz or 1 Hz) stimulation. Functional microRNAs were identified in the hippocampus immediately after VHC-DBS and after a 97-day recording period by sequencing small RNAs bound to Argonaute-2, a component of the miRNA silencing complex.
Results: Low frequency DBS of the VHC significantly delayed the occurrence of the first spontaneous recurrent seizure in the PPS model by ~300%, from 19 to 56 days. No other stimulation regime altered the latency phase. Upregulation of 5 microRNAs during epileptogenesis was suppressed by VHC-stimulation.
Conclusion: We conclude that DBS of the VHC delays epilepsy in the PPS model in rats and is associated with differential regulation of several miRNAs. Additional studies are required to determine whether VHC-regulated miRNAs serve causal roles in the anti-epileptogenic effects of this DBS model.

Introduction

Mesial temporal lobe epilepsy (mTLE) is the most common form of epilepsy that is referred to surgical centers [1]. Development of mTLE is frequently preceded by an initial precipitating injury (IPI) such as complicated febrile seizures or brain trauma [2]. The IPI triggers the process of epileptogenesis which, after a latency period that can last months to years, results in the appearance of

* Corresponding author. Epilepsy Center Frankfurt Rhine-Main, Department of Neurology, University Hospital Frankfurt and Center for Personalized Translational Epilepsy Research (CePTER), Goethe University, Frankfurt, Germany.
E-mail address: laracostard@rcsi.com (L.S. Costard).
[1] Present address: Oscar-Langendorff-Institute of Physiology, Rostock University Medical Center, Rostock, Germany.

https://doi.org/10.1016/j.brs.2019.06.009
1935-861X/

spontaneous recurrent seizures [3]. A common pattern of neuronal damage in mTLE is hippocampal sclerosis (HS), comprising various subtypes with neurodegeneration mainly in CA1, CA3 and the hilus of the dentate gyrus (DG) [4]. Hippocampal sclerosis has been found in the majority of mTLE patients who underwent resective epilepsy surgery [5].

Currently, no anti-epileptogenic therapy exists. Moreover, few mTLE-HS patients become seizure-free under treatment with antiepileptic drugs (AEDs) [6]. Resective epilepsy surgery is an appropriate therapy for pharmacoresistant mTLE-HS patients [7]. However, up to 50% of pharmacoresistant mTLE-HS patients do not qualify for resective surgery (e. g. due to bilateral epileptogenic zones) [8], and 33% of those who undergo surgery do not become permanently seizure free [9]. Electrical deep brain stimulation (DBS) is an alternative treatment option for these patients. Currently, DBS of the anterior nucleus of the thalamus is approved for treatment of pharmacoresistant epilepsy in the European Union and the USA, while responsive neurostimulation (which includes the possibility to stimulate the hippocampus) is approved in the USA only. Stimulation of hippocampal structures has anticonvulsive effects in TLE patients [10]. However, optimal DBS sites are still being explored and mechanisms of action are poorly understood. The increase in efficacy of brain stimulation over time [11] implicates molecular alterations. Indeed, an effect of DBS on gene expression has been recently demonstrated [12].

MicroRNAs (miRNAs) are small, single-stranded RNAs, consisting of 19–25 nucleotides. They regulate protein levels by binding to messenger RNAs (mRNAs), a process mediated by Argonaute (Ago) proteins, resulting in inhibition of translation or destabilization of the target mRNA [13]. It is estimated that at least half of all human genes are regulated by miRNAs [14–16]. Several miRNAs are involved in the pathogenesis of epilepsy, and *in vivo* manipulation of different miRNAs can influence development of seizures in animal models [17–19]. Recently, DBS of the subthalamic nucleus was reported to alter miRNA expression in blood leukocytes from Parkinson's disease patients [20]. Furthermore, miRNAs have been implicated in the neuroprotective mechanism of DBS of the fastigial nucleus in a model of focal cerebral ischemia/reperfusion in rats [21].

We hypothesized that DBS has anti-epileptogenic effects that are mediated in part by altered miRNA expression. To test this idea, we performed two experiments:

1) We tested the effect of various DBS paradigms on the development of epilepsy in a rat model of mTLE-HS. DBS was applied to different hippocampal structures: Fimbria/fornix (FF), perforant pathway (PP), dentate gyrus (DG), or ventral hippocampal commissure (VHC), respectively, with either high (HF, 130 Hz) or low (LF, 5 Hz or 1 Hz) frequency.
2) We analysed effects of select DBS paradigms on Ago-loaded hippocampal miRNAs.

Material and methods

We used a toxin free rat model of mTLE-HS after electrical PP stimulation (PPS) that has been described in detail in Ref. [22]. Key features of the model are a latency period of 2–3 weeks between PPS and the occurrence of the first spontaneous seizure, and the development of the most frequent HS subtype, i. e. ILAE type 1, which comprises hippocampal neuron loss predominantly in CA1 and CA4 [23].

Animals

Animal experiments were approved by the local regulation authority (Regierungspraesidium Giessen) and were conducted in accordance with the with the Directive 2010/63/EU.

Adult male Sprague–Dawley rats (320–450 g) were used in all experiments. Rats were housed in single cages to avoid damage of implants by littermates. Animals had free access to standard pellet food and water. A light/dark cycle of 12 h/12 h was maintained.

Experimental procedures

Surgery

The surgical procedures are described in detail in Ref. [24]. In short, an oval part of the scalp was removed to expose the skull. Drill holes were prepared for electrode implantation and 4 fixing screws. An EEG transmitter (A3028R-FB, Open Source Instruments, Inc., Watertown, MA, USA) was implanted into a skin pouch prepared at the left abdominal site of the rat. Stimulation electrodes (diameter 0.125 mm, Plastics One, Roanoke, VA, USA) were implanted bilaterally into the PP and either the FF or DG, or unilaterally into the VHC. Recording electrodes (diameter 0.25 mm, Plastics One, Roanoke, VA, USA) were implanted bilaterally into the DG. Electrode placement was performed according to the coordinates given in Table 1. After fine-positioning of the electrodes as described in the next paragraph, electrodes were fixed with dental acrylic cement and placed in a pedestal, which was fixed on the skull.

Evoked potentials

To adjust the optimal dorso-ventral (DV) positioning of recording and stimulation electrodes, stimuli of 20 V at 0.5 Hz were applied via the stimulation electrodes during implantation. Evoked potentials were recorded from the DG. Electrode placement of PP electrodes was performed as described in Ref. [25]. For VHC and FF stimulation in rats, evoked potentials were newly established. Electrodes were placed according to coordinates from a rat brain atlas [26] and marked by stimulation with 3 direct current stimuli with 50 V over 30 s, causing local burn marks. Electrode tip positions were validated after transcardial perfusion of the animal with 0.9% NaCl and subsequent fixation perfusion with 4% PFA by preparing 100 μm slices on a cryotome. During surgery, average evoked potentials of 5 pulses were recorded from the DG while stimulating with 10 V and 0.5 Hz and used as reference potentials for subsequent surgeries.

Induction of epileptogenesis by PPS

All stimulations were performed with a stimulus generator (Grass S88, Grass Telefactor/Grass Instruments, USA) and a stimulus isolator (Model SIU5, Grass Instruments, USA).

After surgery, a 7-day recovery phase was allowed before bilateral PPS was applied on 2 consecutive days for 30 min and on the following day for 8 h. The 30 min stimulations on days 1 and 2 induce a state of epileptic tolerance which allows application of 8 h PPS on day 3 without causing self-sustaining status epilepticus or mortality [22]. According to the stimulation paradigm, we will subsequently refer to the model as the 30-30-8-PPS model. The stimulation was performed with the following parameters: pulse duration 0.1 ms, frequency 2 Hz, voltage 20 V, twin pulses with interpulse interval of 40 ms, additional trains of 20 Hz single pulses at 20 V applied once per minute for 10 s [22].

Long-term DBS

High frequency stimulation was performed continuously for 1 week with 130 Hz and 0.1 V based on evidence from Ref. [27]. Low

Table 1
Coordinates for implantation of stimulation and recording electrodes.

Stimulation target	anterior-posterior (AP)	medial-lateral (ML) [mm to sagittal suture]	dorsal-ventral (DV) [mm to skull surface]
DG (recording)	−3 mm of Bregma	±2	≈4.5
DG (stimulation)	−5.2 mm of Bregma	±3	≈4.5
PP (stimulation)	rostral of Lambda	±4.5	≈3.5
FF (stimulation)	−1.6 mm of Bregma	±0.8	≈4.9
VHC (stimulation)	−1.5 mm of Bregma	+0.15	≈4.2

frequency stimulation of FF, DG and PP was performed continuously with 5 Hz and 2 V for 1 week based on evidence from a fornix stimulation trial in humans with mTLE [28]. Low frequency stimulation of the VHC was performed continuously with 1 Hz and 1 V for 2 weeks based on evidence from Ref. [29].

For DBS of the respective target, rats were connected to a swivel commutator and could move freely in their cages. Stimulation was performed with single pulses, biphasic square waves, 0.1 ms pulse duration.

