Experimental Neurology 273 (2015) 92–104

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Rapid changes in expression of class I and IV histone deacetylases during epileptogenesis in mouse models of temporal lobe epilepsy

Rohan Jagirdar [1], Meinrad Drexel [1], Elke Kirchmair, Ramon O. Tasan, Günther Sperk *

Department of Pharmacology, Medical University Innsbruck, 6020 Innsbruck, Austria

ARTICLE INFO

Article history:
Received 18 May 2015
Received in revised form 19 June 2015
Accepted 28 July 2015
Available online 31 July 2015

Keywords:
Epilepsy
Epileptogenesis
Status epilepticus
Intrahippocampal kainic acid
HDAC
Histone acetylation
Epigenetic

ABSTRACT

A prominent role of epigenetic mechanisms in manifestation of epilepsy has been proposed. Thus altered histone H3 and H4 acetylation has been demonstrated in experimental models of temporal lobe epilepsy (TLE). We now investigated changes in the expression of the class I and class IV histone deacetylases (HDAC) in two complementary mouse TLE models. Unilateral intrahippocampal injection of kainic acid (KA) induced a status epilepticus lasting 6 to 24 h, development of spontaneous limbic seizures (2 to 3 days after KA injection) and chronic epilepsy, as revealed by telemetric recordings of the EEGs. Mice were killed at different intervals after KA injection and expression of HDAC mRNAs was investigated by in situ hybridization. We observed marked decreases in the expression of HDACs 1, 2 and 11 (by up to 75%) in the granule cell and pyramidal cell layers of the hippocampus during the acute status epilepticus (2 to 6 h after KA injection). This was followed by increased expression of all class I HDAC mRNAs in all principal cell layers of the hippocampus after 12 to 48 h. In the chronic phase, 14 and 28 days after KA, only modest increases in the expression of HDAC1 mRNA were observed in granule and pyramidal cells. Immunohistochemistry using an antibody detecting HDAC2 revealed results consistent with the mRNA data and indicates also expression in glial cells on the injection side. Similar changes as seen in the KA model were observed after a pilocarpine-induced status epilepticus except that decreases in HDACs 2, 3 and 8 were also seen at the chronic 28 day interval.

The prominent decreases in HDAC expression during status epilepticus are consistent with the previously demonstrated increased expression of numerous proteins and with the augmented acetylation of histone H4. It is suggested that respective putative gene products could facilitate proconvulsive as well as anticonvulsive mechanisms. The increased expression of all class I HDACs during the "silent phase", on the other hand, may be related to decreased histone acetylation, which could cause a decrease in expression of certain proteins, a mechanism that could also promote epileptogenesis. Thus, addressing HDAC expression may have a therapeutic potential in interfering with a status epilepticus and with the manifestation of TLE.

1. Introduction

Temporal lobe epilepsy (TLE) is the most common and difficult to treat form of focal epilepsies. It comprises about 30% of all epilepsies (Fisher et al., 1998). The most common pathology underlying TLE is Ammon's horn sclerosis and sprouting of mossy fibers. Neurodegeneration affects primarily the hilus of the dentate gyrus and hippocampal sectors CA3 and CA1 while other brain areas are less affected (Babb et al., 1984). In some instances dispersion of the granule cell layer is observed (Houser, 1990; Haas et al., 2002). In general, TLE develops after an initial insult, which e.g. can be early febrile seizures, brain trauma or a status epilepticus. However, it may take years from this initial insult until the first clinical symptoms of TLE occur. This time window is often termed as "silent phase" and during this period plastic changes develop in brain circuitries ultimately leading to epilepsy. In theory, this phase would offer an ideal opportunity to suppress the process of epileptogenesis by drug therapy and thereby prevent manifestation of TLE. However, no such "disease modifying" drug has been discovered yet.

A variety of animal models have been developed for investigating changes in molecular and cellular mechanisms that accompany or even may be causatively related to manifestation of epilepsy. These animal models are mostly based on the induction of a status epilepticus by applying repeated electrical stimulation or injections of convulsive toxins, such as kainic acid (KA) or pilocarpine, or by triggering febrile

Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-isoxazole-propionic acid; GluA2, AMPA receptor subunit 2; KA, kainic acid; HDAC, histone deacetylase; TLE, temporal lobe epilepsy; BDNF, brain-derived neurotrophic factor.

* Corresponding author at: Department of Pharmacology, Peter-Mayr-Str. 1a, 6020 Innsbruck, Austria.
E-mail address: guenther.sperk@i-med.ac.at (G. Sperk).

[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.expneurol.2015.07.026

seizures in infant rodents (Sharma et al., 2007). The duration of the silent phase is variable, however, spontaneous seizures develop mostly after 1 to 3 weeks (Rakhade and Jensen, 2009; Williams et al., 2009; Drexel et al., 2012b). Altered gene expression induced by the initial insult (status epilepticus) may be related to molecular and cellular mechanisms leading to the development of epilepsy. These include augmented expression of immediate early genes, growth factors, inflammation associated proteins and neuropeptides (Gass et al., 1992; Esclapez and Houser, 1999; Vezzani et al., 2011; Sperk et al., 2012; Clynen et al., 2014).

These complex epilepsy-induced changes in gene expression of multiple proteins are presumably guided by epigenetic mechanisms, including chromatin modifications (McClelland et al., 2011) such as DNA methylation or histone modifications (Hwang et al., 2013; Kobow and Blumcke, 2014). DNA methylation is mostly targeting CpG dinucleotides of the chromatin and is catalyzed by a family of DNA methyltransferases (Holliday and Pugh, 1975). Histone modifications include acetylation, methylation, phosphorylation and other modifications of specific amino acids in the N-terminal tail of histones (Khorasanizadeh and Ostankovitch, 2014). Some modifications may activate gene expression, while others inhibit. Among these modifications histone methylation and acetylation have been extensively studied in recent years. Histone acetylation is catalyzed by histone acetyltransferases and reversed by histone deacetylases (HDACs). Acetylated histones, H3 and H4, generally reflect a more permissive (open) state of chromatin and thereby increased gene expression, whereas deacetylation mostly suppresses transcription (Kimura et al., 2005). In humans and rodents, multiple HDAC enzymes have been identified. Based on their homology to yeast histone deacetylases they are divided into four major classes (Chuang et al., 2009). Class I HDACs include HDAC1, 2, 3 and 8. Class II HDACs comprise HDAC4, 5, 6, 7, 9 and 10. Class III HDACs are referred to as sirtuins (SIRT1–SIRT7) based on their homology to the yeast HDAC Sir2. They are more general protein deacetylases. Due to its distinct structure, HDAC11 has been attributed to an own class, class IV (Voelter-Mahlknecht et al., 2005).

To investigate a possible role of the expression of HDACs during the development of epilepsy we now investigated changes in the mRNA levels of class I and IV HDACs. We used a mouse model based on unilateral local injection of KA (Riban et al., 2002). The model induces early spontaneous seizure activity (epilepsy) and granule cell dispersion at the site of KA injection. We compared this model with the more broadly used model based on i.p. injection of pilocarpine (Cavalheiro et al., 1996). The concomitant use of both models allowed us to exclude individual aspects of the models (such as the use of different receptor agonists for inducing the status epilepticus) when studying changes in HDAC expression during seizure induction and epileptogenesis in the "silent phase". In an accompanying study we investigated changes in class II HDAC expression in the same animal models (Jagirdar et al., unpublished results). This study revealed significantly different changes for class II HDACs (particularly in relation to granule cell dispersion) as reported here for class I and IV HDACs (see discussion).

2. Materials and methods

2.1. Animals

All animal experiments were conducted according to national guidelines and European Community laws and were approved by the *Committee for Animal Protection* of the Austrian Ministry of Science. In total, we used 91 adult, 10–16 weeks old, male C57Bl6/N mice (25–30 g; Charles River Laboratories, Sulzberg, Germany) for local injection of KA, 18 mice for injection of pilocarpine and 33 control mice. The animals were housed in groups of 3–5 in Sealsafe™ IVC cages (Techniplast GmbH, Hohenpeissenberg, Germany) under standard laboratory conditions (12/12 h light/dark cycle, light turns on at 06:30 AM). They had access to food and water ad libitum.

2.2. Surgical procedures

2.2.1. Anesthesia and presurgical procedures

The mice were given analgesic treatment (5 mg/kg carprofen s.c.; Rimadyl, Pfizer, USA) 60 min prior to the surgery and were then anesthetized by injection of 150 mg/kg ketamine (Ketasol, stock solution: 50 mg/ml, Ogris Pharma Vertriebs-GmbH, Wels, Austria). Anesthesia was maintained with 1–4% of sevoflurane (Sevorane®, Abbott, Vienna, Austria) applied through a veterinary anesthetic mask (VIP 3000, Matrx by Midmark, USA) throughout the entire procedure. The deeply anesthetized mice were fixed in a stereotaxic frame (David Kopf Instruments, California, USA) using ear-bars.

2.2.2. Stereotaxic injections of kainic acid (KA)

KA injection was done unilaterally into the area of the stratum lacunosum/radiatum CA1 of the dorsal hippocampus of the mice. The following stereotaxic coordinates were used: anterioposterior, −1.9 mm; mediolateral, ±1.6 mm; and dorsoventral, −1.9 mm from bregma. The coordinates were determined using the mouse brain atlas by Franklin and Paxinos (2008) and were verified in brain sections from methylene blue injected mice. At the anterioposterior/mediolateral coordinates small holes (0.7 mm in diameter) were drilled into the scull at both sides using a dental drill for applying KA and the recording electrode (both left side) and the reference electrode (contralateral side), respectively.

KA (Ascent Scientific Ltd., North Somerset, United Kingdom; 350 pmoles in 70 nl in a solution of 5 mM in 0.9% NaCl, adjusted to pH 7.0) was infused unilaterally on the right side at a rate of 0.14 μl/min using a 5 μl Hamilton syringe (Neuros™ Hamilton Syringe, 7000.5, Hamilton Bonaduz AG, Switzerland) connected to a syringe pump (QSI™, Stoelting, IL, USA). After injection the needle was withdrawn slowly (5 min). Controls were injected with saline (70 nl).

2.2.3. Implantation of electrodes and of telemetric transmitters

Immediately after infusion of KA, mice were implanted with biopotential transmitters (TA10EA-F20; Data Sciences International, Arden Hills, USA). For this, an incision was made (max. 2 cm, rostrocaudally) at the center of a shaved area at the abdomen and a subcutaneous cavity was formed using Metzenbaum scissors. Then a subcutaneous passage to the skull was formed using a bulb-headed probe (2 mm) and the electrode leads of the transmitter were pulled from the subcutaneous cavity to the opened scalp using a thin forceps. The transmitter was placed into this cavity and the incision was sutured using Supramid (USP 4/0, multifilament, non absorbable) surgical thread (Supramid TC158000, Serag Wiessner, Naila, Germany).

The recording electrode was placed epidurally through the same hole set for infusion of KA. The reference electrode was placed in the same way on the contralateral side. The electrodes were fixed to the scull by setting three stainless steel screws (M1*2, Hummer und Rieß GmbH, Nürnberg, Germany) in an epidural position and by embedding the electrodes and the screws in dental cement (Paladur—Heraeus Kulzer, Henry Schein, Austria). The scalp skin was then closed using Supramid. After surgery the body temperature of the mice was stabilized using a red light lamp. The mice received a second dose of carprofen (5 mg/kg; s.c.) after 24 h.

2.3. EEG-telemetry and video monitoring

Transmitter implanted mice were single housed in single ventilated cages with agarose gels (1 g agarose in 100 ml of 2% sucrose) as a water source in addition to food pellets, oat flakes and water. The EEGs were recorded using an EEG-telemetry system (Dataquest A.R.T., Data Acquisition 4.0 for telemetry systems, Data Sciences International, Arden Hills, USA). EEG signals emitted by the transmitters were captured with receiver plates placed under each cage and transmitted to a computer (Windows XP operating system, Microsoft) through a data

exchange matrix (DSI, Arden Hills, USA). The recorded EEG signals were stored in external hard drives. Behavioral responses were captured by video monitoring using infra-red sensitive Axis 221 network cameras (Axis Communications AB, Lund, Sweden). Mice were kept under 12 h light/dark cycle but with infrared lighting (Conrad Electronics GmbH, Wels, Austria) during the dark phase.

