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Impaired neuronal operation through aberrant intrinsic plasticity in epilepsy

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Objective

ABSTRACT

Patients with temporal lobe epilepsy often display cognitive comorbidity with recurrent seizures. However, the cellular mechanisms underlying the impairment of neuronal information processing remain poorly understood in temporal lobe epilepsy. Within the hippocampal formation neuronal networks undergoe major reorganization, including the sprouting of mossy fibers in the dentate gyrus; they establish aberrant recurrent synapses between dentate granule cells and operate via post-synaptic kainate receptors. In this report we tested the hypothesis that this aberrant local circuit alters information processing of perforant path inputs constituting the major excitatory afferent pathway from enthorinal cortex to dentate granule cells.

Methods

Experiments were performed in dentate granule cells from control rats and rats with temporal lobe epilepsy induced by pilocarpine hydrochloride treatment. Neurons were recorded in patchclamp in whole-cell configuration in hippocampal slices.

Results

Our present data revealed that an aberrant readout of synaptic inputs by kainate receptors triggered a long-lasting impairment of the perforant path input-output operation in epileptic dentate granule cells. We demonstrated that this is due to the aberrant activity-dependent potentiation of the persistent sodium current altering intrinsic firing properties of dentate granule

cells.

Interpretation

We propose that this aberrant activity-dependent intrinsic plasticity which lastingly impairs the information processing of cortical inputs in dentate gyrus may participate to hippocampal-related cognitive deficits, such as those reported in patients with epilepsy.

INTRODUCTION

In temporal lobe epilepsy (TLE), patients often suffer from comorbid disorders including cognitive impairments ¹⁻⁷. Among them, memory deficits have been widely described in patients with TLE^{2,3,8-12} and animal models¹³⁻¹⁷. Many contributing factors have been proposed to explain cognitive dysfunctions ¹ including seizures ^{3,5,6}, interictal epileptiform activities ^{5,12,16,18}, impaired rhythmogenesis and CA1 place cell physiology ^{14,15,17,19-22}, network reorganization ⁴, lesions²³ and antiepileptic treatments⁷. In the hippocampus, considered to play a crucial role in memory processes ²⁴⁻²⁶, neuronal tissue undergoes major reorganization that may play a role in memory dysfunction in TLE; some neurons die, mainly mossy cells and GABA interneurons ²⁷⁻³⁴, while others, which are severed in their inputs or outputs, sprout and form novel aberrant connections ^{32,35-38}. This phenomenon, called reactive plasticity, is well documented in the dentate gyrus (DG) where dentate granule cell (DGC) axons (the mossy fibers, MFs) sprout ³⁵⁻ ^{37,39} and create a reverberant excitatory circuit between DGCs involved in epileptiform bursts in patients with TLE and animal models 35,40-42. Beyond epileptiform activities 42, recurrent MF (rMF) synapses, which operate via kainate receptors (KARs) not present in naïve conditions ⁴³, drive an aberrant sustained firing regime when recruited in the physiological gamma frequency range ⁴⁴. The long-lasting consequence of this local synaptopathic activity on the input-output operation of the major cortical input, the perforant path (PP), in DGCs remains to be addressed. This is a major issue since the DG is considered as a filter or a gate at the entrance of the hippocampus ^{34,45,46}, which converts the dense signals from the upstream entorhinal cortex ⁴⁷⁻⁴⁹ into a sparse and specific code indispensable for the formation and the discrimination of memory items ⁵⁰⁻⁵⁴.

In the present study, we found that an aberrant KAR-mediated synaptic activity lastingly disrupts the PP-evoked DGC sparse firing. This is due to a potentiation of the persistent sodium current (I_{NaP}) leading to an increase of the intrinsic excitability. Therefore, we show that an aberrant readout of synaptic inputs by KARs induces an intrinsic plasticity of I_{NaP} which leads to a longlasting impairment of the input-output operation of the major cortical inputs in DG in TLE. We propose that, beyond epileptifiform discharges, this aberrant activity-dependent intrinsic plasticity disrupts the information processing of cortical inputs in dentate gyrus.

MATERIALS AND METHODS

All experiments were approved by the Institut National de la Santé et de la Recherche Médicale (INSERM) animal care and use agreement (D-13-055-19) and the European community council directive (2010/63/UE). Adult male Wistar rats had access to food and water *ad libitum* and were housed under a 12 h light/dark cycle at 22–24°C.

Animal model of temporal lobe epilepsy

Rats (5-6 weeks of age, 150-350 g, Janvier Breeding Centre, Le Genest-Saint-Isle, France) were injected intraperitoneally (i.p.) with pilocarpine hydrochloride (340 mg/kg dissolved in NaCl 0.9%) 30 min after a low dose of the peripheral cholinergic antagonist scopolamine methyl nitrate (1 mg/kg, i.p.). Approximately 80% of the rats experienced class

IV/V seizures ⁴⁴. After 2-3 h of *status epilepticus*, diazepam (8 mg/kg) was injected (i.p.). After a seizure-free period of several weeks, we selected for recordings and analysis only rats that experienced spontaneous seizures (4-12 months after the pilocarpine injection; chronic epileptic rats, n = 24), which were expected to display a high degree of mossy fiber sprouting ³⁵⁻³⁷. Agematched naïve rats (n = 32) were used as controls.