Control animals were implanted identically with a transmitter and electrodes and also connected to the stimulator via a swivel commutator for corresponding periods (1 or 2 weeks, respectively) but were not stimulated.

Continuous video EEG monitoring

Video and EEG were recorded continuously for up to 97 days. The recording period was chosen because of the maximal predicted battery life of the transmitters (3 months) and the additional 7 d period between implantation and PPS. Data from previous experiments with the same animal model have shown that the recording period if sufficient to evaluate seizure onset and frequency. EEG recordings were performed with an Octal Data Receiver (A3027, Open Source Instruments, Inc., Watertown, MA, USA) with a sampling rate of 512/s. Data were recorded in NDF (Neuroscience Data Format) and converted to EDF (European Data Format) for analysis with EDFbrowser (version 1.57+). Video recording was performed with infrared cameras (IC-7110W, Edimax Technology, Willich, Germany) and sampled with the SecuritySpy software (Ben Software Ltd., London, UK).

The total EEG of all rats was screened visually for appearance of seizure patterns by two experienced reviewers (LC, VN). In accordance with clinical practice in epileptology, seizure patterns were defined as rhythmic activity of at least 10 s which clearly broke background activity, contained epochs of high frequency spikes or spike-wave-complexes, and showed an evolution in frequency and amplitude [30]. Video was used to clarify appearance of artefacts (e.g. chewing, scratching).

Group size calculations

Group size calculations were performed using two-sided t-tests ($\alpha = 0.05$, power 0.8) with data for seizure frequencies and duration of the latency period from Ref. [22]. For the outcome parameter "duration of latency period", group size was $n = 6$, while for outcome parameter "seizure frequency", group size was $n = 5$. For ethical reasons, groups were not completed if it was obvious in the first 2 or 3 animals that there were adverse rather than beneficial effects on the outcome parameter of the applied DBS. The final actual group sizes are listed in the next section.

Experimental groups

In experiment 1, we tested HF-DBS and LF-DBS of various hippocampal targets (PP, DG, FF, VHC). All animals were implanted with PP stimulation electrodes (in order to induce mTLE-HS) and DG recording electrodes (in order to record long term EEG). In all animals (experimental and controls), mTLE was induced by 30-30-8 PPS as described above. Animals in the respective groups were implanted with additional stimulation electrodes in the DG, FF or VHC, respectively (Table 1). Control animals were implanted identically but were not stimulated.

Group identifiers (Table 2) consist of abbreviations for

- the time of DBS: During epileptogenesis (EPG) or manifest epilepsy (ME)
- the DBS target: FF, PP, DG, or VHC
- the stimulation frequency (HF or LF)
- the time point at which the animal was sacrificed (14 d or 97 d).

Experiment 2

After identification of low frequency stimulation of the VHC (VHC-LFS) as anti-epileptogenic, two groups were added to assess immediate effects of 2 weeks of VHC-LFS (Table 3). These animals were killed 14 d after PPS, i. e. immediately after the end of VHC-LFS (if applied). In order to control for the effects of acute symptomatic seizures (as compared to unprovoked spontaneous seizures in epilepsy) and for the mere effect of PPS (as compared to epileptogenesis induced by PPS), we included two additional control groups: One group was implanted following the usual protocol and developed acute symptomatic seizures within a few days after implantation, but did not receive PPS, and no spontaneous seizures (i.e. no epilepsy) occurred later (Ctrl-noPPS-97d). Another group received *per-protocol* PPS but did not develop epilepsy (Ctrl-no-seizure-97d).

Experimental course

After implantation of electrodes and EEG transmitters, rats were allowed to recover for 7 days, before 30-30-8 PPS was applied. To evaluate a possible anti-epileptogenic effect of DBS, stimulation was started one day after the final PPS (i. e. during epileptogenesis) and continued for 7 or 14 days, respectively (Fig. 1, above). To test an anticonvulsive or disease modifying effect, DBS was started 1–3 days after the first spontaneous seizure (i.e. during manifest epilepsy; Fig. 1, below). EEG was recorded for 3 months after DBS. Subsequently, rats were transcardially perfused with 0.9% NaCl. Hippocampi were removed on ice and snap frozen at −80 °C until further use.

MiRNA sequencing

Argonaute 2-immunoprecipitation (Ago2 IP) and small-RNA sequencing were performed in 3 randomly chosen animals per group as previously described in Ref. [18]. For the additional groups of rats perused 14 d after PPS all 6 rats were included for sequencing. Ago2-IP was performed using Ago2-antibody (Cell Signaling, Cat. #2897) and protein A/G beads (Santa Cruz Biotechnology). After Trizol/chloroform extraction of the

Table 2
Treatment and control groups for experiment 1.

Group identifier	n	DBS applied during	DBS target	DBS frequency	Time point of transcardial perfusion
EPG-FF-LF-97d	6	Epileptogenesis	FF	5 Hz	97 d
EPG-FF-HF-97d	3			130 Hz	97 d
EPG-PP-LF-97d	5		PP	5 Hz	97 d
EPG-PP-HF-97d	4			130 Hz	97 d
EPG-DG-LF-97d	2		DG	5 Hz	97 d
EPG-DG-HF-97d	2			130 Hz	97 d
EPG-VHC-LF-97d	6		VHC	1 Hz	97 d
ME-FF-LF-97d	5	Manifest epilepsy	FF	5 Hz	97 d
ME-FF-HF-97d	3			130 Hz	97 d
ME-PP-LF-97d	3		PP	5 Hz	97 d
ME-PP-HF-97d	2			130 Hz	97 d
ME-DG-LF-97d	2		DG	5 Hz	97 d
ME-DG-HF-97d	2			130 Hz	97 d
ME-VHC-LF-97d	5		VHC	1 Hz	97 d
Ctrl-FF-97d	5	n/a	n/a	n/a	97 d
Ctrl-PP-97d	5				97 d
Ctrl-VHC-97d	4				97 d

EPG = epileptogenesis, ME = manifest epilepsy. Ctrl = control. DG = dentate gyrus, FF = fimbria/fornix, PP = perforant pathway, VHC = ventral hippocampal commissure. HF = high frequency, LF = low frequency. 14d: Animal was sacrificed 14 d after induction of epilepsy by PPS; 97d: Animal was sacrificed 97 d after induction of epilepsy by PPS (EPG groups) or 97 d after begin of DBS (ME groups).

Table 3
Additional treatment groups for experiment 2.

Group identifier	n	DBS applied during	DBS target	DBS frequency	Time point of transcardial perfusion
LF-VHC-14d	6	Epileptogenesis	VHC	1 Hz	14 d
Ctrl-VHC-14d	6	n/a	n/a	n/a	14 d
Ctrl-noPPS-97d	3	n/a	n/a	n/a	97 d
Ctrl-no-seizure-97d	3	n/a	n/a	n/a	97 d

For abbreviations, see legend of Table 2.

immunoprecipitated RNA the TruSeq Small RNA Sample Prep Kit (Illumina) was used to prepare sequencing libraries, reducing the all kit reagents to half and increasing PCR cycle number to 15 to accommodate low RNA input. After sequencing, FastX toolkit and cutadapt were used to quality filter and adapter trim the sequencing reads. Filtered data was used to profile mature miRNA sequences from miRBase v21 allowing zero mismatches except for removal of 3'end A and T nucleotides.

Target prediction

Predicted targets (hsa, rno and mmu) for miRNA-129-5p, miRNA-379-3p, miRNA-410-3p, miRNA-431 and miRNA-433-3p were retrieved from TargetScan Release 7.1 [31] and miRDB [32]. To focus on human and rat interactions, we removed targets predicted only in mouse. To calculate a score for predicted miRNA-target interactions (MTIs) (Pscore) as reported previously [33], we first scaled the scores generated by the prediction algorithms (TargetScan's cumulative weighted context score) between 0 and 1 and summed these scores. We multiplied this sum by the number of algorithms in which the MTI was predicted (1 or 2), and finally scaled this between 0 and 1. For visualisation purposes, we further filtered the MTIs based on the following criteria: MTIs were retained if they had a Pscore $\geq$0.75 in either rat or human or if the sum of these Pscores $\geq$1. If any targets included in this filtered list were also targeted by another miRNA, this second MTI was also retained (provided the sum of its Pscores $\geq$0.3), to investigate the interconnectedness between the five miRNAs. 159 MTIs with 128 unique targets were visualised using a Chord diagram, which was generated using the 'circlize' package in R version 3.5.1 [34]. The width of each link corresponds to the sum of the rat and human Pscores for that MTI. Code is available on request. Transcription factors were identified from AnimalTFDB [35].

Statistical analysis

Duration of the latency period and seizure frequencies are provided as means ± standard deviations (text) and as box plots with median and quartiles (figures). For comparison of latency periods and seizure frequency with the respective control groups, the Mann-Whitney-U test was used.

The miRNA expression values obtained by Ago2-IP miRNA sequencing were normalized as reads per million. For each sample individual miRNA read numbers were divided by the total number of miRNA mapping reads in the sample and multiplied by 1 million. This was done to remove differences caused by different number of reads in the various samples. Statistical significance was tested using analysis of variance (ANOVA) using aov in R with false discovery rate (FDR) calculated using the Benjamini-Hochberg procedure using p.adjust in R. Boxplots show median, quartiles and min/max.