2.4. EEG analysis

EEGs were recorded at a sampling rate of 1000 Hz with no a priori filter cut-off and started 30 min after surgery. EEG traces were inspected visually. Increased EEG activity was defined by continuous synchronous high-frequency and high-amplitude oscillations (>2 × baseline amplitude), by obvious progression of spike frequency and a minimum duration of 10 s. Additionally, prolonged periods of increased EEG-activity were checked for post-ictal depression of the EEG signal (below baseline EEG activity) considered as a typical sign of seizures (Bouilleret et al., 1999; Williams et al., 2009).

Seizure-like activity was categorized into "hippocampal paroxysmal discharges" (HPD) and "spontaneous strong seizures". As HPD we defined high frequency oscillations with an amplitude of at least double the baseline and a duration of 10–30 s. Spontaneous strong seizures were characterized by high frequency and high amplitude oscillations (more than 2–3 times baseline). In contrast to HPD, spontaneous strong seizures gradually progressed, lasted for more than 30 s, and were directly followed by post-ictal depression of the EEG-amplitude before returning to baseline.

2.4.1. Motor seizure rating

Recording of EEG signals and video recording were synchronized and were performed continuously for 28 days after KA injection. Seizures defined by EEG were investigated for a behavioral correlate by inspecting the synchronized video recordings and, according to this, were classified as clinical or subclinical seizures. Seizure rating was done in accordance to that of KA-injected rats (Sperk et al., 1983): Stage 1, staring, arrest, chewing; stage 2, unilateral or bilateral tonic movements/seizure; stage 3, rearing without falling; stage 4, rearing with falling, limbic seizures; stage 5, death.

2.5. Pilocarpine-induced seizures

For identifying changes in HDAC expression specifically related to seizure induction and epileptogenesis and not to particular effects of the KA model we investigated changes in HDAC expression also the pilocarpine model of epileptogenesis (Cavalheiro et al., 1996). Male mice (20 to 25 g) were injected with N-methyl scopolamine (170 mg/kg in saline; s.c.) and after 20 min with pilocarpine (Pitsch et al., 2007). All mice rapidly developed seizures leading to a status epilepticus and were treated with 4 mg/kg i.p. diazepam (Gewacalm, Nycomed, Linz, Austria) 40 min after the first stage 3 seizure. Control mice were injected with N-methyl scopolamine followed by saline injections. Seizures were rated according to the same rating scale given above.

2.6. Tissue preparation

For in situ hybridization, KA injected mice were killed in a CO_2-chamber at different time intervals including short-time intervals, 2 h, 4 h, 6 h, 12 h, 24 h and 48 h and long-time intervals 7 days, 14 days and 28 days. In the pilocarpine experiment mice were killed 4 h, 24 h, and 28 days after pilocarpine injection. Mice were then immediately decapitated. The brains were removed and snap frozen in isopentane (−70 °C for 3 min). They were then kept at −70 °C in open tubes for 24 h to allow the isopentane to evaporate and then stored in sealed vials. Coronal 20 μm sections were cut with a cryotome (HM 560 M, Microm GmbH, Walldorf, Germany) and thaw-mounted onto gelatine-coated slides. The sections were stored at −20 °C until further use.

For immunocytochemistry, injected mice were killed at different intervals (4 h, 12 h, 24 h, 14 days) after KA injection by transcardial perfusion with with 4% paraformaldehyde in 50-mmol/l phosphate buffer, pH 7.4 as described previously (Drexel et al., 2012a). Brains were snap-frozen in −70 °C isopentane (Merck, Darmstadt, Germany) for 3 min and stored at −70 °C. Coronary 30-μm-sections were cut on a cryostat microtome (Carl Zeiss AG, Vienna, Austria), collected in 50-mmol/l Tris-buffered saline containing 0.1% NaN_3 (all from Merck), and stored at 4 °C.

2.7. In situ hybridization

2.7.1. Oligonucleotides

Oligonucleotides complementary to the mRNA sequences of the individual HDACs were designed and obtained HPLC purified from Microsynth (Balgach, Switzerland). They are listed in Table 1.

In situ hybridization was performed as described previously in detail (Furtinger et al., 2001). Oligonucleotides (2.5 pmol) were 3′ end-labeled with [^{33}P]α-dATP (75 μCi; 3000 Ci/mmol, SCF-203, Hartmann Analytic, Braunschweig, Germany) and terminal transferase (Roche Diagnostics, Basel, Switzerland), as described previously in detail (Drexel et al., 2012a). Hybridization was performed in 50% formamide, 4× SSC (1× SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.2), 500 μg/ml salmon sperm DNA, 250 μg/ml yeast tRNA, 1× Denhardt's solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 10% dextran sulfate, and 20 mM dithiothreitol (all from Sigma) at 42 °C for 18 h. The slides were washed stringently (50% formamide in 1× SSC, 42 °C) and then rinsed in water briefly, followed by 70% ethanol, and dried. They were then exposed to BioMax MR films (Amersham Pharmacia Biotech, Buckinghamshire, UK) together with [^{14}C]-microscales for 14 to 28 days. The films were developed with Kodak D19 developer. Sections were counterstained with cresyl violet, dehydrated, cleared in butyl acetate, and covered using Eukitt (Merck, Darmstadt, Germany).

2.8. Immunohistochemistry

Paraformaldehyde-fixed sections were processed for immunohistochemistry as described in detail previously (Drexel et al., 2012a). The free-floating mouse brain sections were pre-incubated with mouse Ig

Table 1
Sequences of DNA probes.

Probe	DNA sequence	GenBank no.	Bases
mHDAC1	GAA CTC AAA CAA GCC ATC AAA CAC CGG ACA GTC CTC ACC	NM_008228.2	306–344
mHDAC1	GTT CTG GTT GGT CAT GTT GGA AGG GCT GAT GTG AAG CTT	NM_008228.2	1041–1079
mHDAC2	GAT CAG CAA CAT TCC TAC GAC CTC CTT CAC CTT CAT CCT CAG	NM_008229.2	1479–1520
mHDAC2	AGC ACC AAT ATC CCT CAA GTC TCC TGT TCC AGG	NM_008229.2	827–859
mHDAC3	ACC CTC TCC TCC TTG CCA CTG TAC TTC ATC TGC AGT T	NM_010411.2	1793–1829
mHDAC8	GTC AAA TAT CCC TTC TGT GGC TGG GCA GTC AT	NM_027382.3	383–414
mHDAC8	GTG CAG GGA CAC AGT CAT AAC CTT CGA TGT	NM_027382.3	658–687
mHDAC11	CAG GTC TGT CTG GAC CTT GAC TTA TTG AGG GG	NM_144919.2	1592–1623
mHDAC11	CGA GAT CAA TGA TGG TGG CTC TGG AGA TGC CTT CCA	NM_144919.2	537–572

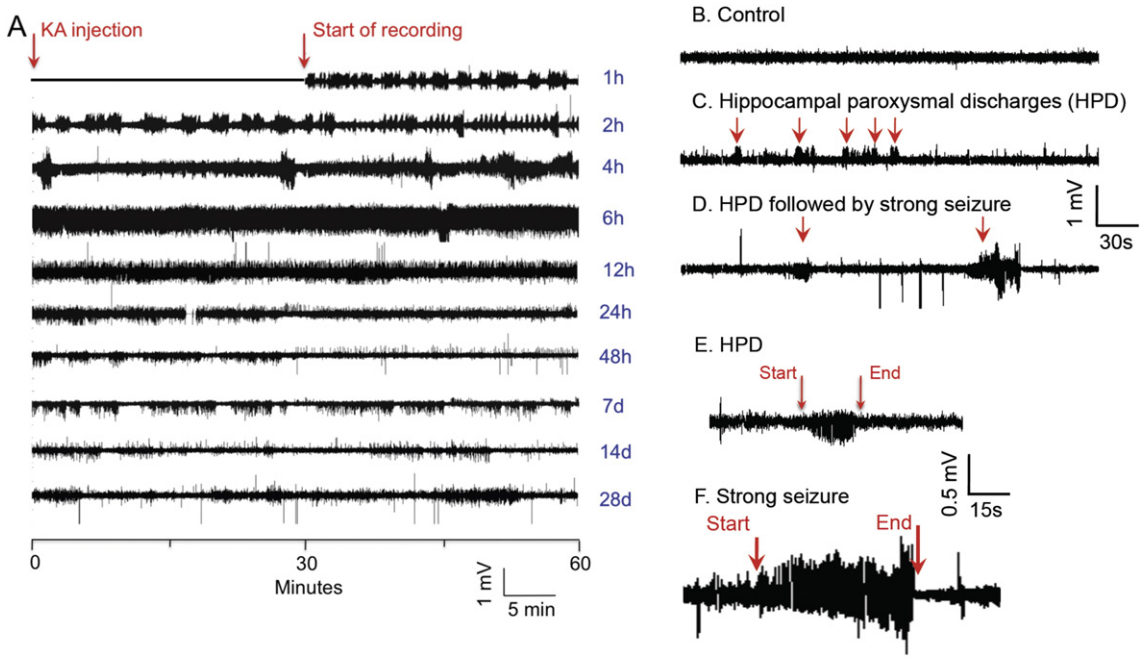


Fig. 1. EEG activity induced by intra-hippocampal injection of kainic acid (KA). A, The initial status epilepticus reaches a maximum after 6 to 10 h. B. Saline injected mouse. C–F, EEGs taken 12 days after the initial status epilepticus. C. Trace showing hippocampal paroxysmal discharges (HPD; arrows); D, trace showing HPD and a strong spontaneous seizure. E and F, larger magnification of a HPD and a strong seizure, respectively. The arrows mark the beginnings and ends. Note the higher amplitude and the post-seizure depression of the strong seizure (F).

blocking reagent (Vector, MKB 2211; 60 min, room temperature) and then with 10% normal horse serum (90 min, room temperature). As primary antibody we used a mouse HDAC2 antibody (5113, Cell Signaling Technology, Danvers, US) at a concentration of 1:2000 with overnight incubation at room temperature. As secondary we used a donkey anti mouse HRP antibody (715-036-151 from Jackson ImmunoResearch; Dianova, Vienna) at a concentration of 1:500 (120 min, room temperature). The reaction product was intensified by incubation with TSA Biotin (60 min, room temperature) followed by staining with Nickel DAB (Drexel et al., 2012a). Western blotting revealed that the antiserum detected equally HDAC1 and HDAC2 in the mouse brain (not shown).

2.9. Quantitative evaluation of in situ hybridization autoradiograms

Quantitative evaluation was done on digitized images of the autoradiographs (eight bit digitized image, 256 gray values) as described previously (Tsunashima et al., 1997). Gray values were measured using the public domain program ImageJ 1.38x (NIH, USA; 255 = white; 0 = black) and converted to relative optical density (ROD). [^{14}C]-micro-scales (Amersham Pharmacia Biotech, Amersham, UK) were exposed together with the slides. They were used to obtain a standard curve and to verify that ROD values were within the linear range.

2.10. Statistical analysis

We performed three independent experiments. They were first evaluated separately. Since no significant differences were observed between individual experiments we pooled the %-values for each interval. The total numbers of mice in the KA experiment were: controls, 23; 2 h, 12; 4 h, 9; 6 h, 10; 12 h, 4; 24 h, 4; 48 h, 3; 7 days, 6; 14 days, 5; 28 days, 7 mice. In the pilocarpine experiment, the following numbers of mice were used: Controls, 10; 4 h, 9; 24 h, 5; 28 d, 4 mice. ROD values were calculated as % of the mean ROD of controls. Data are shown as mean ± SEM. Statistical analysis was done using the GraphPad Prism program (v 5.0d; GraphPad Software Inc., La Jolla, USA). In situ hybridization data were analyzed by one-way ANOVA with post-hoc Dunnett test. Differences in ratings of motor seizures were analyzed using Kruskal–Wallis test with Mann–Whitney post-hoc test.