Slice preparation

Animals were deeply anesthetized with chloral hydrate (70 mg/kg, i.p.) and decapitated. The brain was removed rapidly, the hippocampi were dissected, and transverse 400 µm-thick hippocampal slices were cut using a Leica VT1000S tissue slicer (Leica, Nussloch, Germany) in a solution containing the following (in mM): 132.5 choline chloride, 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 7 MgCl2, 0.5 CaCl2, and 7 D-glucose (2-5 °C). Slices were then transferred for rest at room temperature (> 1 h) in oxygenated normal artificial cerebrospinal fluid (ACSF) containing the following (in mM): 126 NaCl, 3.5 KCl, 1.2 NaH2PO4, 26 NaHCO3, 1.3 MgCl2, 2.0 CaCl2, and 10 D-glucose, pH 7.4.

Electrophysiology

Whole-cell recordings of DGCs from epileptic and control rats were obtained using the "blind" patch-clamp technique and performed in hippocampal acute slices in a submerged chamber perfused with oxygenated ASCF (30-32 °C) at a flow rate of 2-3 ml/min. Glass electrodes (resistance: 6-7 M Ω) were filled with an internal solution containing the following (in mM): 130 KMeSO₄, 5 KCl, 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES-K), 2.5 MgATP, 0.3 NaGTP, 0.2 EGTA, 10 phosphocreatine, and 0.3-0.5% biocytin, pH = 7.25.

Access resistance ranged between 15 and 30 M Ω , and the results were discarded if the access resistance changed by more than 20%. Whole-cell recordings were performed in current-clamp mode (except when stated otherwise) using a Multiclamp 700B amplifier (Axon Instruments, Molecular Devices, Sunnyvale, CA). Data were filtered at 2 kHz, digitized (20 kHz) with a Digidata 1440A (Molecular Devices) to a personal computer, and acquired using Clampex 10.1 software (PClamp, Axon Instruments, Molecular Devices). Recordings have been performed at subthreshold potentials (-49.9 \pm 0.3 mV, n = 92) in the presence of blockers of NMDA (40 μ M D-APV), GABA_A (5 µM gabazine [SR-95531]), and GABA_B (5 µM CGP₅₅₈₄₅) receptors, except when stated otherwise. Excitatory Post-Synaptic Potentials (EPSPs) were evoked by bulk stimulations with bipolar NiCh electrodes (NI-0.7F, Phymep, France). Perforant path (PP) inputs were stimulated by an electrode positioned in the outer two-third of the molecular layer of the dentate gyrus (DG) in control and epileptic rats. PP input stimulations were delivered at 30 Hz, 10-35 µs duration and 10-30 µA intensity. In this condition, the first EPSP in the train was around 6 mV (1st EPSP amplitude: control, 6.3 ± 0.2 mV, n = 54, vs. epileptic, 6 ± 0.3 mV, n = 33, p =0.436).

Conditioning protocol

The conditioning trains (CT) were delivered by an electrode positioned in the inner molecular layer of the DG to activate associational/commissural (A/C) inputs in control rats (A/C-CT) or recurrent mossy fibers (rMF) inputs in epileptic rats (rMF-CT). One CT consisted in a short 30 Hz-train of stimulations for around 1.5 s duration. CTs were interleaved by long-lasting resting period of 8 s and were applied for few minutes (around 8 min) at a subthreshold potential (-49.4 \pm 0.4 mV, n = 92); this conditioning period comprised a total CT duration of around 60 s (except when stated otherwise). Electrical stimulations were delivered at 15-35 µs

duration and 10-35 μ A intensity. In this condition, the first EPSP in the train was around 5 mV (control, 5.2 ± 0.2 mV, n = 19, vs. epileptic, 5 ± 0.3 , n = 36, p = 0.501).

In a set of experiments, the conditioning protocol consisted in trains of waveforms of current simulating trains of EPSPs (termed α EPSPs) delivered to DGCs from control rats through the recording pipette. First, a custom-made program (Matlab, Mathworks, Natik, MA) was used to generate fast and slow α EPSPs, mimicking EPSP_{AMPA} and EPSP_{KA} respectively. α EPSPs had an exponentially rising and falling waveform according to the following formula:

$$w(t) = \left(1 - \exp\left(-\frac{t}{\tau_{on}}\right)\right) \times \left(\exp\left(-\frac{t}{\tau_{off}}\right)\right)$$

For $\alpha \text{EPSP}_{AMPA}$, τ_{on} was set to 0.5 ms, and τ_{off} to 10 ms. For αEPSP_{KA} , τ_{on} was set to 2 ms, and τ_{off} to 70 ms as previously described ⁵⁵. We then used a custom-made program (Matlab) to generate 30 Hz-trains of either pure $\alpha \text{EPSP}_{AMPA}$ or pure αEPSP_{KA} , including summation and short-term depression profiles as observed in previous work ⁴⁴. Trains of αEPSPs had a waveform using the following formula:

$$\alpha(t) = \sum_{i=1}^{N} d_i \times \left(1 - \exp\left(-\frac{t - t_i}{\tau_{on}}\right)\right) \times \left(\exp\left(-\frac{t - t_i}{\tau_{off}}\right)\right) \times \mathbf{1}_{[t_i, \mathrm{T}]}(t)$$