Results

Low frequency stimulation of the VHC delays epilepsy

The aim of the first experiment was to investigate possible anti-epileptogenic and anti-epileptic effects of DBS of 4 different hippocampal targets in a rat model of mTLE. Low frequency DBS of the VHC had an anti-epileptogenic effect, increasing the latency period from 19 ± 11 to 56 ± 23 days ($p = 0.004$; Fig. 2). None of the other DBS paradigms significantly altered the latency period.

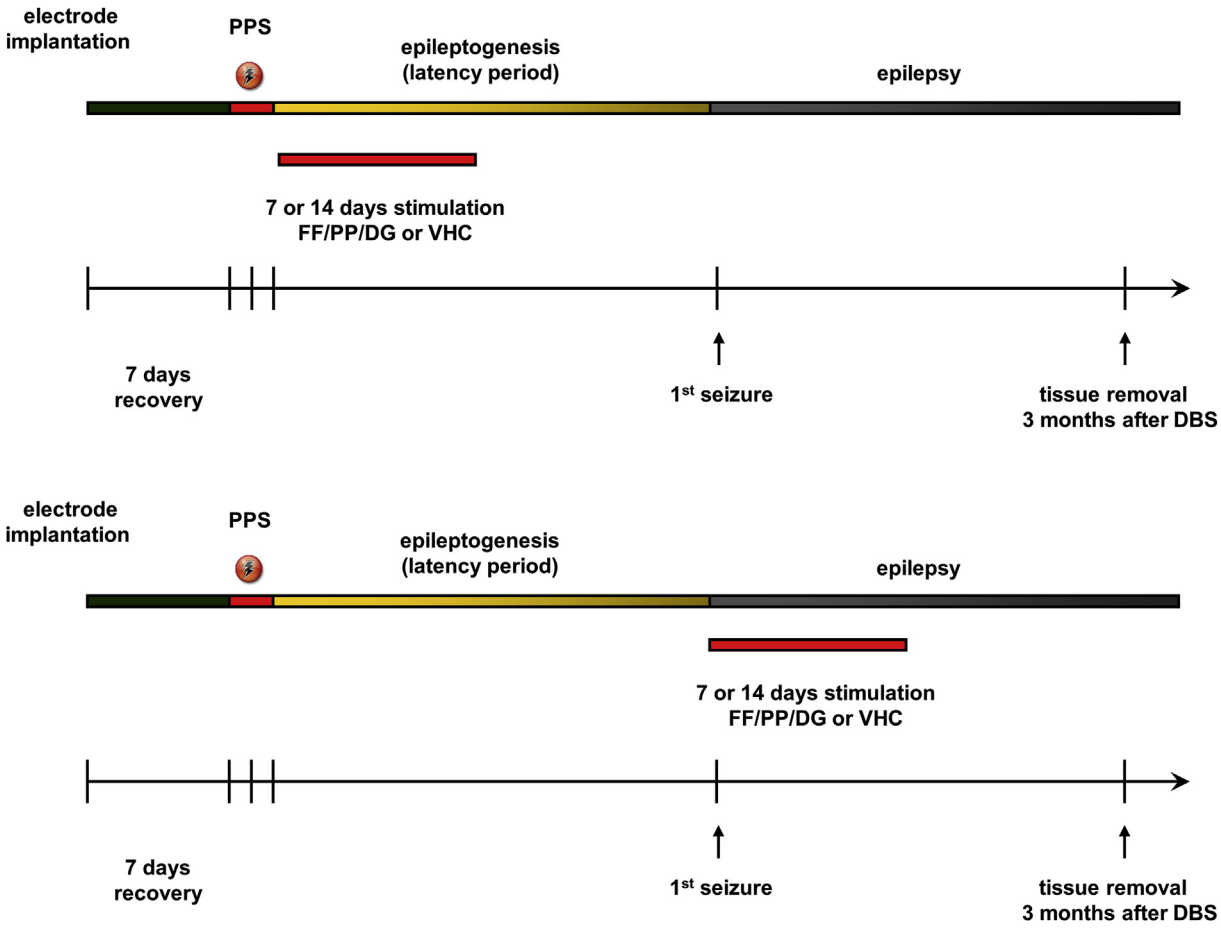

Fig. 1. Experimental course of experiment 1 to validate the anti-epileptogenic (above) and anticonvulsive (below) effect of DBS. Seven days after electrode 30-30-8 PPS was applied. DBS of one out of 4 targets was applied either starting one day after PPS (to test for anti-epileptogenic effects) or 1–3 days after the first spontaneous seizure (to test for anti-epileptic effects).

Application of the tested stimulation paradigms during manifest epilepsy (ME) did not significantly alter seizure frequency measured during or after DBS as compared to PP/FF/DG controls (Fig. 3). We found a significantly lower seizure frequency in ME-VHC-LF-97d (0.3 ± 0.2 seizures/d) compared to the matching control group (Ctrl-VHC-97d; 1.3 ± 0.7 seizures/d, $p = 0.028$). However, this is likely caused by an unusually high seizure frequency in the Ctrl-VHC-97d group as compared to other control rats without VHC electrodes (Ctrl-PP-97d: 0.3 ± 0.3 seizures/d; Ctrl-FF-97d: 0.4 ± 0.1 seizures/d).

Epileptogenesis is associated with select alterations of miRNA expression

The effect of PPS on functional (Ago-loaded) miRNA has not previously been explored. In the 6 experimental groups, RNAseq analysis of the hippocampus of rats subjected to PPS detected 200 hippocampal miRNAs with an expression of >100 rpm. This represents approximately half of all annotated rat miRNAs [36].

Twenty-four miRNAs were differentially regulated between epileptogenesis (14 d after induction of epilepsy) and manifest epilepsy (97 d after induction of epilepsy, Table 4). Immediately after 14 d of VHC-LFS, 8 miRNAs were differentially expressed as compared to non-VHC-stimulated controls. About 3 months after 14 d of VHC-LFS, 15 miRNAs were differentially regulated between VHC-stimulated animals and controls.

A principle component analysis (PCA) shows clustering of the respective treatment groups (Fig. 4). Furthermore, later time points group together and are separated from rats that were either perfused early, did not receive PPS or did not develop spontaneous seizures. This pattern suggests an effect of epileptogenesis on miRNA expression.

Upregulation of 5 miRNAs was suppressed by VHC stimulation

VHC-LFS was found to block changes to a set of five miRNAs (129-5p, 410-3p 431, 433-3p and 379-3p) normally upregulated during epileptogenesis (for relative changes see Fig. 5, absolute expression is shown in Fig. 6). Hippocampal miRNA expression data from the same animal model during and after epileptogenesis confirmed the pathological upregulation of miRNA-129-5p and miRNA-433-3p in manifest epilepsy, whereas miRNA-379-3p, miRNA-410-3p and miRNA-431 were not significantly regulated in manifest epilepsy (unpublished data).

MiRNAs suppressed by VHC stimulation are predicted to regulate N-glycan biosynthesis and growth factor signalling

Pathway enrichment analysis (miRPath v.3 [37]) of miRNAs 129-5p, 379-3p, 410-3p, 431 and 433-3p (Table 5) suggested that these miRNAs may be involved in the regulation of N-Glycan

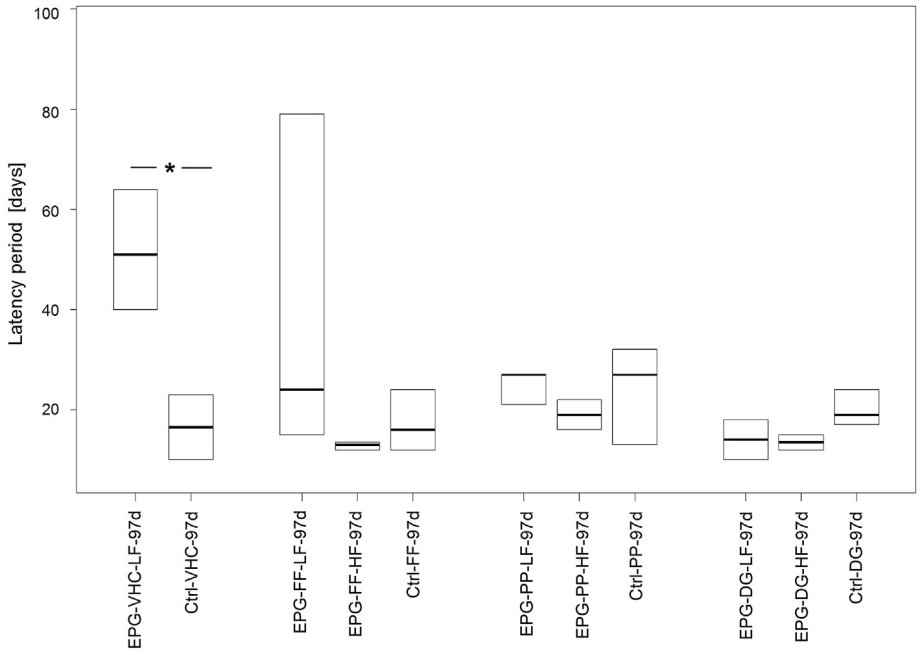

Fig. 2. Duration of the latency period after stimulation during epileptogenesis (EPG). Boxplots show median and quartiles. The latency period did not differ between control groups. Low frequency DBS of the VHC (EPG-VHC-LF-97d) significantly prolonged the average latency period (*p = 0.004). The other investigated DBS paradigms did not alter the latency period. EPG = epileptogenesis, VHC = ventral hippocampal commissure, Ctrl = control, LF = low frequency, FF = fimbria/fornix, HF = high frequency, PP = perforant pathway, DG = dentate gyrus.