3. Results

3.1. Intra-hippocampal KA model

3.1.1. EEG and behavioral data

We performed continuous telemetric EEG recordings until the mice were killed; in 8 mice recordings were done for 28 days. Concomitantly to EEG recordings video monitoring was performed in 12 mice for 14 days. In agreement with data published by (Bouilleret et al., 1999;

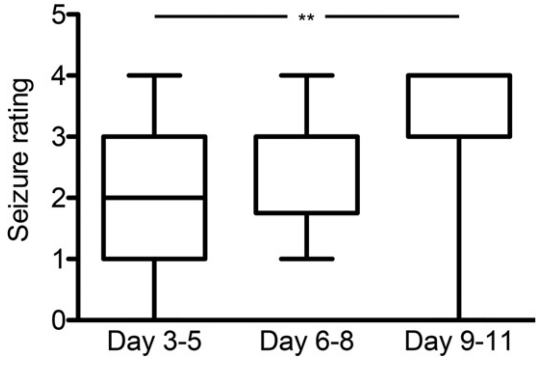


Fig. 2. Development of motor seizures after injection of kainic acid (KA). Strong EEG seizures (Fig. 1F) were recorded telemetrically 3 days after injection of KA and thereafter at a rate of 0 to 2 per 24 h. Using the behavioral video recordings, we evaluated the motor seizures occurring concomitantly with each strong EEG seizure. The following seizure rating scale was used: stage 0, no motor signs; stage 1, staring, arrest, chewing; stage 2, unilateral or bilateral tonic movements/seizure; stage 3, rearing without falling; stage 4, rearing with falling, limbic seizures; stage 5, death. Ratings of motor seizures that occurred concomitantly with strong EEG seizures were pooled for three days (days 3–5, days 6–8, days 9–11) and summed up. Median seizure rating observed on days 9–11 was significantly higher than that observed during days 3–5 (**$p < 0.01$, Kruskal-Wallis test with Mann–Whitney post-hoc test).

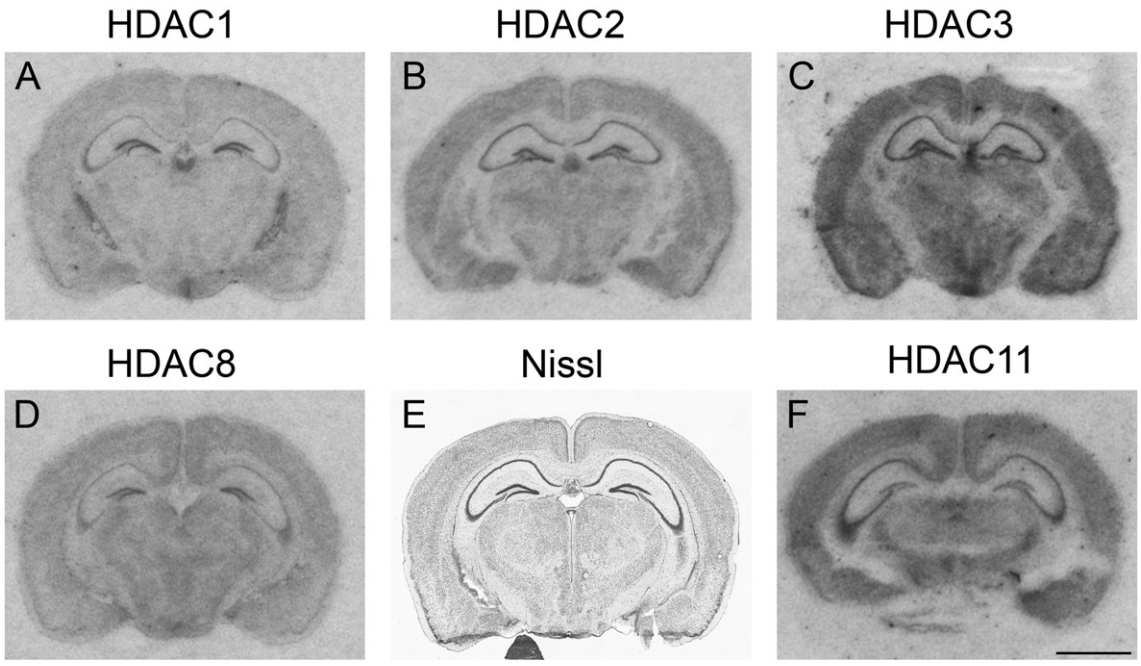


Fig. 3. Expression patterns of mRNAs encoding class I HDACs 1–3 and 8 as well as class IV HDAC 11 in control mice. The images represent photomicrographs of autoradiographs after in situ hybridization with the respective radiolabeled antisense probes. Panels A–D, class I HDACs; panel F, class IV HDAC; panel E depicts a corresponding Nissl staining taken from the Allen Brain Atlas. GC, granule cell layer. Scale bar in F: 2 mm.

Riban et al., 2002; Pallud et al., 2011), EEG changes were characterized by a trias of seizure-like events, the acute status epilepticus, frequent hippocampal paroxysmal discharges (HPD) and rare spontaneous strong seizures (Fig. 1).

3.2. Status epilepticus

Although still deeply anesthetized, all KA-injected mice developed a status epilepticus seen in the EEG (Fig. 1A). At this time episodes of

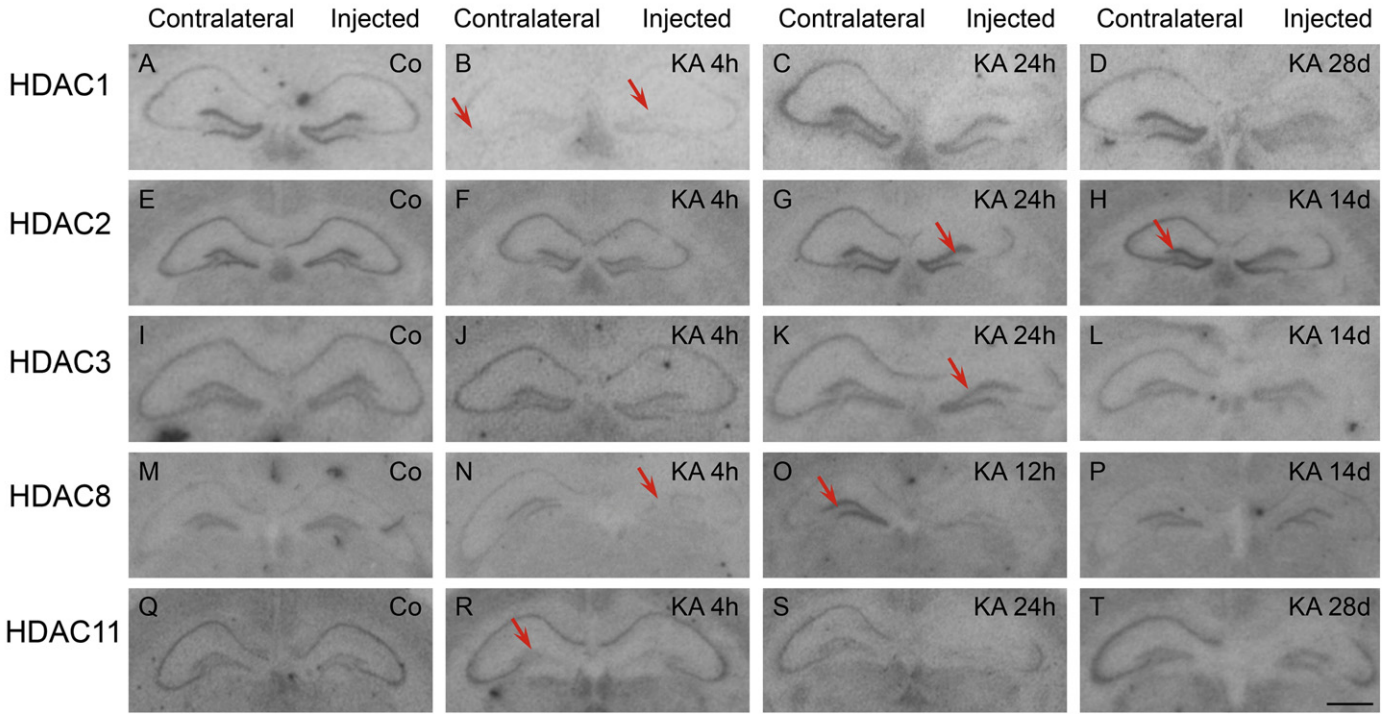
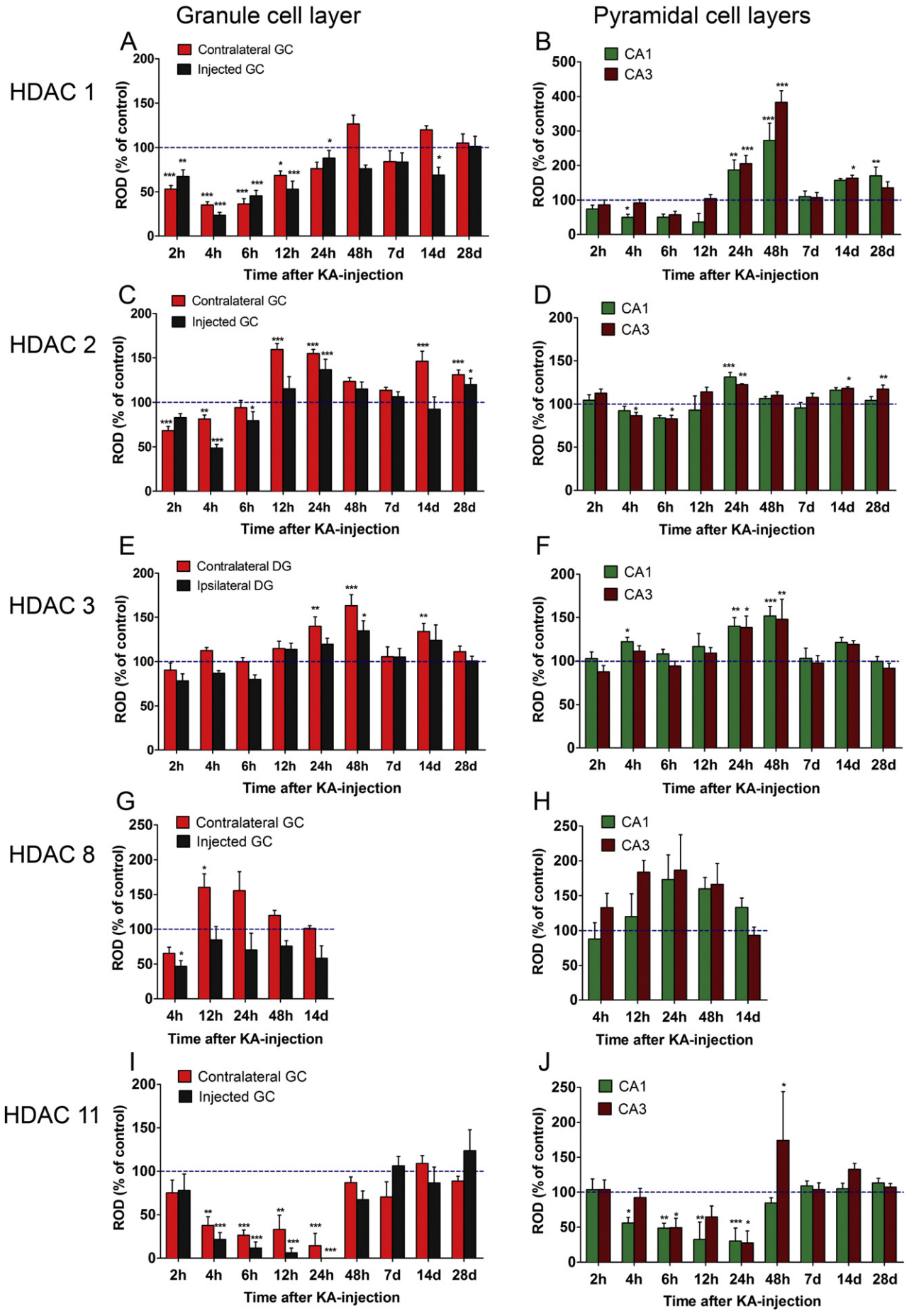


Fig. 4. Expression of class I HDAC1, 2, 3 and 8 and class IV HDAC11 mRNAs after unilateral injection of KA into the hippocampus. Shown are photomicrographs of autoradiographs from controls (Co) and at selected time points after KA injection after in situ hybridization with radiolabelled antisense probes for the individual HDACs. The right CA1 sector (stratum radiatum/lacunosum moleculare) was injected with KA which is reflected by neurodegenerative changes in the CA1 and CA3 sectors of the right hippocampus. Arrows indicate: in B, decreased expression of HDAC1 throughout the ipsi- and contralateral hippocampus 4 h after KA; G, K: increased expression of HDAC2 (in G) and HDAC3 (in K) in the injected granule cell layer after 24 h; N, O: decreased expression of HDAC8 in the ipsilateral (N) but increased expression in the contralateral (O) granule cell layer after 4 h and 12 h, respectively; R: early decrease in HDAC11 expression in the granule cell layer. Compare quantitative evaluation shown in Fig. 5. Scale bar in T: 1 mm.