Each α EPSP occurred at $t = t_i$ with $t_i = i/f$. *i* varied from 1 to *N* where *N* referred to the number of α EPSPs. The frequency of α EPSPs *f* was set to 30 Hz, t_N to 1.5 s and *N* has been inferred to 44 α EPSPs. The recording window *T* was set to 2 s. Trains of α EPSPs followed an intra-train depression profile using the sequence (d_i) defined as $d_1 = 1$, $d_2 = 0.7$, $d_3 = 0.65$

and $d_k = 0.6$ for $4 \le k \le N$, which modulated each α EPSP amplitude. The summation profile included every previous term of the sum according to the following function: $\mathbf{1}_{[t_i,T]}(t) = 1$ if $t \in [t_i, T]$, $\mathbf{1}_{[t_i,T]}(t) = 0$ otherwise. α EPSP_{AMPA}-CT simulated A/C-CT in control condition as it generated a sparse firing pattern in DGCs from control rats (Fig 4). Contrariwise, α EPSP_{KA}-CT mimicked rMF-CT in epileptic condition because it produced a sustained firing pattern in DGCs from control rats (Fig 4).

In TLE, DGCs exhibit ongoing synaptic activity comprising 50% EPSP_{AMPA} and 50% EPSP_{KA}⁴³. In order to simulate this pathophysiological condition, we finally implemented the train of α EPSPs model to generate waveforms composed of $N/2 = 22 \alpha$ EPSP_{AMPA} and $N/2 = 22 \alpha$ EPSP_{KA}. There were *N*! possible different sequences of α EPSPs and the generation of any particular pattern is equiprobable. We thus generated 10 different patterns and used them together as CT (mixed α EPSP_{AMPA/KA}-CT). Each pattern was repeated 5 times (50 trials in total), changing the sequence from a cell to another. Mixed α EPSPs-CT generated sustained firing patterns in control DGCs (Fig 5A, D, 6.09 ± 0.4 Hz, *n* = 11) as observed for α EPSP_{KA}-CT in control condition as well as with rMF-CT in DGCs from epileptic rats (Kruskal-Wallis, *p* = 0.983).

Data analysis

Signals were analyzed off-line using Clampfit 10.1 (Molecular Devices) and MiniAnalysis (Synaptosoft, Decatur, GA). PP-DGC firing rate was measured in response to successive short 30 Hz-train of stimulations (around 1.5 s duration) interleaved by long-lasting resting period of 8 s at subthreshold potentials (-49.9 \pm 0.3 mV). The PP-DGC firing rate was averaged before (for around 10 min) and after (up to 30 min) CT. The time course of PP-DGC firing rate, before and after CT, was evaluated by averaging the firing rate every two minutes.

Trains of PP-inputs were also recorded in voltage-clamp mode at -70 mV (close to the resting membrane potential) to assess EPSCs amplitude without any effect of subthreshold active conductances (Fig 2).

To evaluate firing frequency versus injected current (f/I) relationships, DGC firing rate was measured in response to depolarizing squared pulses of current of increasing intensity (500 ms, 20 to 160 pA, 20 pA steps) from -70 mV as a baseline membrane potential, before and after CT. We applied linear regressions to individual f/I curves and collected the neuronal gain, defined as the slope of the f/I curve ^{56,57}. The rheobase was measured as the minimal current amplitude necessary to evoke an action potential. Basic membrane properties such as resting membrane potential, input resistance, membrane time constant, action potential amplitude, threshold and half-width were measured and compared between DGCs from control and epileptic rats (Table S1) as well as between before, after CT and after subsequent application of 20 nM TTX (Tables S2 and S3).

In one set of experiments, recordings were performed in voltage-clamp mode to measure the persistent sodium current (I_{NaP}) in DGCs from epileptic rats. The current was evoked by successive voltage steps of incremental amplitude (500 ms, from -80 to -40 mV, 5 mV step, from a holding potential of -70 mV) following a 50 ms pre-step depolarization to -30 mV in order to inactivate the transient sodium current. Current was averaged from 5 trials for each voltage point before and after rMF-CT. The latter was recorded in current-clamp mode. We then used bath application of 1 μ M TTX and the current evoked by the same voltage-steps protocol was subtracted to isolate the subthreshold TTX-sensitive I_{NaP} component before and after rMF-CT. Furthermore, I_{NaP} amplitude after rMF-CT was subtracted to its amplitude before rMF-CT to assess I_{NaP} plasticity.