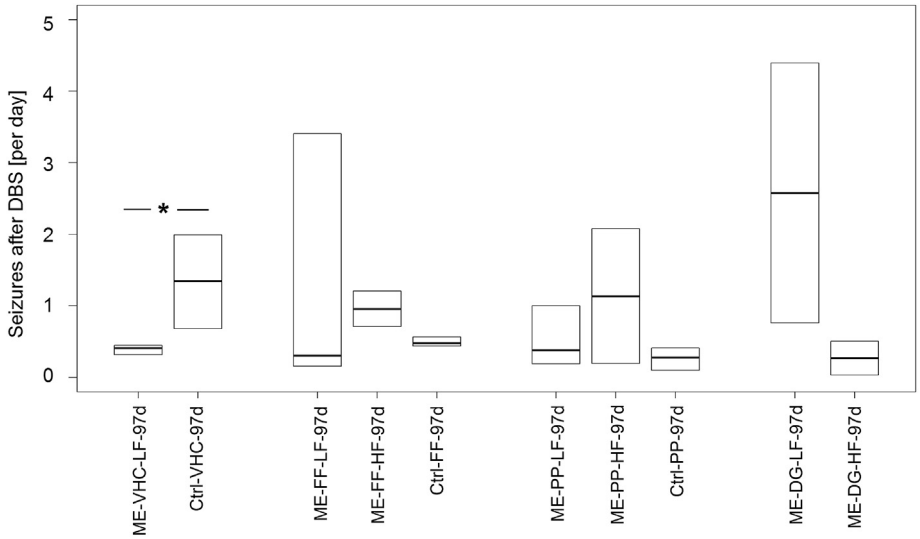

Fig. 3. Seizure frequency after stimulation during manifest epilepsy. Boxplots show median and quartiles. Seizure duration was significantly lower after 14 d of VHC LFS (ME-VHC-LF-97d) as compared to animals which were implanted with VHC electrodes but not VHC stimulated (Ctrl-VHC-97d, *p = 0.028), but not compared to control groups without VHC electrodes (Ctrl-FF-97d, Ctrl-PP-97d). EPG = epileptogenesis, VHC = ventral hippocampal commissure, Ctrl = control, LF = low frequency, FF = fimbria/fornix, HF = high frequency, PP = perforant pathway, DG = dentate gyrus.

biosynthesis, MAPK and Ras signaling pathways, and pathways associated with amphetamine addiction.

To further investigate the functional role of these miRNAs, we analysed their predicted gene targets and derived a score for each interaction from TargetScan [31] and miRDB [32] prediction algorithms (see Methods). The top-scoring predicted targets of miRNAs 129-5p, 379-3p, 410-3p, 431 and 433-3p are shown in Fig. 7, all of which are expressed in the brain (Human protein atlas, https://www.proteinatlas.org/humanproteome/brain; [37]).

Discussion

The present study demonstrates for the first time that DBS of the VHC can delay the development of epilepsy in a rat model. We also show that DBS produces a highly specific regulation of a set of miRNAs including miRNAs previously implicated in seizure control as well as novel miRNAs not previously associated with epilepsy. Together, the findings support important roles for DBS in modulation of epileptogenesis and identify potential molecular mechanisms.

Table 4
Significant relative miRNA changes in epileptogenesis and VHC-LFS.

Significant regulation between epileptogenesis and manifest epilepsy		Significant regulation immediately after VHC-DBS		Significant regulation ≈3 months after VHC-DBS	
Downregulated in Ctrl-VHC-97 d vs.Ctrl-VHC-14d		Downregulated in LF-VHC-14d vs. Ctrl-VHC-14d		Downregulated in EPG-LF-VHC-97d vs. Ctrl-VHC-97d	
miRNA	relative change	miRNA	relative change	miRNA	relative change
187-3p	−54%	129-2-3p	−45%	132-3p	−51%
129-2-3p	−38%	24-3p	−27%	431	−44%
3085	−37%	let-7b-5p	−20%	410-3p	−39%
24-3p	−30%			129-5p	−38%
191a-5p	−28%			341	−37%
326-3p	−27%			369-3p	−33%
148b-3p	−26%			337-5p	−31%
103-3p	−17%			487b-3p	−30%
				136-5p	−30%
				323-3p	−29%
				433-3p	−28%
				379-3p	−28%
				181a-1-3p	−22%
Upregulated in Ctrl-VHC-97 d vs. Ctrl-VHC-14d		**Upregulated in LF-VHC-14d vs. Ctrl-VHC-14d**		**Upregulated in EPG-LF-VHC-97d vs.Ctrl-VHC-97d**	
miRNA	**relative change**	**miRNA**	**relative change**	**miRNA**	**relative change**
485-3p	129%	181a-1-3p	28%	let-7a-5p	16%
136-5p	122%	let-7d-3p	27%	let-7f-5p	14%
379-3p	73%	let-7e-5p	19%		
1224	72%	let-7d-5p	19%		
132-3p	62%	let-7a-1-3p	18%		
181a-1-3p	62%				
410-3p	59%				
129-5p	59%				
431	50%				
let-7d-3p	48%				
150-5p	46%				
487b-3p	34%				
27b-5p	32%				
433-3p	27%				
101a-3p	26%				
let-7c-3-3p	24%				
let-7c-3-3p	24%				

P-values are calculated as described by [64].

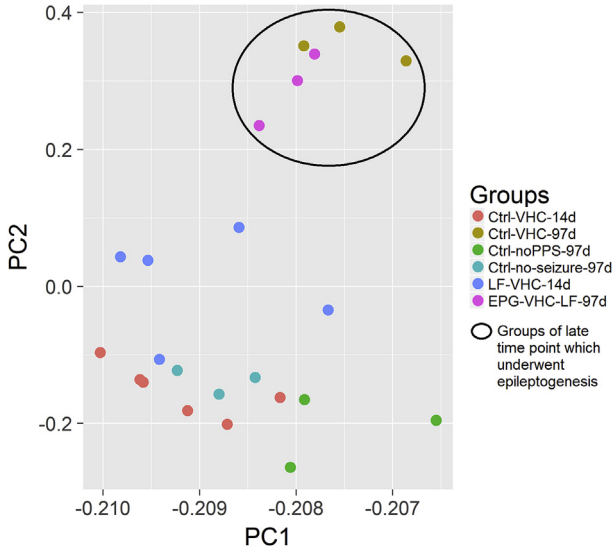

Fig. 4. PCA of miRNA expression data from LF-VHC-stimulated animals at 2 time points (14 d or 97 d after induction of epilepsy) and 4 control groups. The PCA shows clustering of samples from similar groups and a separation of the late time point groups which underwent epileptogenesis (circle) from the early time point groups and non-epileptic control groups (Ctrl-noPPS and Ctrl-no-seizures).

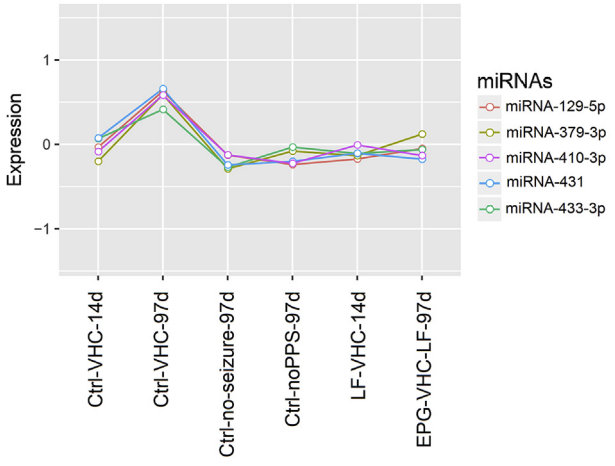

Fig. 5. Expression pattern of 5 miRNAs (miRNA-129-5p, 410-3p 431, 433-3p and 379-3p) which were upregulated in manifest epilepsy and whose upregulation was prevented by VHC-LFS. MiRNAs were grouped according to similar expression patterns by cluster analysis calculating the Euclidean distance between clusters. Expression values are normalized to have a mean expression value of 0. Expression of all 5 miRNAs is higher in the Ctrl-VHC-97d group compared to all other groups (Ctrl-VHC-14d, Ctrl-no-seizure, Ctrl-noPPS, LF-VHC-14d, EPG-VHC-LF-97d).