Granule cell layer
Pyramidal cell layers
HDAC 1
HDAC 2
HDAC 3
HDAC 8
HDAC 11
A
B
C
D
E
F
G
H
I
J
Contralateral GC
Injected GC
Contralateral DG
Ipsilateral DG
CA1
CA3
ROD (% of control)
Time after KA-injection
2h
4h
6h
12h
24h
48h
7d
14d
28d

seizures (about 10 per h), lasting about 2 min were observed. The frequency of these seizure spells progressed to continuous seizure activity after 2 h. Thereafter continuous EEG discharges were observed that started to become somewhat less frequent after 12 h and were significantly reduced in amplitude and frequency thereafter (Fig. 1A). After 24 to 36 h the frequent status epilepticus-like discharges turned into HPD (Fig. 1 C–D). Motor seizures were suppressed by the initial anesthesia. Thus only rare mild motor convulsions were observed in the video recordings.

3.3. Hippocampal paroxysmal discharges (HPD)

The spike/wave discharges seen during status epilepticus turned into short paroxysmal discharges after 48 h characterized by short periods of increased EEG-activity but without obvious behavioral changes (Pallud et al., 2011). They were generally not preceded by spike activity and revealed no postictal depression as observed after spontaneous strong seizures (Fig. 1D and 1F). The mean rate of HPD was 50.7 ± 3.30 per 24 h, the mean amplitude 0.77 ± 0.02 mV and their mean duration 17.5 ± 0.50 s (n = 25). The number of HPD during light (22.9 ± 4.99 per 12 h; n = 7) was not different from that during dark (25.5 ± 5.61 per 12 h; n = 7). They were observed at the same rate during the entire recording period (28 days).

3.4. Spontaneous strong seizures

About 48 h after KA injection, we observed rare strong seizures in the EEGs of the continuously monitored mice in addition to the rather frequent paroxysmal discharges. We monitored 224 spontaneous strong seizures in 7 mice. They were characterized by a high-amplitude EEG activity (1.4 ± 0.06 mV) lasting for more than 30 s (45.0 ± 3.93 s), and – in contrast to HPD – were always followed by a post-ictal flattening of the EEG signal (Fig. 1D and F). Frequency of strong seizures was 1.54 ± 0.14 per 24 h and they tended to occur more frequently (not significant) during night (0.85 ± 0.127 per 12 h; n = 7) than during day (0.61 ± 0.084 per 12 h; n = 7).

3.5. Motor seizures

Motor seizures occurring concomitantly with strong EEG seizures were rated in the video recordings. No changes in motor behavior were observed concomitantly with HPD (number of observations: 50 in 7 mice). In total 60 spontaneous strong seizures were video-analyzed for the concomitant behavioral correlate. Among all strong EEG seizures 91.7% were accompanied by motor seizures of stages 1 to 4. During days 3 to 11 the severity of motor seizures monitored in association with strong EEG seizures increased from median stage 2 at days 3–5 to median stage 4 seizures at days 9 to 11 (Fig. 2; $p < 0.01$).

3.6. Histopathology

At all intervals investigated losses in CA1 and CA3 pyramidal neurons and of interneurons were observed at the injection site as early as 4 h after KA injection. At the early intervals (4 h to 12 h after KA injection) the granule cell layer was not affected. At later intervals we observed modest (24 h) and thereafter (after 14 and 28 days) pronounced granule cell dispersion in the injected hippocampus as reported previously (Haas et al., 2002; Riban et al., 2002; Pallud et al., 2011) and shown by us elsewhere (Jagirdar et al., unpublished results). No signs of histopathological changes were observed in the contralateral hippocampus. Also no neurodegenerative changes were seen in other brain areas such as the amygdala or entorhinal cortex.

3.7. Distribution of HDAC mRNAs in the control hippocampus

Fig. 3 shows the mRNA distribution of class I HDACs 1, 2, 3, 8 (Fig. 3A–D) and class IV HDAC11 (Fig. 3F) in the dorsal hippocampus of control mice. HDAC mRNAs were well detected in the hippocampus of control mice. Since the individual HDAC probes can bind at somewhat different affinity a direct comparison of the extent of expression between different HDAC mRNAs has to be judged with caution. The expression patterns are clearly different between individual HDAC species. Thus, whereas HDAC2, 3 and 11 mRNAs seem to be equally distributed in all principal cell layers of the hippocampus, HDACs 1 and 8 are stronger expressed in the granule cells of the dentate gyrus than in the Ammon's horn. We observed HDAC1, 2, 3 and 11 mRNA expression also in other brain areas e.g., the cerebral cortex in the same section (Fig. 3). The relative intensities of expression as well as the distribution of individual HDAC mRNAs was in agreement to that reported for the rat hippocampus recently (Broide et al., 2007) and with the distribution of HDAC 2 protein shown by immunohistochemistry (Yao et al., 2013).

3.8. Changes in expression of Class I HDACs (HDAC1, 2, 3 and 8) after intra-hippocampal KA injection

Images of HDAC mRNAs at different intervals after KA injection are shown in Fig. 4. For considering also a possible local effect of the KA injection, HDAC mRNA expression was analyzed in the granule cell layers of the injected and of the contralateral hippocampus compared to respective control values (Fig. 5 left column). In addition, changes in the pyramidal layers contralateral to the KA injection, which were not affected by neurodegeneration, were analyzed (Fig. 5 right column). In contrast to changes seen in the expression of class II HDACs (Jagirdar et al., unpublished results), changes in class I and IV mRNA levels were rather similar in the ipsi- and contralateral granule cell layer and in the contralateral pyramidal cell layers (Figs. 4, 5).

3.8.1. HDAC1

As shown in Figs. 4 and 5A, expression of class I HDAC1 mRNA becomes markedly reduced 2, 4, 6 and 12 h after KA injection, both in the ipsi- and in the contralateral granule cell layer (Figs. 4B, 5A; at 4 h: 23.8 ± 3.21%, $p < 0.001$; and 35.2 ± 3.68% of controls, $p < 0.001$, ipsi- and contralateral to the injection, respectively). A trend for decreases in HDAC1 mRNA levels was also observed in pyramidal cell layers at the intervals 2 to 12 h after induction of the status epilepticus (Figs. 4B, 5B; to 51 and 58% of control in CA1 and CA3, respectively; not significant). At this time continuous seizure activity was detected in the EEG (Fig. 1A).

In the granule cell layers, HDAC1 mRNA levels recovered to control values after 24 h and remained unchanged on both sides up to 28 days (except for the injected side after 14 days) (Figs. 4B, 5A). In contrast, in the contralateral pyramidal cell layers we observed pronounced increases after 24 to 48 h (e.g., 48 h; CA1, 272.4 ± 49.94%, $p < 0.001$; CA3, 382.6 ± 34.47% of control, $p < 0.001$). Thereafter HDAC1 mRNA levels returned to control values also in the pyramidal cell layers, except for 57 and 63% increases in sectors CA3 after 14 days and in sector CA1 after 28 days, respectively (Figs. 4C, D, 5B). Behaviorally, the initial

Fig. 5. Time course of changes in expression of class I HDACs (HDAC1, 2, 3 and 8) and class IV HDAC11 after unilateral injection of KA into the hippocampus. The figures show changes in the mRNA expression as relative optic densities (ROD) expressed as % of controls (means ± SEM). The left column depicts changes in mRNA expression in the granule cell layer (GC) comparing the injected (black bars) with the contralateral side (red bars). The right column shows changes in the pyramidal layers (green bars, CA1; brown bars, CA3) contralateral to the injection side. The contralateral side is not affected by cell loss due to the local injection of KA. N numbers: Controls, 23; 2 h, 12; 4 h, 9; 6 h, 10; 12 h, 4; 24 h, 4; 48 h, 3; 7 days, 6; 14 days, 5; 28 days, 7. Statistical analysis was done by one-way ANOVA with Dunnett's post-hoc test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. controls.

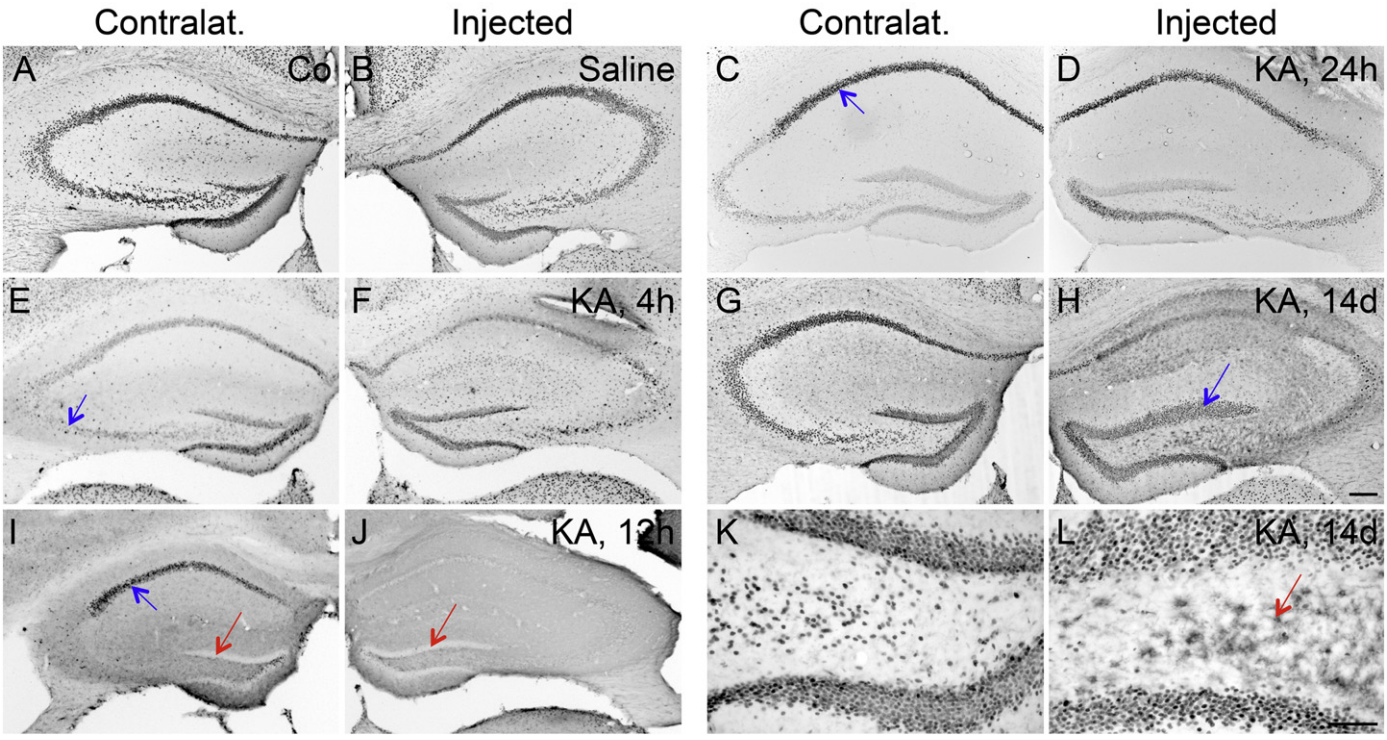


Fig. 6. Time course of expression of HDAC2 immunoreactivity after KA-induced seizures. HDAC2-IR was primarily seen in neurons and rarely in astrocytes. Note the slight general reduction in IR in the contralateral hippocampus (there was no neurodegeneration) after 4 h (E, F; blue arrow). Note the strongly increased expression of the protein in sector CA1 of the contralateral hippocampus 12 and 24 h after KA (blue arrows in I and C, but also on the injected side, D). Interestingly, 12 h after KA there was an entire loss of the protein in dentate granule cells, both on the injected and on the contralateral side (red arrows in I, J). After 14 days HDAC2 protein recovered in all principal cells. Note the beginning of granule cell dispersion (blue arrow in H). There seemed to be also some expression in astrocytes in the hilus of the injected dentate gyrus (Fig. 8L). In some instances wide-spread labeling of glia was observed in the injected hippocampus at later intervals (28 d after KA injection; not shown). We have no proof yet whether this labeling is specific. Scale bars: 200 μm in H for A–H; 60 μm in L, for K, L.