Statistical analysis

Numerical values are given either as population data (individual + mean values) or as means \pm standard error (SEM). Linear regressions and gaussian fits were performed with Origin 7.0 (OriginLab, Northampton, MA). The normal distribution of data was assessed with the Shapiro-Wilk test. Statistical significance was assessed using two-tailed paired and unpaired Student's *t* tests, the one-way ANOVA (with or without repeated measures) and Tukey HSD post-hoc tests for pairwise comparisons, as well as the non-parametric Wilcoxon signed rank test, Mann-Whitney rank-sum test, Kruskal-Wallis one-way ANOVA, Friedman ANOVA and Kolmogorov-Smirnov tests when appropriate (Systat 11, Systat Software, Richmond, CA; Statistica, StatSoft, Tulsa, OK). Correlations were assessed using Spearman's rank correlation coefficient (Matlab, Mathworks, Natik, MA). The level of significance was set at *p* < 0.05. *n* refers to the number of cells, *N* to the number of interspike intervals (ISIs).

Neuron staining

Neurons were labeled after intracellular diffusion of 0.3-0.5% biocytin added to the pipette solution. At the end of experiments, hippocampal slices were post-fixed overnight at room temperature with Antigenfix. The morphology of stained neurons was revealed using 1/500 cy3 conjugated streptavidin incubated overnight at room temperature and then counterstained 30 min with green fluorescent Nissl NeuroTrace®. Biocytin fluorescence was visualized and images were acquired using a TCS SP5 X confocal microscope (Leica Microsystems, Wetzlar, Germany). 3D DGCs reconstructions have been performed using Neurolucida (MBF Bioscience, Williston, VT). All neurons included in the present study had their soma located in the granular layer of the dentate gyrus and possessed all morphological features of DGCs.

Drugs and chemical compounds

TTX, pilocarpine hydrochloride, scopolamine methyl nitrate, and biocytin were purchased from Sigma (St. Louis, MO), ACSF and intracellular compounds from Sigma and Carlo Ebra Reagents (Rodano, Italy), gabazine (SR-95531), D-APV, CGP₅₅₈₄₅, from Tocris Bioscience (Bristol, UK), UBP310 from Tocris Bioscience and Abcam (Cambridge, UK), BAPTA tetrapotassium salt from Molecular Devices (Eugene, OR, USA), diazepam from Roche (Basel, Switzerland), Antigenfix from DiaPath (Martingo, Italy), cy3 conjugated streptavidin from Jackson Laboratory (Bar Harbor, ME) and Nissl NeuroTrace® from Invitrogen/Life (Carlsbad, CA).

RESULTS

Long-lasting disruption of PP-evoked DGC sparse firing induced by synaptopathic activity in TLE

Previous *in vivo* recordings have revealed that DGCs are exposed to barrages of excitatory synaptic inputs; this is caused by trains of high-frequency fast AMPAR-mediated synaptic inputs (interevent intervals around 30 ms) which last up to few seconds and which are relayed from the entorhinal cortex via the perforant path (PP) ⁴⁷⁻⁴⁹. We first compared the input-output operation of DGCs exposed to trains of excitatory potential synaptic potentials (EPSPs) in control and epileptic conditions. The trains of EPSPs were evoked by stimulation of the PP (PP-EPSPs, Fig 1A) at 30 Hz (1.5 s duration). PP-EPSPs of small amplitude (1st EPSP amplitude: control, 6.3 ± 0.2 mV, n = 54, vs. epileptic, 6 ± 0.3 mV, n = 33, p = 0.436) were evoked to match the spontaneous EPSPs recorded *in vivo* ⁴⁹. Trains of PP-EPSPs were regularly evoked at low frequency (0.1 Hz) and recorded at subthreshold potential (around -50 mV, see methods) in order

to evaluate the input-output operation of DGCs. In the first sets of experiment AMPA/kainate receptor-mediated EPSP were pharmacologically isolated in the presence of D-APV (40 µM), gabazine (5 µM) and CGP₅₅₈₄₅ (5 µM) to inhibit NMDA and GABAergic receptors. In these conditions, we observed that trains of PP-EPSPs caused a similar sparse DGC firing in hippocampal slices from control $(1.12 \pm 0.08 \text{ Hz}, n = 11)$ and epileptic rats $(1.17 \pm 0.07 \text{ Hz}, n = 10.08 \text{ Hz})$ 15, p = 0.622) (Fig 1) as reported in naïve animals *in vivo*⁴⁹. Moreover, recordings in voltageclamp mode revealed no significant difference of the excitatory postsynaptic current (EPSC) strength between control (1st EPSC amplitude: -70 ± 6 pA, n = 11) and epileptic conditions (-81 \pm 9 pA, n = 7, p = 0.119) (Fig 2A, B). We have previously reported that rMF synapses, operating via KARs, drive an aberrant sustained firing regime in DGCs when recruited in the gamma frequency range ⁴⁴. To evaluate the long-lasting consequence of this synaptopathic activity on DGC input-output operation, the PP-evoked DGC (PP-DGC) firing pattern was compared before and after associational/commissural fibers (A/C)- or rMF-evoked EPSPs used as conditioning trains (CT) in control or epileptic conditions, respectively (Fig 1, see Materials and Methods). CT consisted in short 30 Hz-train of stimulations for around 1.5 s duration. CT, interleaved by longlasting resting period of 8 s, were applied for few minutes (around 8 min) at a subthreshold potential (around -50 mV, see Methods); this conditioning period comprised a total CT duration of around 60 s (except when stated otherwise).