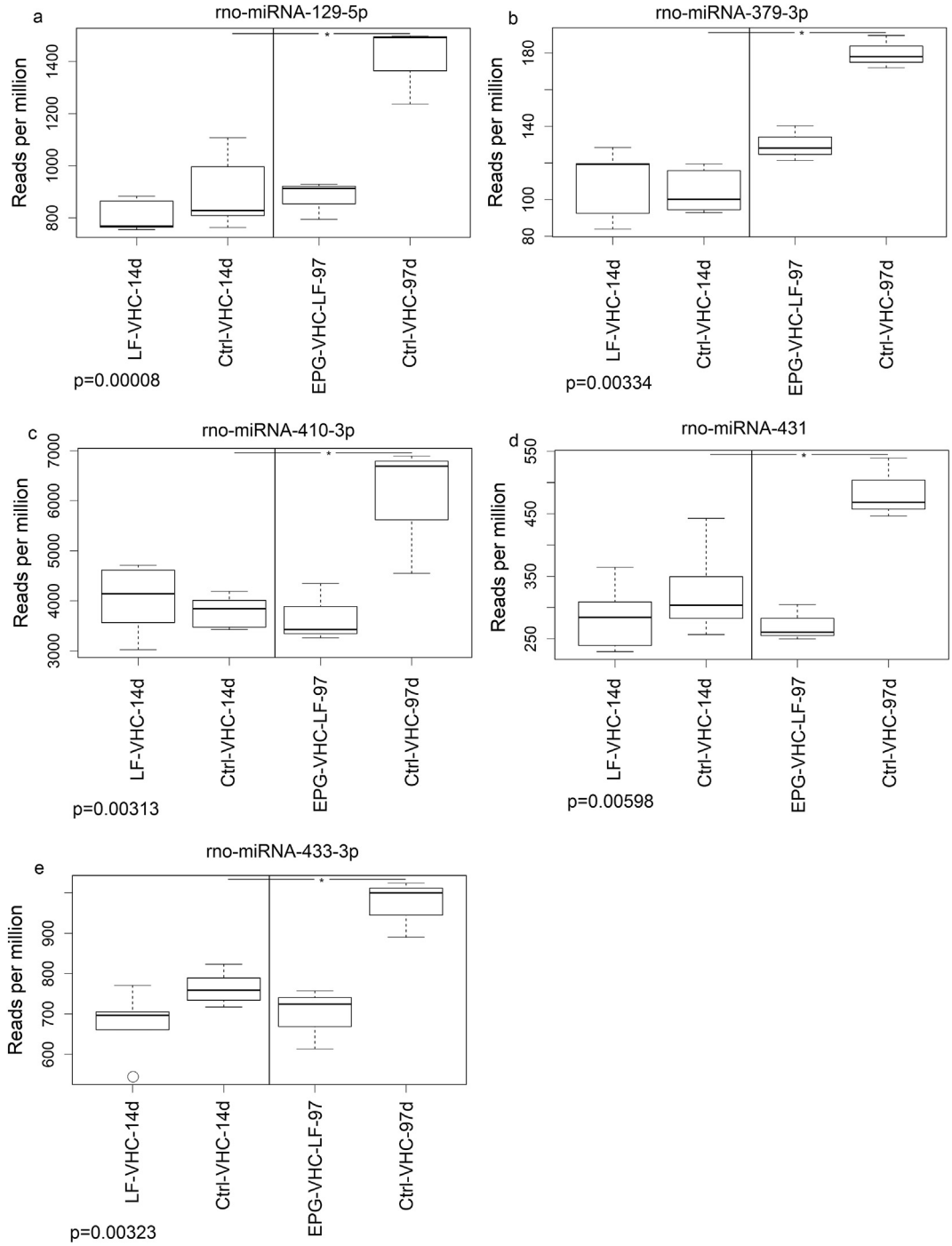

Fig. 6. Quantitative expression profiles of the 5 miRNAs from Fig. 5. All 5 miRNAs were significantly upregulated during epileptogenesis in Ctrl-VHC-97d compared to Ctrl-VHC-14d animals. The upregulation was prevented by VHC-LFS (LF-VHC-14d vs. EPG-VHC-LF-97d). Boxplots show median and quartiles, whiskers indicate minimum and maximum.

Effects of DBS on epileptogenesis and manifest epilepsy

Low frequency DBS of the VHC significantly prolonged the latency period and can therefore be considered anti-epileptogenic. To our knowledge, an anti-epileptogenic effect of VHC stimulation has not yet been validated. Various DBS paradigms targeting hippocampal structures or the amygdala have been shown to modify the epileptogenesis process [38–43]. However, only a few possible underlying mechanisms such as increased binding of ligands to benzodiazepine receptors [40] or activation of adenosine receptors [41] have been discussed.

Low frequency DBS of the VHC has previously been shown to produce anticonvulsive effects in a rat model of manifest epilepsy induced by amygdala stimulation, and in a genetic mouse model of epilepsy [29,44]. In accordance with these data, we found a significantly lower seizure frequency compared to control animals with implanted but unstimulated VHC electrodes. However, no significant difference in seizure frequency appeared between VHC stimulated rats and control groups without VHC electrodes. We

Table 5
Pathway enrichment analysis of chronically regulated miRNAs 129-5p, 379-3p, 410-3p, 431 and 433-3p.

Pathway	p-value	miRNAs	Predicted mRNAs
N-Glycan biosynthesis	0.00003	miRNA 129-5p	Man1a1
		miRNA 431	Man1a2
		miRNA 410-3p	Man1c1
			ALG12
MAPK signaling pathway	0.00007	miRNA 410-3p	Ntrk2, sos1, Nlk, Dusp2, Nr4a1, Ntf3, Rap1a, Lamtor3, Pdgfra, Fgfr2
		miRNA 431	Tab1, Rapgef2
		miRNA 129-5p	Prkx, Map4k4, Dusp10, c-Fos, Fgf13, Cacng2, ELK4, ETS Elk4, Tab 2
		miRNA 379-3p	Dusp10, Ppp3r1, Dusp8
		miRNA 433-3p	Rps6ka5
Amphetamine addiction	0.00023	miRNA 129-5p	Prkx, Fos, Valm1, Creb5, Gria2, Slc18a2
		miRNA 410-3p	Creb5, Hdac1, Gria2
		miRNA 379-3p	Ppp3r1
Ras signaling pathway	0.00554	miRNA 410-3p	Sos1, Vegfa, Ets1, RAP1A, Gng5, Pdgfra, Fgfr2
		miRNA 129-5p	Prkx, Ets1, Igf1, Kitlg, Fgf13, Calm1, Ralbp1, Pak7, Plce1
		miRNA 433-3p	Pak 4
		miRNA 431	RAB5B, Rab5b

assume that the implantation of VHC electrodes itself might be proconvulsive and that this effect is abrogated by electrical stimulation of the implanted VHC electrodes.

Five Hz FF stimulation prolonged the latency period in some rats, but latency was very variable in this group. The FF is a relatively small structure, and implantation of stimulation electrodes into the FF is a newly established method. We verified implantation coordinates in additional animals, but this was not possible in animals that were used for miRNA sequencing. Hence, imperfect placement of the electrodes cannot be excluded with absolute confidence and might have contributed to deviations in the results. There was no association between latency and age of rats at the time of surgery, size of population spikes during PP electrode implantation or onset of acute symptomatic seizures.

Direct translation of the anti-epileptogenic effect of VHC stimulation into clinical medicine poses several difficulties: Reliable biomarkers of human epileptogenesis are currently not established. Furthermore, compared to rodents, the VHC virtually disappeared during human phylogeny [45]. The dorsal hippocampal commissure, is preserved in humans, and its electrical stimulation attenuated interictal and ictal EEG activity in epileptic humans; however, the effect was rather attributed to co-stimulation of the nearby fornix [29]. Non-invasive brain stimulation paradigms such as transcutaneous vagal nerve stimulation or transcranial direct current stimulation are anticonvulsive and have few side effects [46–48] and are therefore applicable in patients at high risk for epileptogenesis. If the molecular alterations induced by VHC stimulation could be replicated by non-invasive stimulation techniques, this could prepare the ground for translation of anti-epileptogenesis into clinical use.

More importantly, our results raise the possibility of indirect translation. Rather than exerting anti-epileptogenic effects by stimulating the brain of humans at risk for epileptogenesis, it might be possible to replicate the anti-epileptogenic effects of VHC stimulation by direct manipulation of miRNAs that were altered by VHC stimulation, overcoming anatomical differences in the brains of humans and rats.

MiRNA regulation after VHC-LFS

MiRNAs 129-5p, 379-3p, 410-3p, 431 and 433-3p were upregulated in control animals with manifest epilepsy. This upregulation was prevented by VHC-LFS.

The five miRNAs were not regulated in animals that experienced acute symptomatic seizures shortly after electrode implantation but did not develop epilepsy later. Hence, we assume that regulation of these miRNAs is not simply seizure-induced but rather associated with actual epileptogenesis.