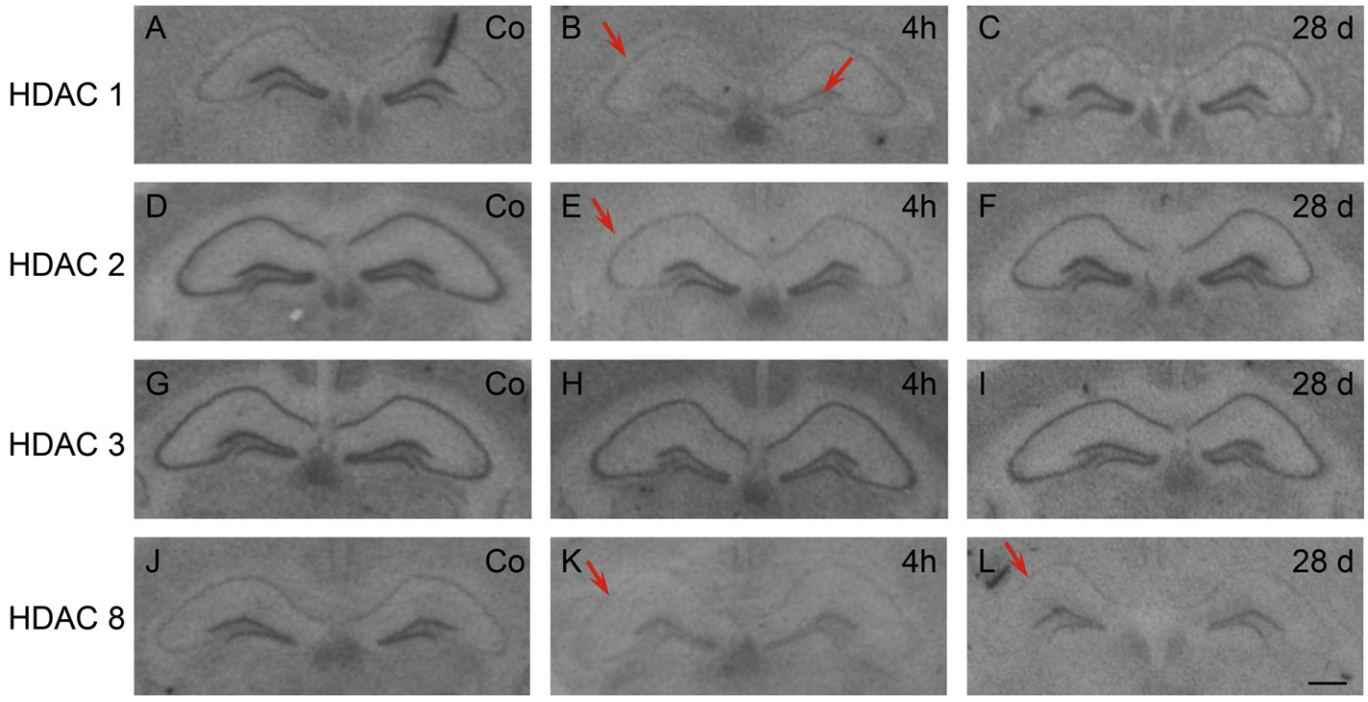


Fig. 7. Expression of HDAC 1, 2, 3, 8 mRNAs after pilocarpine-induced status epilepticus. Shown are photomicrographs of autoradiographs after in situ hybridization with radiolabelled antisense probes for the individual HDACs of controls (Co) and selected time points after pilocarpine injection (4 h and 28 days). Arrows indicate: in B, decreased expression of HDAC1 in the dentate granule cell layer and in the hippocampal pyramidal cell layer 4 h after pilocarpine; E: decreased expression of HDAC2; K, L, decreased expression of HDAC8 4 h and 28 days after KA. Compare quantitative evaluation shown in Fig. 8. Scale bar in L: 500 μm.

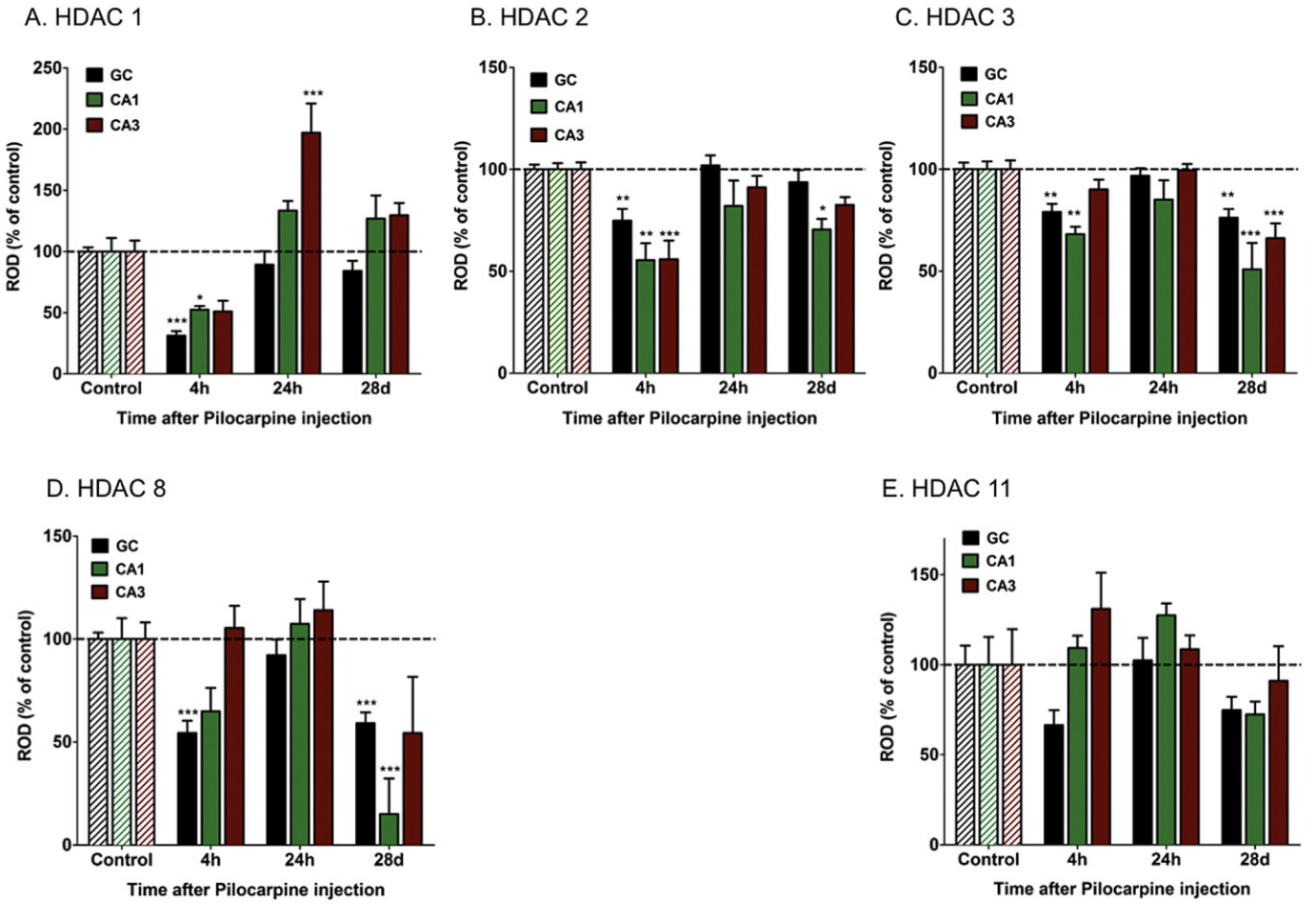


Fig. 8. Time course of changes in expression of HDAC 1, 2, 3, 8 and 11 mRNAs after pilocarpine-induced status epilepticus. The figures show changes in the mRNA expression as relative optic densities (ROD) expressed as % of controls (means ± SEM). Black bars represent granule cell (GC) mRNA, green and brown bars pyramidal cell layers of sectors CA1 and CA3, respectively. Numbers of mice: Controls, 10; 4 h, 9; 24 h, 4; 28 days, 7. Statistical analysis was done by one-way ANOVA with Dunnett's post-hoc test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. controls.

status epilepticus (seen in the EEG) faded away 12 to 24 h after KA and the interval thereafter was characterized by 1 to 2 spontaneous strong EEG-seizures per day (Fig. 1).

3.8.2. HDAC2

Also HDAC2 mRNA expression was significantly reduced in granule cells on both sides 2 to 4 h after KA injection (Fig. 5C). In contrast to HDAC1, HDAC2 mRNA levels were then significantly increased 12 h after KA contralateral to the KA injection (Fig. 5C; 159.6 ± 6.66% of controls, $p < 0.001$), after 24 h in both granule cell layers (Figs. 4G, 5C; ipsilateral to KA injection: 136.7 ± 11.69% of controls, $p < 0.001$; contralateral, 154.7 ± 5.02%, $p < 0.001$) and approached control values after 48 h. HDAC2 mRNA levels were again increased in the granule cell layer of the contralateral hippocampus after 14 days (Figs. 4H, 5C; 142.9 ± 5.78% of controls, $p < 0.001$) and bilaterally after 28 days (Fig. 5C; ipsilateral: 115.6 ± 2.98%, $p < 0.05$; contralateral: 133.2 ± 5.34% of controls, $p < 0.001$).

In sectors CA1 and CA3 of the pyramidal cell layer changes in HDAC2 mRNA levels were characterized by modest decreases (by about 10 to 15%) 4 and 6 h (Figs. 4F, 5D; significant for CA3, $p < 0.05$) after KA injection and by small increases (Figs. 4G, 5D; CA1, 122.7 ± 0.78% of control; $p < 0.01$) after 24 h. We observed also modest increases in sector CA3 at the late intervals after 14 and 28 days (Fig. 5D).

3.8.3. HDAC3

In contrast to the prominent changes seen for HDACs 1 and 2, HDAC3 mRNA was not significantly changed in the granule cell layers at the early intervals (2 to 12 h after KA). It revealed, however, significant increases in its levels at 24 (Fig. 5E; 139.8 ± 10.60% of control; $p < 0.01$) and 48 h intervals (163.4 ± 12.39% of control; $p < 0.001$) in the granule cell layer of the contralateral hippocampus (Fig. 5E). In pyramidal layers CA1 and CA3 (contralateral to KA injection), HDAC3 mRNA expression paralleled that in the contralateral granule cell layer with significant increases after 24 (Figs. 4K, 5E; CA1: 140.0 ± 9.84% of control, $p < 0.01$; CA3: 138.5 ± 13.10%, $p < 0.05$) and 48 h (Fig. 5F; CA1, 151.7 ± 11.30% of control, $p < 0.001$; CA3: 147.9 ± 23.25%, $p < 0.01$) that returned to control levels thereafter (Fig. 5E, F).