As previously described ⁴⁴, trains of A/C-EPSPs or rMF-EPSPs triggered sparse or sustained firing regimes in DGCs from control or epileptic rats, respectively (A/C-CT: 1.42 ± 0.11 Hz, n = 11, vs. rMF-CT: 5.83 ± 0.23 Hz, n = 15, p < 0.0001, Student) (Fig 1B to D and Fig 4B). We observed that the PP-DGC firing rate was not modified after A/C-CT in control rats (1.11 ± 0.12 Hz, n = 11, p = 0.594) (Fig 1B, E and F). By contrast, the PP-DGC firing rate was strikingly increased after rMF-CT by around 200% in epileptic rats (3.48 ± 0.39 Hz, n = 15, p =

0.0007) for at least 30 minutes (n = 6, p = 0.018) (Fig 1C, E and F). This long-lasting change of PP-DGC firing rate was neither due to a change of the basic membrane properties (Table S1 and S2) nor associated with an increase of the PP synaptic strength after CT (Fig 2). Contrarily, we observed a similar depression of PP synaptic inputs efficacy after A/C-CT in control rats (1st EPSC amplitude before CT, -70 ± 6 pA vs. after CT, -55 ± 9 pA, n = 11, p = 0.008), or after rMF-CT in epileptic rats (1st EPSC amplitude before CT. -81 ± 9 pA vs. after CT. -55 ± 7 pA. n = 6. p = 0.0011, Fig 2A, C); the depression of PP inputs was 25 ± 8 % in control conditions vs. 33 ± 3 % in epileptic condition (p = 0.447, Fig 2A, E). Furthermore, we also observed a similar depression of PP synaptic inputs within trains before or after CT in control rats (ratio last/1st EPSC, before CT: 0.25 ± 0.04 vs. after CT: 0.25 ± 0.05 , p = 0.936) or CT in epileptic rats (before CT: 0.16 ± 0.03 vs. after CT: 0.18 ± 0.03 , p = 0.552, Fig 2A, B, D). Moreover, the short-term depression of PP synaptic inputs within trains was not significantly different between control and epileptic conditions before or after CT (before CT: p = 0.14; after CT: p = 0.299, Fig 2D). In a set of experiments, we additionally tested the efficacy of shorter conditioning period to trigger plasticity of PP-DGC firing rate. For these experiments, we applied CT (interleaved by period of 8 s) during around 2 min (comprising a total CT duration of around 15 sec). In this condition, we still observed a striking increased of PP-DGC firing rate after rMF-CT by around 200% in epileptic rats for at least 30 minutes (before CT: 1.28 ± 0.08 Hz vs. after CT: 3.93 ± 0.4 Hz, n =5, p < 0.0001, not shown).

To test the role of KARs in the potentiation of PP-DGC firing rate induced by rMF-CT, experiments were performed in the presence of the KAR blocker UBP310 ⁵⁸. We observed that UBP310 (5 μ M) prevented the rMF-DGC sustained firing (rMF-CT without UBP310: 5.83 \pm 0.23 Hz, n = 15 vs. with UBP310: 1.62 \pm 0.26 Hz, n = 11, p = 0.00002) (Fig 3A, I) and the subsequent rMF-CT-dependent potentiation of the PP-DGC firing rate in epileptic rats (before

CT: 1.06 ± 0.09 Hz vs. after CT: 1 ± 0.08 Hz, n = 12, p = 0.790) (Fig 3A, B, J). Unblocking NMDA receptors (NMDARs), by removing D-APV, did not modify the PP-DGC firing regime after A/C-CT in control (before CT: 1.51 ± 0.12 Hz vs. after CT: 1.35 ± 0.14 Hz, n = 8, p =0.353; A/C-CT: 2.32 ± 0.14 Hz) (Fig 3C, D). By contrast, the PP-DGC firing rate was still strongly increased after rMF-CT in the absence of NMDA receptor antagonist in epileptic rats (before CT: 1.42 ± 0.15 Hz vs. after CT: 3.26 ± 0.28 Hz, n = 12, p = 0.0001; rMF-CT: $5.85 \pm$ 0.37 Hz) (Fig 3E, F, I, J). Finally, we observed that rMF-CT-dependent potentiation of PP-DGC firing rate could be also observed under less pharmacological constrained conditions, i.e. in the absence of both NMDA and GABA receptors antagonists in epileptic rats (in ACSF condition; before CT: around 1 Hz vs. after CT: around 3.5 Hz, n = 2, not shown). Taken together, these experiments demonstrate that KARs but not NMDARs play a key role in the increased PP-DGC firing rate induced by rMF-CT in epileptic conditions. Many forms of activity-dependent synaptic and intrinsic plasticities are a Ca^{2+} -dependent process ⁵⁹. To test the role of intracellular Ca^{2+} in the rMF-CT-dependent potentiation of PP-DGC firing rate, recordings were performed with patch pipette containing the calcium chelator BAPTA (20 mM, see Materials and Methods); AMPA/kainate receptor-mediated EPSPs were pharmacologically isolated in the presence of D-APV (40 µM), gabazine (5 µM) and CGP55845 (5 µM). The rMF-CT induced a similar DGC firing rate in the absence or in the presence of BAPTA (CT-No BAPTA: 5.83 ± 0.23 Hz, n = 15vs CT-with BAPTA: 6.83 ± 1.12 Hz, n = 10, p = 0.279) (Fig 3G, I). By contrast, BAPTA prevented the rMF-CT-dependent potentiation of PP-DGC firing rate in epileptic rats (before CT: 0.99 ± 0.11 Hz vs. after CT: 0.92 ± 0.09 Hz, n = 10, p = 0.515) (Fig 3G to J). Therefore, the plasticity of PP-DGC firing pattern induced by rMF-CT is a Ca²⁺-dependent process. Previous studies have reported I_{NaP} as a likely candidate in the modulation of firing rate ^{44,56}. We then challenged the role of I_{NaP} in the long-lasting change of PP-DGC firing pattern. We showed that