The role of miRNA-129-5p in neuronal excitability and in TLE has been investigated extensively. MiRNA-129-5p was upregulated in pilocarpine-induced epilepsy in mice [49], during early epileptogenesis and in manifest epilepsy in the 30-30-8-PPS model, and in resected hippocampal tissue from mTLE patients [18,50]. Antagonization of miRNA-129-5p prior to intra-amygdala kainic acid-induced status epilepticus had an anticonvulsive effect [18]. Prevention of the pathological upregulation of miRNA-129-5p by VHC-LFS might contribute to the anti-epileptogenic effect of this DBS paradigm. Possible mechanisms involved in the action of miRNA-129-5p comprise regulation of the potassium channel Kv1.1 [51] and synaptic downscaling, which might have a maladaptive role in epileptogenesis and was completely prevented by antagonising miRNA-129-5p [18]. The latter process involved regulation of the miRNA-129-5p targets Atp2b4 (a calcium pump) and doublecortin.

Upregulation of miRNA-433-3p was described in a pilocarpine, PPS and amygdala-stimulation model in rats [49,52,53]. On the other hand, miRNA-433-3p was downregulated after acute seizures induced by 6 Hz stimulation in mice [54], during epileptogenesis in a pilocarpine model [54,55] and in hippocampal tissue from TLE patients compared to autopsy control tissue [56]. Thus, the role of miRNA-433-3p in epileptogenesis and epilepsy is less clear than the role of miRNA-129-5p.

Conflicting results have been reported for miRNA-379-3p (upregulation in the present study, downregulation during epileptogenesis in a pilocarpine model [55]) and for miRNA-410-3p (upregulation in chronic epilepsy after pilocarpine-induced SE [49], but not in earlier data from the 30-30-8-PPS model (unpublished data)). MiRNA-379-3p and miRNA-410-3p both belong to the miRNA-379-410 cluster, which also includes miRNA-134-5p. MiRNA-134-5p influences the shape of dendritic spines [57]. Furthermore, silencing of miRNA-134-5p was anticonvulsive after induced status epilepticus in mice [58] and reduced induced epileptiform activity in brain slices [59].

For miRNA-431, upregulation during epileptogenesis was found in pilocarpine models of rats [49] and mice [54], as well as in a mouse kainate model [60]. These findings are consistent with our results. However, overall hippocampal expression levels of miRNAs

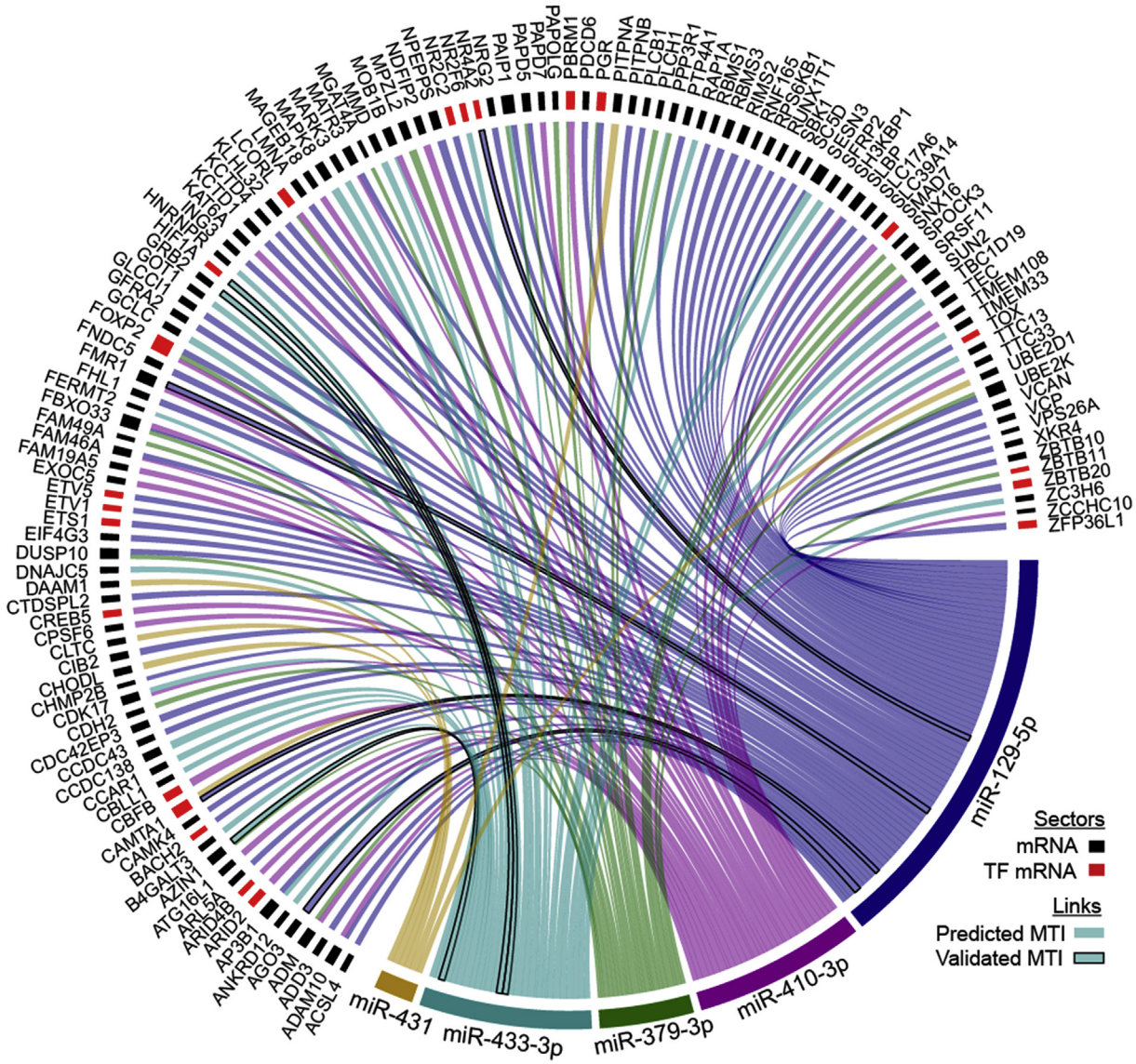

Fig. 7. Chord diagram showing predicted targets of the 5 miRNAs whose upregulation during epileptogenesis was prevented by VHC-LFS. The miRNAs and their mRNA targets are represented by coloured sectors around the outside of the circle (transcription factors are coloured red). Sectors are linked if a miRNA-target interaction (MTI) is predicted between the miRNA and mRNA target, as described in the Methods. The width of each link corresponds to the MTI Pscore associated with that link (MTIs with wider links have higher scores). MTIs that are also validated by strong experimental evidence (Western Blot, reporter assay, or qRT-PCR as classified by miRTarBase Release 6.0 [61] are highlighted by links with black borders. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

379-3p and 431-5p were very low, questioning their relevance for pathophysiological processes in the development of epilepsy.

Possible mechanisms of VHC stimulation

Several of the top-scoring predicted targets of the 5 miRNAs whose regulation was suppressed by VHC stimulation have been previously associated with epilepsy, including ADAM10 (Disintegrin and metallopeptidase domain 10), ADD3 (Gamma-adducin), CAMTA1 (Calmodulin-binding transcription activator 1), CDH2 (Cadherin 2), DNAJC5 (DnaJ homolog subfamily C member 5), FMR1 (fragile X mental retardation 1), GFRA2 (glial cell line derived neurotrophic factor family receptor alpha 2), KAT6A (Histone acetyltransferase KAT6A), NRG2 (Pro-neuregulin-2, membrane-bound isoform) and PLCB1 (Phospholipase C, beta-1) (http://carpedb.ua.edu/and [61]). In addition, we identified novel putative targets that may be involved in epileptogenesis. According to the performed pathway analysis, N-Glycan biosynthesis and growth factors might be affected by the miRNAs upregulated in PPS rats but not in VHC stimulated PPS rats. Both pathways have been associated with epilepsy in prior studies [62,63].

Our target prediction analyses of miRNAs with a presumptive role in anti-epileptogenic or anticonvulsive treatment effects might offer insights into less considered mechanisms of such effects.

Limitations of the study

Causing and preventing experimental epileptogenesis poses a dilemma: A long latency period is desirable to investigate epileptogenic mechanisms in detail but significantly prolongs time and efforts in screening and treatment experiments. The 30-30-8-PPS model provides not only the histological pattern of HS ILAE type 1 but also a latency period of 2–4 weeks, sufficient to test weeks-long interventions like DBS. On the other hand, throughput is

low, complicating screening of different DBS paradigms. Many DBS variables can be adjusted (e. g. target structure, duration, voltage/current, frequency, polarity, pulse form, single pulse duration, number of phases, intermittent vs. continuous stimulation), allowing for a virtually unlimited number of stimulation paradigms. Therefore, we aimed to align the tested DBS paradigms as closely as possible to established evidence. There is clinical evidence for an anticonvulsant effect of 5 Hz fornix stimulation in human TLE patients [28], so we adopted this paradigm for DBS of FF, PP and DG. For VHC DBS, on the other hand, there is evidence of anticonvulsant efficacy only for a 2 weeks stimulation with 1 Hz [29]. The need to follow prior evidence led to differences in frequencies and duration between the tested paradigms. Because even small adjustments could possibly make substantial clinical differences, we cannot exclude that stimulating FF, PP or DG for longer periods (e. g. 2 weeks) or with different frequencies (e. g. 5 Hz) could have influenced epileptogenesis differently. Hence, a direct comparison between efficacy of FF-, PP- and DG-DBS on the one hand with VHC-DBS on the other hand is not possible from our data.