3.8.4. HDAC8

HDAC8 was only weakly expressed. The quantitative evaluation was done only in one of three experiments covering the time points 4, 12, 24 and 48 h and 14 days after KA injection (Fig. 5G, H). At the 4 h interval we observed a 53.3 ± 8.25% ($p < 0.05$) and 34.7 ± 8.73% (not significant) decrease of HDAC8 mRNA levels in the ipsilateral and contralateral granule cell layers, respectively (Figs. 4N, 5G). Twelve and 24 h after KA injection HDAC8 mRNA levels were markedly increased in the contralateral granule cell layer, statistically significant for the 12 h interval (160.4 ± 19.27% $p < 0.05$) (Figs. 4O, 5G).

Similarly as for HDAC3, 12 to 48 h after KA in contralateral pyramidal cell layers of sectors CA1 and CA3 up to 60 and 86% increased HDAC8 mRNA levels were observed, respectively. Due to the small sample size in the experiment the numbers are, however, statistically not significant (Fig. 5H).

3.9. Expression of class IV HDAC11

HDAC11 mRNA is prominently expressed throughout the entire hippocampus (Figs. 3, 4Q). Its levels were dramatically decreased (by 35.4% to 93.8%; P-values 0.05 to 0.001) throughout the entire hippocampus (granule cell and pyramidal cell layers) 6 to 24 h after unilateral KA injection (Figs. 4R, S, 5I, J). They recovered rapidly thereafter and were similar to control levels 2 to 28 days after KA injection (Figs. 4T, 5I). In the dentate gyrus the early decreases mostly exceeded 75% and reached more than 93% in the granule cell layer of the injected hippocampus after 12 and 24 h (Fig. 5I; 12 h: granule cell layer: ipsilateral, 6.1 ± 5.53% of control, $p < 0.001$; contralateral: 33.2 ± 16.32% of control, $p < 0.01$). In the pyramidal cell layer of sectors CA1 and CA3 decreases in HDAC11 mRNA levels were 40 to 70% between 4 and 24 h after KA injection (Fig. 5J; e.g., 24 h: CA1, 30.2 ± 18.65% of control, $p < 0.001$; CA3, 27.4 ± 17.26% of control, $p < 0.05$).

3.10. Changes in HDAC protein after intra-hippocampal injection of KA

To verify the observed changes in the expression of HDAC mRNAs on the protein level we attempted to perform immunohistochemistry. We first tested several antibodies by western blotting and observed for most of them several unspecific bands and obtained low quality immune histochemical labeling. The comparatively high concentration of HDAC2 protein and two valuable antibodies (antibody 5223 from Cell Signaling and ab51823 from Abcam) then allowed clear detection of HDAC2.

As shown in Fig. 6A, B, HDAC2 is highly expressed in nuclei of neurons. Its distribution in the hippocampus follows that of its mRNA distribution (Fig. 3). Rarely HDAC2-immunoreactivity (IR) was also seen in astrocytes. Taken the contralateral hippocampus (there was no neurodegeneration) we observed a slight general reduction in HDAC2-IR (Fig. 6E, F). This was contrasted by a pronounced increased expression of the protein in sector CA1 of the contralateral hippocampus 12 and 24 h after KA injection (Fig. 6I, C, but also D). Interestingly 12 h after KA injection there was an entire loss of the protein in dentate granule cells, both on the injected and on the contralateral side (Fig. 6I, J). After 14 d HDAC2 protein had recovered in all principal cells. HDAC2-IR indicated expression of the protein in astrocytes of the dentate hilus of injected hippocampus (Fig. 6L) In some instances wide-spread labeling of glia was observed in the injected hippocampus at later intervals (28 days after KA injection; not shown). We have no proof yet whether this labeling is specific.

3.11. Pilocarpine model

3.11.1. Behavior and neuropathology

In order to separate changes of HDAC expression that are due to seizure activity and epileptogenesis from those that are generated by stimulation of KA receptors we investigated the expression of HDAC mRNAs also in the pilocarpine model of epilepsy. As described by before (Cavalheiro et al., 1996; Pitsch et al., 2007), pilocarpine induced a status epilepticus characterized by tonic-clonic seizures and full limbic convulsions with rearing and falling over in all mice investigated. The status epilepticus was interrupted by injection of diazepam 45 min after the first limbic seizure. We performed no EEG recordings in these mice. Histopathological changes were characterized by (20–40%) loss of CA1 and CA3 pyramidal neurons seen at the 24 h and 28 day intervals.

3.11.2. Expression of HDAC mRNAs in the pilocarpine model of TLE

Also in the pilocarpine model of TLE changes in the expression of individual HDACs followed distinctly different patterns, both in the regional expression and in the temporal patterns. In contrast to our findings after intra-hippocampal KA injection, changes in the individual HDAC transcripts after pilocarpine-induced status epilepticus were similar in different hippocampal subfields.

3.11.3. Class I HDAC 1, 2, 3 and 8 mRNAs

As shown in Figs. 7 and 8, mRNA levels of class I HDACs 1, 2, 3 and 8 were decreased in the pyramidal cell layer of sector CA1 and in dentate granule cells during the status epilepticus at 4 h after pilocarpine injection. These decreases were more pronounced for HDAC1, 2 and 8 mRNAs (by 25 to 70%) than for HDAC3 (20 to 30%) (Figs. 7B, E, H, K, 8A–D; granule cell layer: HDAC1, 31.2 ± 3.81% of control, $p < 0.001$; HDAC2, 74.8 ± 5.87% of control, $p < 0.01$; HDAC3, 79.1 ± 3.91% of control, $p < 0.01$; HDAC8, 54.4 ± 5.95% of control, $p < 0.01$). No changes were observed in HDAC3 and 8 mRNA expression at the 4 h interval in the pyramidal cell layer of sector CA3. After 24 h, HDAC2, 3 and 8 mRNA levels had returned to control values but, interestingly, increased for HDAC1 in the pyramidal cell layer (Fig. 8A; CA3: 197.0 ± 23.90% of control, $p < 0.001$). At the late interval, 28 days after the initial status epilepticus, HDAC1 mRNA levels tended to increase (not significant) in the sectors CA1 and CA3, whereas those of HDAC2 tended to decrease (significant only for sector CA1). HDAC3 and HDAC8 mRNA levels were significantly decreased in all principal cell layers of the hippocampus by 25 to 50% and 40 to 85%, respectively (Fig. 8C, D).

3.11.4. Class IV HDAC 11 mRNA

HDAC11 mRNA levels were not significantly altered, although there was a tendency for a decrease in the granule cell layer (by about 35%) at the 4 h interval, and in CA1 pyramidal and in the granule cells after 28 days (Fig. 8E).

4. Discussion

4.1. Seizure behavior of mice after unilateral intra-hippocampal injection of KA

Changes in the EEG were similar as previously reported (Bouilleret et al., 1999; Riban et al., 2002; Pallud et al., 2011). They were characterized by a trias of epilepsy-like events, 1) the acute status epilepticus, 2) frequent HPD and 3) rare spontaneous strong seizures reflecting manifest epilepsy in the mice. All three events appear to be generated in the hippocampus, respectively the dentate gyrus (Pallud et al., 2011). Continuous telemetric recordings used in our study allowed a further characterization of their onset and frequency. Interestingly, based on EEG-recordings the initial KA-induced status epilepticus lasted about 4 to 24 h and was most severe after around 4 to 8 h although behavioral seizures were modest and rare. It was followed by frequent HPD (around 50 per 24 h) observed during the entire 28 day recording period. Strong spontaneous seizures were considerably less frequent (around 1.5 per 24 h). In contrast to other TLE models (Drexel et al., 2012b) the onset of spontaneous strong seizures was uniform and fast (already 3 days after KA). An interesting finding was that strong EEG-seizures were initially accompanied primarily by stage 2 behavioral seizures (median) and they rapidly (at days 9 to 11) developed to full motor seizures (median 4). This different interval between onset of strong EEG seizures and of full motor seizures may be part of a rather brief period of epileptogenesis. It may relate also to the expression patterns of some HDAC mRNAs that were distinctly different at the 24 to 48 h interval.

4.2. Early changes in HDAC mRNA expression during acute KA-induced status epilepticus

Notably the class I HDACs, HDAC1 and 2 were down-regulated on both sides in the dentate gyrus 2 to 6 h after KA injection and in sectors CA1 and CA3 after 4 and 6 h. This was paralleled by even more prominent down-regulation of class IV HDAC11 in dentate granule cells and hippocampal pyramidal cells. Interestingly, changes in IR did not entirely match changes in mRNA expression of HDAC2. There was a general decrease in HDAC2 protein in the hippocampus during the status epilepticus reflecting that in mRNA expression. With some delay,

however, we observed an even stronger decrease in HDAC2 protein in the granule cell layers of both sides when mRNA levels recovered already there. The subsequently increased expression in the pyramidal cell layer and the recovery and modest increases in all principal layers after 14 days was similar on protein and mRNA level. These changes may be clearly related to the acute generalized EEG seizures during the status epilepticus. Strikingly, these rapid decreases in HDAC mRNA levels were restricted to class I and IV HDACs; they were not seen for class IIa and IIb mRNAs (Jagirdar et al., unpublished results). Early changes in the pilocarpine model were very similar to those in the kainate model, especially in respect to the pronounced decreases in HDAC1, 2 and 8 mRNA expression in all hippocampal subfields during the status epilepticus (4 h) although the early decrease in HDAC2 mRNA levels seen in all principal layers of the hippocampus after pilocarpine were not seen in the KA model.

Decreased mRNA levels have been suggested to occur in the course of status epilepticus due to the marked activation of transcription (Wasterlain, 1972). Here we observe decreases in individual HDAC mRNAs (HDAC1, and 11) but not in HDAC3 mRNA. HDAC5 mRNA expression is even increasing at the same time (Jagirdar et al., unpublished results). The decreased expression of class I HDACs in both seizure models coincides with the previously reported hyperacetylation of histone H4 at *c-fos* and *c-jun* promoters and increased *c-fos* and *c-jun* expression 0.5 to 3 h after a status epilepticus induced by i.p. KA injection in mice (Sng et al., 2006). Six h after KA injection, acetylated histones approached control levels again. Acetylated H4 co-precipitated with *c-fos* and *c-jun* DNA and therefore may have facilitated the observed increases in immediate early gene expression. Pretreatment with the histone acetyltransferase inhibitor curcumin suppressed H4 acetylation, *c-fos* and *c-jun* expression and acute seizure activity (Sng et al., 2006). These experiments clearly indicate a role of gene products expressed upon H4 acetylation. The fast decreases in class I HDAC expression in our KA and pilocarpine treated mice may contribute to the increased levels of hyperacetylated H4. This is supported by the fact that treatment with the HDAC inhibitor trichostatin A also augments *c-fos* and *c-jun* expression both in control rats and after KA-induced status epilepticus (Sng et al., 2005). Like H4 acetylation also down-regulation of class I HDACs (in our experiments) was transient indicating a possible causative relationship of the initial decrease in class I HDAC expression and the hyperacetylation of H4.

Consequences of seizure-induced changes in H4 acetylation, however, highly depend on the individual promoter involved. Thus, expression of brain derived neurotrophic factor (BDNF) is rapidly induced in granule cells and pyramidal cells of the hippocampus after experimentally induced status epilepticus in rats (Isackson et al., 1991; Dugich-Djordjevic et al., 1992). Interestingly, seizure-induced H4 acetylation may be differently affected at different BDNF promoters (Huang et al., 2002). Downstream promoters (P1 and P2) for splice variants 1 and 2 are associated with *hyper*acetylated H4 whereas the upstream P4 promoter (corresponding to splice variant 4) co-precipitated with *hypo*acetylated H4 three hours after pilocarpine-induced status epilepticus. This indicates that differential histone H3 and H4 acetylation/deacetylation at different promoters of the same gene may regulate expression of different BDNF splice variants upon epileptic seizures (Timmusk et al., 1993; Huang et al., 2002; Tsankova et al., 2004). BDNF splice variants appear to be differently targeted into different subcellular compartments of hippocampal principal neurons, such as soma, dendrites or axons (Chiaruttini et al., 2008) and over-expression of BDNF may exert both pro- and anti-convulsive/-epileptogenic actions (Simonato et al., 2006).