inhibition of I_{NaP} by a low dose of TTX (20 nM) ^{44,55,60}, bath applied 15 min after CT, fully restored the PP-DGC firing rate (after CT: 3.66 ± 0.54 Hz, vs. after CT + TTX: 1.17 ± 0.07 Hz, n = 9, p = 0.008) back to its basal level in epileptic rats (before CT: 1.15 ± 0.09 Hz vs. after CT + TTX: 1.17 ± 0.07 Hz, n = 9, p = 0.953) (Fig 1C, F) without change of basic membrane properties (Table S3). This strongly suggests that I_{NaP} is involved in the long-lasting increase of firing rate following the rMF-CT in TLE (see also below).

The present data revealed that a KAR-dependent CT triggers a long-lasting increase of PP-DGC firing rate in TLE. We have previously reported that KAR-mediated EPSPs (EPSP_{KA}) represent around half of the excitatory synaptic drive in DGCs in TLE⁴³. To assess whether a barrage comprising mixed events was sufficient to trigger a change of PP-DGC firing rate, simulated trains of EPSPs (α EPSPs, see Methods) were tested in naive rats (Fig 4). First, we showed that $\alpha \text{EPSP}_{\text{AMPA}}$ -CT mimicked A/C-CT in control rats (control, $\alpha \text{EPSP}_{\text{AMPA}}$ -CT: 1.48 ± 0.38 Hz, n = 7 vs. A/C-CT: 1.42 ± 0.11 Hz, n = 11, p = 0.184) (Fig 4A, B) and that this type of CT, as A/C-CT, did not modify PP-DGC firing rate (before CT: 0.94 ± 0.1 Hz vs. after CT: 1.09 ± 0.17 Hz, n = 9, p = 0.498) (Fig 4C, D, F). By contrast, trains of solely $\alpha EPSP_{KA}$, which triggered a DGC firing regime in control rats similar to that elicited by activation of rMFs in epileptic rats (control - αEPSP_{KA} -CT: 5.94 ± 0.27 Hz, n = 21 vs. epileptic - rMF-CT: 5.83 ± 0.23 Hz, n = 15, p = 0.755) (Fig 4A, B), induced a striking increase of the PP-DGC firing rate (before CT: 0.99 ± 0.04 Hz vs. after CT: 3.01 ± 0.18 Hz, n = 21, p = 0.0001) (Fig 4C to G). This was reversed in the presence of 20 nM TTX to abolish I_{NaP} (PP-DGC firing rate after CT: 2.87 ± 0.22 Hz vs. after CT + TTX: 1.03 ± 0.07 Hz, n = 13, p = 0.002) (Fig 4E, F). This long-lasting increase of PP-DGC firing rate triggered by $\alpha EPSP_{KA}$ -CT was also a Ca²⁺-dependent process since it was prevented when recordings were performed in the presence of 20 mM BAPTA in the patch pipette (before CT: 0.95 ± 0.07 Hz vs. after CT: 0.83 ± 0.01 Hz, n = 6, p = 0.146; during CT: 5.14 ± 0.63 Hz) (Fig.

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4G). Then, we tested CT using simulated trains of EPSPs comprising half of α EPSP_{KA} and α EPSP_{AMPA} randomly distributed (mixed α EPSP) (Fig 5A, see Materials and Methods). We observed that this type of CT was still able to trigger a significant increase of PP-DGC firing rate (before CT: 0.88 ± 0.03 Hz vs. after CT: 2.82 ± 0.3 Hz, *n* = 11, *p* = 0.003; during CT: 6.09 ± 0.4 Hz) (Fig 5B, C). Therefore, we propose that a synaptic barrage comprising a fraction of EPSP_{KA} ⁴³ is sufficient to trigger a long-lasting increase of the PP-DGC firing rate.

We then examined the relationship between the plasticity of PP-DGCs firing rate and the firing regime imposed during CT. We assessed this issue by analysing data pooled from different experimental conditions, both from naïve and epileptic rats (Fig 5D). This clearly revealed that the increase of PP-DGCs firing rate is closely correlated to the DGC firing regime imposed during the CT in control and epileptic rats (Fig 5D, n = 78; R = 0.72; p = 1.6e-13, spearman's rank correlation coefficient). Taken together, these experiments show that the sustained firing regime imposed by EPSP_{KA} during the CT is an important determinant in the long-lasting increase of PP-DGC firing rate in TLE.