The total number of interventions that can be tested is restricted by the long duration of a complete experimental cycle (>3 months per animal). Overlap of miRNA regulation in epileptogenesis should be investigated in different animal models to facilitate further experiments on miRNA manipulations *in vivo*.

Conclusion

Low frequency DBS of the VHC has an anti-epileptogenic effect, delaying onset of epilepsy, and is associated with differential regulation of several miRNAs. Manipulation of one or more of the five identified miRNAs could be an appropriate way to target complex processes like epileptogenesis. If miRNA manipulation can replicate the effect of DBS should be investigated by *in vivo* knock down.

Funding

This work was supported by the European Union's 'Seventh Framework' Programme (FP7) under Grant Agreement no. 602130 (EpimiRNA).

Declarations of interest

None.

References

[1] Téllez-Zenteno JF, Hernández-Ronquillo L. A review of the epidemiology of temporal lobe epilepsy. Epilepsy Res Treat 2012;2012. https://doi.org/10.1155/2012/630853.
[2] Wieser H-G, ILAE Commission on Neurosurgery of Epilepsy. ILAE Commission Report. Mesial temporal lobe epilepsy with hippocampal sclerosis. Epilepsia 2004;45:695–714. https://doi.org/10.1111/j.0013-9580.2004.09004.x.
[3] French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, et al. Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. Ann Neurol 1993;34:774–80. https://doi.org/10.1002/ana.410340604.
[4] Blümcke I, Thom M, Aronica E, Armstrong DD, Bartolomei F, Bernasconi A, et al. International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: a task force report from the ILAE commission on diagnostic methods. Epilepsia 2013;54:1315–29. https://doi.org/10.1111/epi.12220.
[5] Blumcke I, Spreafico R, Haaker G, Coras R, Kobow K, Bien CG, et al. Histopathological findings in brain tissue obtained during epilepsy surgery. N Engl J Med 2017;377:1648–56. https://doi.org/10.1056/NEJMoa1703784.
[6] Pohlen MS, Jin J, Tobias RS, Maheshwari A. Pharmacoresistance with newer anti-epileptic drugs in mesial temporal lobe epilepsy with hippocampal sclerosis. Epilepsy Res 2017;137:56–60. https://doi.org/10.1016/j.eplepsyres.2017.09.012.
[7] Wiebe S, Blume WT, Girvin JP, Eliasziw M. A randomized, controlled trial of surgery for temporal-lobe epilepsy. N Engl J Med 2001;345:311–8. https://doi.org/10.1056/NEJM200108023450501.
[8] Engel, Jr, MD, PhD, J, Michael P. McDermott, PhD, Samuel Wiebe, MD, John T. Langfitt, PhD, John M. Stern, MD, Sandra Dewar, RN, Michael R. Sperling, MD, Irenita Gardiner, RN, Giuseppe Erba, MD, Itzhak Fried, MD, PhD, Margaret Jacobs, BA, Harry V. Vinters, MD, Scott Mintzer, MD, Karl Kieburtz, MD, MPH, and for the early randomized surgical epilepsy trial (ERSET) study group. Early surgical therapy for drug-resistant temporal lobe epilepsy a randomized trial. JAMA n.d.;307:922–930. doi:10.1001/jama.2012.220.
[9] Téllez-Zenteno JF, Dhar R, Wiebe S. Long-term seizure outcomes following epilepsy surgery: a systematic review and meta-analysis. Brain J Neurol 2005;128:1188–98. https://doi.org/10.1093/brain/awh449.
[10] Li MCH, Cook MJ. Deep brain stimulation for drug-resistant epilepsy. Epilepsia 2018;59:273–90. https://doi.org/10.1111/epi.13964.
[11] Gooneratne IK, Green AL, Dugan P, Sen A, Franzini A, Aziz T, et al. Comparing neurostimulation technologies in refractory focal-onset epilepsy. J Neurol Neurosurg Psychiatry 2016;87:1174–82. https://doi.org/10.1136/jnnp-2016-313297.
[12] Chen Y-C, Zhu G-Y, Wang X, Shi L, Jiang Y, Zhang X, et al. Deep brain stimulation of the anterior nucleus of the thalamus reverses the gene expression of cytokines and their receptors as well as neuronal degeneration in epileptic rats. Brain Res 2017;1657:304–11. https://doi.org/10.1016/j.brainres.2016.12.020.
[13] Gebert LFR, MacRae IJ. Regulation of microRNA function in animals. Nat Rev Mol Cell Biol 2018;1. https://doi.org/10.1038/s41580-018-0045-7.
[14] Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are MicroRNA targets. Cell 2005;120:15–20. https://doi.org/10.1016/j.cell.2004.12.035.
[15] Park K, Kim K-B. miRTar hunter: a prediction system for identifying human microRNA target sites. Mol Cells 2013;35:195–201. https://doi.org/10.1007/s10059-013-2165-4.
[16] Xie X, Lu J, Kulbokas EJ, Golub TR, Mootha V, Lindblad-Toh K, et al. Systematic discovery of regulatory motifs in human promoters and 3' UTRs by comparison of several mammals. Nature 2005;434:338–45. https://doi.org/10.1038/nature03441.
[17] Jimenez-Mateos EM, Arribas-Blazquez M, Sanz-Rodriguez A, Concannon C, Olivos-Ore LA, Reschke CR, et al. microRNA targeting of the P2X7 purinoceptor opposes a contralateral epileptogenic focus in the hippocampus. Sci Rep 2015;5:17486. https://doi.org/10.1038/srep17486.
[18] 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. EMBO J 2017;36:1770–87. https://doi.org/10.15252/embj.201695748.
[19] Reschke CR, Silva LFA, Norwood BA, Senthilkumar K, Morris G, Sanz-Rodriguez A, et al. Potent anti-seizure effects of locked nucleic acid antagomirs targeting mir-134 in multiple mouse and rat models of epilepsy. Mol Ther Nucleic Acids 2017;6:45–56. https://doi.org/10.1016/j.omtn.2016.11.002.
[20] Soreq L, Salomonis N, Bronstein M, Greenberg DS, Israel Z, Bergman H, et al. Small RNA sequencing-microarray analyses in Parkinson leukocytes reveal deep brain stimulation-induced splicing changes that classify brain region transcriptomes. Front Mol Neurosci 2013;6:10. https://doi.org/10.3389/fnmol.2013.00010.
[21] Feng L-B, Pang X-M, Zhang L, Li J-P, Huang L-G, Su S-Y, et al. MicroRNA involvement in mechanism of endogenous protection induced by fastigial nucleus stimulation based on deep sequencing and bioinformatics. BMC Med Genomics 2015;8. https://doi.org/10.1186/s12920-015-0155-4.
[22] Norwood BA, Bumanglag AV, Osculati F, Sbarbati A, Marzola P, Nicolato E, et al. Classic hippocampal sclerosis and hippocampal-onset epilepsy produced by a single "cryptic" episode of focal hippocampal excitation in awake rats. J Comp Neurol 2010;518:3381–407. https://doi.org/10.1002/cne.22406.
[23] Blümcke I, Thom M, Aronica E, Armstrong DD, Bartolomei F, Bernasconi A, et al. International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: a Task Force report from the ILAE Commission on Diagnostic Methods. Epilepsia 2013;54:1315–29. https://doi.org/10.1111/epi.12220.
[24] Bumanglag AV, Sloviter RS. Minimal latency to hippocampal epileptogenesis and clinical epilepsy after perforant pathway stimulation-induced status epilepticus in awake rats. J Comp Neurol 2008;510:561–80. https://doi.org/10.1002/cne.21801.
[25] Andersen P, Holmqvist B, Voorhoeve PE. Entorhinal activation of dentate granule cells. Acta Physiol Scand 1966;66:448–60. https://doi.org/10.1111/j.1748-1716.1966.tb03223.x.
[26] Paxinos, Watson. The rat brain in stereotaxic coordinates. 1998.
[27] Wyckhuys T, Raedt R, Vonck K, Wadman W, Boon P. Comparison of hippocampal Deep Brain Stimulation with high (130 Hz) and low frequency (5 Hz) on afterdischarges in kindled rats. Epilepsy Res 2010;88:239–46. https://doi.org/10.1016/j.eplepsyres.2009.11.014.
[28] Koubeissi MZ, Kahriman E, Syed TU, Miller J, Durand DM. Low-frequency electrical stimulation of a fiber tract in temporal lobe epilepsy. Ann Neurol 2013;74:223–31. https://doi.org/10.1002/ana.23915.
[29] Rashid S, Pho G, Czigler M, Werz MA, Durand DM. Low frequency stimulation of hippocampal commissures reduces seizures in chronic rat model of