Besides transient overexpression of BDNF and of immediate early genes *c-fos* and *c-jun* (Dragunow and Robertson, 1987) acute seizures increase gene expression of numerous neuropeptides and proteins associated e.g., with inflammation, neurodegeneration, neuronal plasticity or neurotransmission (Forstermann et al., 1982; Sperk, 1994; Gorter et al., 2006; Vezzani et al., 2011). Decreased expression of HDACs may be one mechanism facilitating expression of these genes. The respective gene products may support pro-convulsive (Forstermann et al., 1982) or endogenous anticonvulsive mechanisms (Sperk et al., 1992; Esclapez and Houser, 1999; Szabo et al., 2000). Thus, increased expression of the T-type calcium channel α1-subunit Ca*v*3.2 after pilocarpine-induced status epilepticus leads to increased cellular T-type Ca^{++} currents and an increase in intrinsic burst firing. Since these functional changes are absent in Ca*v*3.2 knock out mice they may represent a specific seizure-promoting mechanism (Becker et al., 2008).

In contrast, treatment with the anticonvulsive class I HDAC inhibitor valproic acid increases expression of neuropeptide Y in the reticular nucleus thalami of rats suggesting that the expression of neuropeptide Y may be under the control of HDACs (Brill et al., 2006). Neuropeptide Y becomes over-expressed by recurrent seizures and represents a potent endogenous anticonvulsive principle (Vezzani and Sperk, 2004). Thus, down-regulation of HDACs may lead to an anticonvulsive effect by increasing neuropeptide Y expression in the same way as their inhibition by treatment with valproic acid.

4.3. Transient up-regulation of class I HDACs 12 to 48 h after seizures induced by intrahippocampal injection of KA

Subsequent to their pronounced down-regulation, class I HDACs 1, 2 and 3 were significantly up-regulated in the contralateral granule cell and pyramidal cell layers 24 and 48 h after KA injection. This finding is consistent with increased expression of HDAC2 protein in the temporal neocortex of patients with temporal lobe epilepsy and in the dentate gyrus of rats in acute (1 day) and chronic phase (60 days) after lithium/pilocarpine induced status epilepticus (Huang et al., 2012; Chen et al., 2013).

The changes in HDAC expression could be merely counter-regulation of their preceding down-regulation and interrupt or even reverse the increased gene expression of the corresponding proteins and peptides. Increased expression of HDACs may lead to decreased expression of certain genes and thereby promote epileptogenesis. Thus, expression of the AMPA receptor subunit GluA2 is suppressed in animal models of epilepsy resulting in potent pro-convulsive effects (Grooms et al., 2000; Gorter et al., 2006). Decreased expression of the AMPA receptor subunit GluA2 (but not of GluA1) after KA induced seizures leads to GluA2 lacking AMPA receptors revealing increased Ca^{++} influx upon stimulation and thus increased vulnerability to seizure propagation and excitotoxic brain damage (Grooms et al., 2000; Gorter et al., 2006). Huang et al. (2002) reported decreased acetylation of the H4 histone at the GluA2 promoter in the hippocampal CA3 sector as early as 3 h after pilocarpine-induced status epilepticus. Also these experiments indicate a role of histone acetylation/deacetylation. In our experiments up-regulation of HDACs 1 to 3 in granule cell and pyramidal cell layers occurred at a somewhat later interval (12 to 24 h after KA injection). As shown elsewhere (Jagirdar et al., unpublished results) we observed up-regulation of class II HDAC5 in granule and pyramidal cell layers already 4 to 6 h lasting up to 12 and 48 h after KA injection. Thus in principle increased expression of class I HDACs and of class II HDAC5 could be responsible for the reduced acetylation of histone H4 at the GluA2 promoter and for reduced GluA2 expression. Interestingly, the HDAC inhibitor trichostatin A prevented and quickly reversed deacetylation of GluA2-associated histones (Huang et al., 2002). Trichostatin A also blunted seizure-induced downregulation of GluA2 mRNA in CA3 indicating a mechanism on the level of histone deacetylation rather than decreased activity of histone acetyltransferases. Also decreased expression of $GABA_A$ receptor subunits δ and α5 after KA and pilocarpine-induced status epilepticus, mediating tonic inhibition, may contribute to epileptogenesis (Schwarzer et al., 1997; Tsunashima et al., 1997; Peng et al., 2004; Drexel et al., 2013). These changes in the constitution of $GABA_A$ receptors are, however, not transient but long lasting.

There was an obvious difference between the kainate and the pilocarpine models in respect to the up-regulation of HDACs 1 to 3 at the 24 h interval. It was clearly seen in the KA model but not after pilocarpine injection. Interesting is also the transient nature of this effect. The most likely explanation is that we did not pick up changes in the pilocarpine model due to a slightly different time course of seizure induced changes in HDAC expression. Whereas we investigated the 24 h and 48 h intervals after KA injection we examined it only 24 h after pilocarpine injection. It is noteworthy that the process of epileptogenesis appears to be faster in the KA model. Spontaneous strong EEG seizures occurred already after 3 days and were associated with full motor seizures between 6 and 11 days. In contrast, in the mouse pilocarpine model development of spontaneous strong EEG seizures lasts between 4 and 42 days (Cavalheiro et al., 1996). Thus it may be that if increased expression of HDACs is related to epileptogenesis it may occur at different intervals in the KA and pilocarpine models.

Changes in the expression of HDACs may, however, also induce different epilepsy-related changes, such as apoptosis, neuronal sprouting, or regulation of axon formation resulting in different cognitive or neuropsychological changes observed in TLE. Thus, overexpression of HDAC2, but not of HDAC1, decreases dendritic spine density, synapse number, and synaptic plasticity in the CA1 sector of the hippocampus as well as memory formation in mice (Guan et al., 2009). Conversely, HDAC2 knock out results in increased synapse number and memory facilitation, similar to chronic treatment with HDAC inhibitors in mice. And, the reduced synapse number and learning impairment of HDAC2 overexpressing mice can be ameliorated by chronic treatment with HDAC inhibitors (Guan et al., 2009). In contrast, viral vector-induced overexpression of HDAC1 in the hippocampus of mice had no effect on short- and long-term memory acquisition but promoted contextual fear extinction (Bahari-Javan et al., 2012). Increases in HDAC1 and 2 expression, however, were transient at the 24 to 48 h intervals after KA injection and thus may not substantially influence the neuropsychological behavior of the epileptic mice. Less prominent increases in HDAC1 and 2 mRNA levels were also observed at the late intervals (14 and 28 days after KA injection). They may be related to cognitive and neuropsychological changes observed in chronically epileptic mice, including impaired spatial memory and decreased anxiety (Chauviere et al., 2009; Jones et al., 2009). The functional consequence (epilepsy?) of these increases in HDAC expression may be experimentally counteracted by HDAC inhibition e.g., with valproic acid.

4.4. Long-time changes in HDAC expression in TLE models

Main findings at the 14 and 28 d intervals were a significant up-regulation of HDAC1 and 2 mRNAs in the KA model and down-regulation of HDACs 3 and 8 in the pilocarpine model. Both changes were statistically significant and may relate the chronic epileptic state in these animals. Since these changes are distinctly different in the two models they argue also for differences in their pathophysiology. Major differences between the two models are the frequent HPD and the granule cell dispersion observed after intra-hippocampal kainate but not after pilocarpine. How these could relate to the observed differences in HDAC expression will require further investigations.

In conclusion, our data demonstrate pronounced and distinctively different changes in the expression of class I and IV HDACs in subfields of the hippocampus after an acute status epilepticus, during epileptogenesis and in chronic TLE. They suggest a crucial role of gene expression of HDACs in the epigenetic regulation of seizure-induced expression of neuropeptides and proteins and thereby promote the development of epilepsy as well as the formation of endogenous anticonvulsive mechanisms.

5. Limitations of our study

Our study concentrated on patterns of the expression of HDACs on the mRNA level. It is rather descriptive and with the exception of HDAC2 we have no indication yet whether presumed changes in protein expression follow that of mRNA levels. Also the link to functional changes affecting regulation of expression of different proteins and their role in epileptogenesis is of course missing. This was far beyond the aim of this initial unbiased approach. Since there is a clear balance between the actions of histone acetyl transferases and HDACs, knowledge on epilepsy-related changes in the expression of histone acetyl transferases would be as pertinent as that for HDACs. HDACs may, however, act also on other targets than on histones, notably on cytoplasmatic proteins. We address this question in a submitted manuscript (Jagirdar, unpublished). On the other hand we refer to a variety of studies indicating changes in the acetylation state of specific promoters of genes presumably related to epileptogenesis (e.g., Huang et al., 2012; Chen et al., 2013). This gives clear indication for the relevance of the observed dynamic changes in HDAC mRNA expression. A direct link of the here described changes in the expression of HDACs to these epigenetic mechanisms, however, has still to be corroborated.

Acknowledgments

We thank Anneliese Bukovac and Elisabeth Gasser for technical assistance and Dr. Heide Hörtnagl for proof reading the manuscript. The work was supported by the European Science Foundation (Eurocores Programme; EuroEPIGENOMICS) and the Austrian Research Fund (Project I 664).

References

Babb, T.L., Lieb, J.P., Brown, W.J., Pretorius, J., Crandall, P.H., 1984. Distribution of pyramidal cell density and hyperexcitability in the epileptic human hippocampal formation. Epilepsia 25, 721–728.
Bahari-Javan, S., Maddalena, A., Kerimoglu, C., Wittnam, J., Held, T., Bahr, M., Burkhardt, S., Delalle, I., Kugler, S., Fischer, A., Sananbenesi, F., 2012. HDAC1 regulates fear extinction in mice. J. Neurosci. 32, 5062–5073.
Becker, A.J., Pitsch, J., Sochivko, D., Opitz, T., Staniek, M., Chen, C.C., Campbell, K.P., Schoch, S., Yaari, Y., Beck, H., 2008. Transcriptional upregulation of Cav3.2 mediates epileptogenesis in the pilocarpine model of epilepsy. J. Neurosci. 28, 13341–13353.
Bouilleret, V., Ridoux, V., Depaulis, A., Marescaux, C., Nehlig, A., Le Gal La Salle, G., 1999. Recurrent seizures and hippocampal sclerosis following intrahippocampal kainate injection in adult mice: electroencephalography, histopathology and synaptic reorganization similar to mesial temporal lobe epilepsy. Neuroscience 89, 717–729.
Brill, J., Lee, M., Zhao, S., Fernald, R.D., Huguenard, J.R., 2006. Chronic valproic acid treatment triggers increased neuropeptide y expression and signaling in rat nucleus reticularis thalami. J. Neurosci. 26, 6813–6822.
Broide, R.S., Redwine, J.M., Aftahi, N., Young, W., Bloom, F.E., Winrow, C.J., 2007. Distribution of histone deacetylases 1–11 in the rat brain. J. Mol. Neurosci. 31, 47–58.
Cavalheiro, E.A., Santos, N.F., Priel, M.R., 1996. The pilocarpine model of epilepsy in mice. Epilepsia 37, 1015–1019.
Chauviere, L., Rafrafi, N., Thinus-Blanc, C., Bartolomei, F., Esclapez, M., Bernard, C., 2009. Early deficits in spatial memory and theta rhythm in experimental temporal lobe epilepsy. J. Neurosci. 29, 5402–5410.
Chen, Y., Xie, Y., Wang, H., Chen, Y., 2013. SIRT1 expression and activity are up-regulated in the brain tissue of epileptic patients and rat models. Nan Fang Yi Ke Da Xue Xue Bao 33, 528–532.
Chiaruttini, C., Sonego, M., Baj, G., Simonato, M., Tongiorgi, E., 2008. BDNF mRNA splice variants display activity-dependent targeting to distinct hippocampal laminae. Mol. Cell. Neurosci. 37, 11–19.
Chuang, D.M., Leng, Y., Marinova, Z., Kim, H.J., Chiu, C.T., 2009. Multiple roles of HDAC inhibition in neurodegenerative conditions. Trends Neurosci. 32, 591–601.
Clynen, E., Swijsen, A., Raijmakers, M., Hoogland, G., Rigo, J.M., 2014. Neuropeptides as targets for the development of anticonvulsant drugs. Mol. Neurobiol. 50, 626–646.
Dragunow, M., Robertson, H.A., 1987. Kindling stimulation induces c-fos protein(s) in granule cells of the rat dentate gyrus. Nature 329, 441–442.
Drexel, M., Kirchmair, E., Wieselthaler-Holzl, A., Preidt, A.P., Sperk, G., 2012a. Somatostatin and neuropeptide Y neurons undergo different plasticity in parahippocampal regions in kainic acid-induced epilepsy. J. Neuropathol. Exp. Neurol. 71, 312–329.
Drexel, M., Preidt, A.P., Sperk, G., 2012b. Sequel of spontaneous seizures after kainic acid-induced status epilepticus and associated neuropathological changes in the subiculum and entorhinal cortex. Neuropharmacology 63, 806–817.
Drexel, M., Kirchmair, E., Sperk, G., 2013. Changes in the expression of GABAA receptor subunit mRNAs in parahippocampal areas after kainic acid induced seizures. Front Neural Circuits 7, 142.