Synaptopathic activity induces an increase of I_{NaP} and intrinsic excitability in DGCs in TLE

Our data have revealed that the blockade of I_{NaP} fully canceled the long-lasting change of PP-DGC firing triggered by rMF-CT or α EPSP_{KA}-CT (see above). Therefore, we postulated that this long-lasting change could result from a potentiation of I_{NaP} . To directly assess this hypothesis, we performed a set of experiments in which I_{NaP} was measured in voltage-clamp mode before and after CT in epileptic DGCs (Fig 6, see Materials and Methods). I_{NaP} is a Na⁺ current that activates below spike threshold and slowly inactivates ⁶¹. Therefore, I_{NaP} was activated using a protocol consisting in depolarizing steps (ranging from -80 to -40 mV, see Methods) (Fig 6A). The current evoked by the same protocol in 1 μ M TTX was then subtracted

to isolate the TTX-sensitive slowly inactivating I_{NaP} (Fig 6B). As previously reported ⁵⁵, we found that epileptic DGCs displayed a moderate I_{NaP} component. This component was clearly potentiated (Fig 6B) by around 60% following CT (before CT: -32.4 pA vs. after CT: -51.6 pA, n = 6, p = 0.003 at -40 mV) leading to an increase of the TTX-sensitive inward rectification (Fig 6A, C to E). This significant potentiation of I_{NaP} (by around 20 pA at -40 mV) lasted at least 30 min following CT (mean I_{NaP} 22-30 min after CT compared to 10 min before CT: + 67.35 %, n =5, p = 0.009, Fig 6F). In keeping with this, subthreshold membrane depolarization evoked by a current pulse was enhanced by around 200% after CT in epileptic (+221.5 \pm 64.6%, n = 7, p =0.008) but not in control rats (+20.4 \pm 24.7%, n = 9, p = 0.705) (Fig 7A, B). This augmentation of subthreshold membrane depolarization was canceled by subsequent application of 20 nM TTX $(+63.2 \pm 37.1\%$ compared to before CT, n = 7, p = 0.127) (Fig 7A, B). Because the rMF-CT triggered an increase of I_{NaP} , we hypothesized that this could lead to a change of DGCs intrinsic excitability. To test this, incremental current pulses were applied to determine the gain of the frequency/current (f/I) relationship, and the rheobase for action potential generation (see Methods). We observed that rMF-CT in epileptic rats triggered an increase of the f/I gain by around 70% and a reduction of the rheobase by around 20% (Fig 7C, D and Table S4). By contrast, A/C-CT in control DGCs or rMF-CT in the presence of UBP310 (5 μ M) in TLE did not modify the intrinsic excitability (Fig 7C, D and Table S4). In keeping with this, CT comprising only $\alpha EPSP_{KA}$ or mixed $\alpha EPSP_{AMPA}/_{KA}$, but not $\alpha EPSP_{AMPA}$ -CT, induced an enhancement of the f/I gain and a reduction of the rheobase (Fig 7C, E and Table S4). Blocking I_{NaP} by bath application of 20 nM TTX returned the f/I gain and the rheobase to their basal level following rMF-Ct and αEPSP_{KA}-CT (Fig 7C to E and Table S4). However, 20 nM TTX had no significant effect on *f/I* gain and rheobase in control DGCs following A/C-CT (Fig 7C to D and Table S4).

Together, these findings demonstrate that rMF-CT operating via KARs induces a potentiation of I_{NaP} , leading to an increase of intrinsic excitability and PP-DGC firing rate.

DISCUSSION

In the present study, we have discovered a novel form of activity-dependent intrinsic plasticity, the potentiation of I_{NaP} triggered in specific conditions of firing regime in DGCs in TLE. Remarkably, we have demonstrated that these conditions are achieved by the readout of aberrant KAR-operated synapses in DGCs. We show that this intrinsic plasticity disrupts the fundamental sparse firing of DGCs and leads to a long-lasting impairment of the normal processing of their PP inputs in TLE, a major pathway involved in learning and memory.

Ion channel dysfunctions are involved in different forms of epilepsy including genetic epilepsies and acquired channelopathies ^{62,63}. In the cortex and the hippocampus, acquired channelopathies increase neuronal excitability via a basal enhancement of Na⁺ and Ca²⁺ or decreases of K⁺ and *h* currents in the hippocampus and the neocortex ⁶⁴⁻⁶⁶. By contrast in TLE, acquired channelopathies downscale DGCs excitability by increasing I_h , the inward rectifier K⁺ current (Kir) ⁶⁷⁻⁷⁰ and reducing Ca²⁺ currents ⁷¹. It has been proposed that these basal changes of ion channel activities may constitute a compensatory protective mechanism to preserve the gate function of DG. This is in keeping with reports showing that, in spite of an increased excitability in the enthorinal cortex ⁷², the DG filter function upon the stimulation of PP inputs is spared in TLE ³⁴. Accordingly, we found that PP-DGC sparse firing, as well as intrinsic excitability, is well preserved in our basal conditions (before CT) in TLE. Remarkably, we now show that a transient KAR-dependent CT lastingly disrupts the sparse firing of DGCs in TLE via the intrinsic plasticity of I_{NaP} .