temporal lobe epilepsy. Epilepsia 2012;53:147–56. https://doi.org/10.1111/j.1528-1167.2011.03348.x.
[30] Fisher RS, Scharfman HE, deCurtis M. How can we identify ictal and interictal abnormal activity? Adv Exp Med Biol 2014;813:3–23. https://doi.org/10.1007/978-94-017-8914-1_1.
[31] Agarwal V, Bell G, Nam J, Bartel D. Predicting effective microRNA target sites in mammalian mRNAs. eLife; 2015. https://elifesciences.org/articles/05005. [Accessed 29 September 2018].
[32] Wong N, Wang X. miRDB: an online resource for microRNA target prediction and functional annotations. Nucleic Acids Res 2015;43:D146–52. https://doi.org/10.1093/nar/gku1104.
[33] 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. Sci Rep 2017;7:3328. https://doi.org/10.1038/s41598-017-02969-6.
[34] Gu Z, Gu L, Eils R, Schlesner M, Brors B. Circlize Implements and enhances circular visualization in R. Bioinforma Oxf Engl 2014;30:2811–2. https://doi.org/10.1093/bioinformatics/btu393.
[35] Zhang H-M, Liu T, Liu C-J, Song S, Zhang X, Liu W, et al. AnimalTFDB 2.0: a resource for expression, prediction and functional study of animal transcription factors. Nucleic Acids Res 2015;43:D76–81. https://doi.org/10.1093/nar/gku887.
[36] 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. Annu Rev Genet 2015;49:213–42. https://doi.org/10.1146/annurev-genet-120213-092023.
[37] Vlachos IS, Zagganas K, Paraskevopoulou MD, Georgakilas G, Karagkouni D, Vergoulis T, et al. DIANA-miRPath v3.0: deciphering microRNA function with experimental support. Nucleic Acids Res 2015;43:W460–6. https://doi.org/10.1093/nar/gkv403.
[38] Cuellar-Herrera M, Neri-Bazan L, Rocha LL. Behavioral effects of high frequency electrical stimulation of the hippocampus on electrical kindling in rats. Epilepsy Res 2006;72:10–7. https://doi.org/10.1016/j.eplepsyres.2006.07.002.
[39] Goodman JH, Berger RE, Tcheng TK. Preemptive low-frequency stimulation decreases the incidence of amygdala-kindled seizures. Epilepsia 2005;46:1–7. https://doi.org/10.1111/j.0013-9580.2005.03804.x.
[40] López-Meraz ML, Neri-Bazán L, Rocha L. Low frequency stimulation modifies receptor binding in rat brain. Epilepsy Res 2004;59:95–105. https://doi.org/10.1016/j.eplepsyres.2004.02.005.
[41] Mohammad-Zadeh M, Mirnajafi-Zadeh J, Fathollahi Y, Javan M, Jahanshahi A, Noorbakhsh SM, et al. The role of adenosine A1 receptors in mediating the inhibitory effects of low frequency stimulation of perforant path on kindling acquisition in rats. Neuroscience 2009;158:1632–43. https://doi.org/10.1016/j.neuroscience.2008.11.008.
[42] Sun H-L, Zhang S-H, Zhong K, Xu Z-H, Zhu W, Fang Q, et al. Mode-dependent effect of low-frequency stimulation targeting the hippocampal CA3 subfield on amygdala-kindled seizures in rats. Epilepsy Res 2010;90:83–90. https://doi.org/10.1016/j.eplepsyres.2010.03.011.
[43] Van Nieuwenhuyse B, Vonck K, Raedt R, Meurs A, Wadman W, Boon P. Deep brain stimulation early during epileptogenesis modifies disease progression in the hippocampus. Epilepsia 2012;53:26–7.
[44] Kile KB, Tian N, Durand DM. Low frequency stimulation decreases seizure activity in a mutation model of epilepsy. Epilepsia 2010;51:1745–53. https://doi.org/10.1111/j.1528-1167.2010.02679.x.
[45] Gloor P, Salanova V, Olivier A, Quesney LF. The human dorsal hippocampal commissure. An anatomically identifiable and functional pathway. Brain J Neurol 1993;116(Pt 5):1249–73.
[46] Assenza G, Campana C, Assenza F, Pellegrino G, Di Pino G, Fabrizio E, et al. Cathodal transcranial direct current stimulation reduces seizure frequency in adults with drug-resistant temporal lobe epilepsy: a sham controlled study. Brain Stimulat 2017;10:333–5. https://doi.org/10.1016/j.brs.2016.12.005.
[47] Bauer S, Baier H, Baumgartner C, Bohlmann K, Fauser S, Graf W, et al. Transcutaneous vagus nerve stimulation (tVNS) for treatment of drug-resistant epilepsy: a randomized, double-blind clinical trial (cMPsE02). Brain Stimul Basic Transl Clin Res Neuromodulation 2016;9:356–63. https://doi.org/10.1016/j.brs.2015.11.003.
[48] Bikson M, Grossman P, Thomas C, Zannou AL, Jiang J, Adnan T, et al. Safety of transcranial direct current stimulation: evidence based update 2016. Brain Stimulat 2016;9:641–61. https://doi.org/10.1016/j.brs.2016.06.004.
[49] Roncon P, Soukupovà 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. Sci Rep 2015;5:14143. https://doi.org/10.1038/srep14143.
[50] Miller-Delaney SFC, Bryan K, Das S, McKiernan RC, Bray IM, Reynolds JP, et al. Differential DNA methylation profiles of coding and non-coding genes define hippocampal sclerosis in human temporal lobe epilepsy. Brain 2015;138:616–31. https://doi.org/10.1093/brain/awu373.
[51] Sosanya NM, Huang PPC, 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;202:53–69. https://doi.org/10.1083/jcb.201212089.
[52] Bot AM, Dębski KJ, Lukasiuk K. Alterations in miRNA levels in the dentate gyrus in epileptic rats. PLoS One 2013;8:e76051. https://doi.org/10.1371/journal.pone.0076051.
[53] 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. Neurobiol Dis 2014;62:508–20. https://doi.org/10.1016/j.nbd.2013.10.026.
[54] Kretschmann A, Danis B, Andonovic L, Abnaof K, Rikxoort M van, Siegel F, et al. Different MicroRNA profiles in chronic epilepsy versus acute seizure mouse models. J Mol Neurosci 2014;55:466–79. https://doi.org/10.1007/s12031-014-0368-6.
[55] Risbud RM, Porter BE. Changes in microRNA expression in the whole hippocampus and hippocampal synaptoneurosome fraction following pilocarpine induced status epilepticus. PLoS One 2013;8:e53464. https://doi.org/10.1371/journal.pone.0053464.
[56] McKiernan RC, Jimenez-Mateos EM, Sano T, Bray I, Stallings RL, Simon RP, et al. Expression profiling the microRNA response to epileptic preconditioning identifies miR184 as a modulator of seizure-induced neuronal death. Exp Neurol 2012;237:346–54. https://doi.org/10.1016/j.expneurol.2012.06.029.
[57] Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, et al. A brain-specific microRNA regulates dendritic spine development. Nature 2006;439:283–9. https://doi.org/10.1038/nature04367.
[58] Jimenez-Mateos EM, Engel T, Merino-Serrais P, McKiernan RC, Tanaka K, Mouri G, et al. Silencing microRNA-134 produces neuroprotective and prolonged seizure-suppressive effects. Nat Med 2012;18:1087–94. https://doi.org/10.1038/nm.2834.
[59] Morris G, Brennan GP, Reschke CR, Henshall DC, Schorge S. Spared CA1 pyramidal neuron function and hippocampal performance following antisense knockdown of microRNA-134. Epilepsia 2018;59:1518–26. https://doi.org/10.1111/epi.14475.
[60] Schouten M, Bielefeld P, Fratantoni SA, Hubens CJ, Piersma SR, Pham TV, et al. Multi-omics profile of the mouse dentate gyrus after kainic acid-induced status epilepticus. Sci Data 2016;3:160068. https://doi.org/10.1038/sdata.2016.68.
[61] Wang J, Lin Z-J, Liu L, Xu H-Q, Shi Y-W, Yi Y-H, et al. Epilepsy-associated genes. Seizure 2017;44:11–20. https://doi.org/10.1016/j.seizure.2016.11.030.
[62] Freeze HH, Eklund EA, Ng BG, Patterson MC. Neurological aspects of human glycosylation disorders. Annu Rev Neurosci 2015;38:105–25. https://doi.org/10.1146/annurev-neuro-071714-034019.
[63] Scharfman HE. The neurobiology of epilepsy. Curr Neurol Neurosci Rep 2007;7:348–54.
[64] Vlachos IS, Zagganas K, Paraskevopoulou MD, Georgakilas G, Karagkouni D, Vergoulis T, Dalamagas T, Hatzigeorgiou AG. DIANA-miRPath v3.0: deciphering microRNA function with experimental support. Nucleic Acids Res 2015;43:W460–6. https://doi.org/10.1093/nar/gkv403.