Dugich-Djordjevic, M.M., Tocco, G., Lapchak, P.A., Pasinetti, G.M., Najm, I., Baudry, M., Hefti, F., 1992. Regionally specific and rapid increases in brain-derived neurotrophic factor messenger RNA in the adult rat brain following seizures induced by systemic administration of kainic acid. Neuroscience 47, 303–315.
Esclapez, M., Houser, C.R., 1999. Up-regulation of GAD65 and GAD67 in remaining hippocampal GABA neurons in a model of temporal lobe epilepsy. J. Comp. Neurol. 412, 488–505.
Fisher, P.D., Sperber, E.F., Moshe, S.L., 1998. Hippocampal sclerosis revisited. Brain Dev. 20, 563–573.
Forstermann, U., Heldt, R., Knappen, F., Hertting, G., 1982. Potential anticonvulsive properties of endogenous prostaglandins formed in mouse brain. Brain Res. 240, 303–310.
Franklin, K.B.J., Paxinos, G., 2008. The Mouse Brain in Stereotaxic Coordinates. Elsevier, Amsterdam.
Furtinger, S., Pirker, S., Czech, T., Baumgartner, C., Ransmayr, G., Sperk, G., 2001. Plasticity of Y1 and Y2 receptors and neuropeptide Y fibers in patients with temporal lobe epilepsy. J. Neurosci. 21, 5804–5812.
Gass, P., Herdegen, T., Bravo, R., Kiessling, M., 1992. Induction of immediate early gene encoded proteins in the rat hippocampus after bicuculline-induced seizures: differential expression of KROX-24, FOS and JUN proteins. Neuroscience 48, 315–324.
Gorter, J.A., van Vliet, E.A., Aronica, E., Breit, T., Rauwerda, H., Lopes da Silva, F.H., Wadman, W.J., 2006. Potential new antiepileptogenic targets indicated by microarray analysis in a rat model for temporal lobe epilepsy. J. Neurosci. 26, 11083–11110.
Grooms, S.Y., Opitz, T., Bennett, M.V., Zukin, R.S., 2000. Status epilepticus decreases glutamate receptor 2 mRNA and protein expression in hippocampal pyramidal cells before neuronal death. Proc. Natl. Acad. Sci. U. S. A. 97, 3631–3636.
Guan, J.S., Haggarty, S.J., Giacometti, E., Dannenberg, J.H., Joseph, N., Gao, J., Nieland, T.J., Zhou, Y., Wang, X., Mazitschek, R., Bradner, J.E., DePinho, R.A., Jaenisch, R., Tsai, L.H., 2009. HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459, 55–60.
Haas, C.A., Dudeck, O., Kirsch, M., Huszka, C., Kann, G., Pollak, S., Zentner, J., Frotscher, M., 2002. Role for reelin in the development of granule cell dispersion in temporal lobe epilepsy. J. Neurosci. 22, 5797–5802.
Holliday, R., Pugh, J.E., 1975. DNA modification mechanisms and gene activity during development. Science 187, 226–232.
Houser, C.R., 1990. Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. Brain Res. 535, 195–204.
Huang, Y., Doherty, J.J., Dingledine, R., 2002. Altered histone acetylation at glutamate receptor 2 and brain-derived neurotrophic factor genes is an early event triggered by status epilepticus. J. Neurosci. 22, 8422–8428.
Huang, Y., Zhao, F., Wang, L., Yin, H., Zhou, C., Wang, X., 2012. Increased expression of histone deacetylases 2 in temporal lobe epilepsy: a study of epileptic patients and rat models. Synapse 66, 151–159.
Hwang, J.Y., Aromolaran, K.A., Zukin, R.S., 2013. Epigenetic mechanisms in stroke and epilepsy. Neuropsychopharmacology 38, 167–182.
Isackson, P.J., Huntsman, M.M., Murray, K.D., Gall, C.M., 1991. BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: temporal patterns of induction distinct from NGF. Neuron 6, 937–948.
Jones, N.C., Kumar, G., O'Brien, T.J., Morris, M.J., Rees, S.M., Salzberg, M.R., 2009. Anxiolytic effects of rapid amygdala kindling, and the influence of early life experience in rats. Behav. Brain Res. 203, 81–87.
Khorasanizadeh, S., Ostankovitch, M., 2014. Zooming into epigenetic regulatory elements in health and disease. J. Mol. Biol. 426, 3327–3329.
Kimura, A., Matsubara, K., Horikoshi, M., 2005. A decade of histone acetylation: marking eukaryotic chromosomes with specific codes. J. Biochem. 138, 647–662.
Kobow, K., Blumcke, I., 2014. Epigenetic mechanisms in epilepsy. Prog. Brain Res. 213, 279–316.
McClelland, S., Flynn, C., Dube, C., Richichi, C., Zha, Q., Ghestem, A., Esclapez, M., Bernard, C., Baram, T.Z., 2011. Neuron-restrictive silencer factor-mediated hyperpolarization-activated cyclic nucleotide gated channelopathy in experimental temporal lobe epilepsy. Ann. Neurol. 70, 454–464.
Pallud, J., Haussler, U., Langlois, M., Hamelin, S., Devaux, B., Deransart, C., Depaulis, A., 2011. Dentate gyrus and hilus transection blocks seizure propagation and granule cell dispersion in a mouse model for mesial temporal lobe epilepsy. Hippocampus 21, 334–343.
Peng, Z., Huang, C.S., Stell, B.M., Mody, I., Houser, C.R., 2004. Altered expression of the delta subunit of the GABAA receptor in a mouse model of temporal lobe epilepsy. J. Neurosci. 24, 8629–8639.
Pitsch, J., Schoch, S., Gueler, N., Flor, P.J., van der Putten, H., Becker, A.J., 2007. Functional role of mGluR1 and mGluR4 in pilocarpine-induced temporal lobe epilepsy. Neurobiol. Dis. 26, 623–633.
Rakhade, S.N., Jensen, F.E., 2009. Epileptogenesis in the immature brain: emerging mechanisms. Nat. Rev. Neurol. 5, 380–391.
Riban, V., Bouilleret, V., Pham-Le, B.T., Fritschy, J.M., Marescaux, C., Depaulis, A., 2002. Evolution of hippocampal epileptic activity during the development of hippocampal sclerosis in a mouse model of temporal lobe epilepsy. Neuroscience 112, 101–111.
Schwarzer, C., Tsunashima, K., Wanzenbock, C., Fuchs, K., Sieghart, W., Sperk, G., 1997. GABA(A) receptor subunits in the rat hippocampus II: altered distribution in kainic acid-induced temporal lobe epilepsy. Neuroscience 80, 1001–1017.
Sharma, A.K., Reams, R.Y., Jordan, W.H., Miller, M.A., Thacker, H.L., Snyder, P.W., 2007. Mesial temporal lobe epilepsy: pathogenesis, induced rodent models and lesions. Toxicol. Pathol. 35, 984–999.
Simonato, M., Tongiorgi, E., Kokaia, M., 2006. Angels and demons: neurotrophic factors and epilepsy. Trends Pharmacol. Sci. 27, 631–638.
Sng, J.C., Taniura, H., Yoneda, Y., 2005. Inhibition of histone deacetylation by trichostatin A intensifies the transcriptions of neuronal c-fos and c-jun genes after kainate stimulation. Neurosci. Lett. 386, 150–155.
Sng, J.C., Taniura, H., Yoneda, Y., 2006. Histone modifications in kainate-induced status epilepticus. Eur. J. Neurosci. 23, 1269–1282.
Sperk, G., 1994. Kainic acid seizures in the rat. Prog. Neurobiol. 42, 1–32.
Sperk, G., Lassmann, H., Baran, H., Kish, S.J., Seitelberger, F., Hornykiewicz, O., 1983. Kainic acid induced seizures: neurochemical and histopathological changes. Neuroscience 10, 1301–1315.
Sperk, G., Marksteiner, J., Gruber, B., Bellmann, R., Mahata, M., Ortler, M., 1992. Functional changes in neuropeptide Y- and somatostatin-containing neurons induced by limbic seizures in the rat. Neuroscience 50, 831–846.
Sperk, G., Wieselthaler-Holzl, A., Pirker, S., Tasan, R., Strasser, S.S., Drexel, M., Pifl, C., Marschalek, J., Ortler, M., Trinka, E., Heitmair-Wietzorrek, K., Ciofi, P., Feucht, M., Baumgartner, C., Czech, T., 2012. Glutamate decarboxylase 67 is expressed in hippocampal mossy fibers of temporal lobe epilepsy patients. Hippocampus 22, 590–603.
Szabo, G., Kartarova, Z., Hoertnagl, B., Somogyi, R., Sperk, G., 2000. Differential regulation of adult and embryonic glutamate decarboxylases in rat dentate granule cells after kainate-induced limbic seizures. Neuroscience 100, 287–295.
Timmusk, T., Palm, K., Metsis, M., Reintam, T., Paalme, V., Saarma, M., Persson, H., 1993. Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron 10, 475–489.
Tsankova, N.M., Kumar, A., Nestler, E.J., 2004. Histone modifications at gene promoter regions in rat hippocampus after acute and chronic electroconvulsive seizures. J. Neurosci. 24, 5603–5610.
Tsunashima, K., Schwarzer, C., Kirchmair, E., Sieghart, W., Sperk, G., 1997. GABA(A) receptor subunits in the rat hippocampus III: altered messenger RNA expression in kainic acid-induced epilepsy. Neuroscience 80, 1019–1032.
Vezzani, A., Sperk, G., 2004. Overexpression of NPY and Y2 receptors in epileptic brain tissue: an endogenous neuroprotective mechanism in temporal lobe epilepsy? Neuropeptides 38, 245–252.
Vezzani, A., French, J., Bartfai, T., Baram, T.Z., 2011. The role of inflammation in epilepsy. Nat. Rev. Neurol. 7, 31–40.
Voelter-Mahlknecht, S., Ho, A.D., Mahlknecht, U., 2005. Chromosomal organization and localization of the novel class IV human histone deacetylase 11 gene. Int. J. Mol. Med. 16, 589–598.
Wasterlain, C.G., 1972. Breakdown of brain polysomes in status epilepticus. Brain Res. 39, 278–284.
Williams, P.A., White, A.M., Clark, S., Ferraro, D.J., Swiercz, W., Staley, K.J., Dudek, F.E., 2009. Development of spontaneous recurrent seizures after kainate-induced status epilepticus. J. Neurosci. 29, 2103–2112.
Yao, Z.G., Zhang, L., Huang, L., Zhu, H., Liu, Y., Ma, C.M., Sheng, S.L., Qin, C., 2013. Regional and cell-type specific distribution of HDAC2 in the adult mouse brain. Brain Struct. Funct. 218, 563–573.