In physiological conditions, activity-dependent intrinsic plasticity can modulate neuronal input-output functions and contributes significantly to the establishment of specific memory traces ^{59,73}. Intrinsic plasticity can also be involved in homeostatic mechanisms to normalize neuronal firing as shown in vitro and in vivo 57,73,74. Different types of currents have been reported to contribute to intrinsic plasticity including I_{NaT} ⁷⁵⁻⁷⁷, I_{h} ⁷⁸ and Kv1 channel activity ⁷⁹ affecting the spike threshold. Intrinsic plasticity can also lead to an increase of f/I gain due to a reduction of the afterhyperpolarization (AHP) outward current $(I_{AHP})^{59}$ via the activation of metabotropic glutamate receptors ⁵⁹ or the metabotropic function of KARs ⁸⁰⁻⁸⁴. In TLE, we observed that the intrinsic plasticity is associated with a reduction of the rheobase and an increase of f/I gain in DGCs. These changes are primarily driven by I_{NaP} because this current is potentiated following CT and its blockade afterwards fully restores the rheobase, the f/I gain and the PP-DGC firing rate. Moreover, we have previously shown that aberrant $EPSP_{KA}$ did not affect I_{AHP} in DGCs in TLE⁴⁴. From our knowledge, this is the first demonstration of an activity-dependent potentiation of I_{NaP} leading to a long-lasting change of intrinsic excitability in mammals. A learning-induced somatic depolarization involving I_{NaP} was previously reported in neurons of the snail Lymnaea stagnalis⁸⁵. Interestingly, in basal conditions (i.e. before CT), we observed a similar firing rate between control and epileptic DGCs. In keeping with this, I_{NaP} is not significantly different when comparing epileptic DGCs with control ones ⁵⁵. This suggests that KAR-dependent intrinsic plasticity, observed during tens of minutes, presumably remains a transient phenomenon at hourly or daily time scales. Numerous studies have investigated the mechanisms underlying the induction of intrinsic plasticity and have reported that NMDARs, a rise of intracellular Ca²⁺ and downstream intracellular kinases play crucial roles ^{73,76,78}. In our experimental conditions, NMDARs are not a key component since their blockade did not prevent the increase of excitability by CT in DGCs in comparison with the blockade of KARs. However, the potentiation of the PP-DGC firing rate, induced by rMF-CT in epileptic DGCs and by the α EPSP_{KA}-CT in control DGCs, is a Ca²⁺-dependent process, since it is fully prevented in presence of the calcium chelator BAPTA in the recording pipette. The Ca²⁺ influx is likely to result from the activation of voltage-dependent calcium channels ⁸⁶⁻⁸⁸ during the sustained firing regime driven by rMF-CT. Along this line, we observed that the increase of the PP-DGC firing rate is correlated with the firing regime imposed by CT. Indeed, rMF-CT, which imposes a sustained firing regime via KARs, but not A/C-CT, triggers a long-lasting increase of the PP-DGC firing nate and intrinsic excitability. Accordingly, waveforms of current (α EPSP_{KA}), mimicking aberrant EPSP_{KA} and driving an elevated firing regime, are sufficient to induce an intrinsic plasticity in control DGCs. This suggests that intrinsic plasticity of *I*_{NaP} could also play a role in physiological conditions in DGCs. Additional experiments should be performed to further shed light on neuronal activities triggering this type of intrinsic plasticity in control conditions.

What could be the physiological consequences of this activity-dependent plasticity in TLE? A sparse activity in the DG is an important feature for the formation and the discrimination of memory items in the hippocampus ^{50,52,54}. The DG is commonly considered to be a gate and a "low-pass filter" at the entrance of the hippocampus ^{34,45}. It converts dense upstream cortical signals into a sparse and specific code ⁴⁹⁻⁵⁴. DGCs achieve this sparsification thanks to a set of morphological, intrinsic and synaptic properties making them extremely reluctant to fire ^{34,89-92}. On the other hand, DGCs use a rate coding scheme by which they modify their firing rate in response to an environmentally relevant stimulus ^{50,52,93}. A recent study has demonstrated that hilar mossy cell ablation, causing a transient DGC hyperexcitability (without rMF sprouting), impairs pattern separation ⁹⁴. In TLE, DG undergoes major network reorganisation. This includes *de novo* appearance of dense recurrent excitatory circuit ^{32,35-38}, interneuron loss and the dysfunction of surviving dentate somatostatin and basket interneurons ^{95,96} which tend to increase

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neuronal excitability and may interfere with the normal information processing in DG. Moreover, a computer model indicates that acquired channelopathies, which downscale hyperexcitable networks by transcriptional upregulation of 'leak' channels in DGCs in TLE 68,70 , restore pattern separation ability of DG 97 . We now report that ectopic KAR-mediated activity triggers a long-lasting disruption of the sparse firing of PP inputs due to an intrinsic plasticity of I_{NaP} in TLE. We propose that this aberrant long-lasting plasticity may contribute to the impairment of DG functions such as gate function and pattern separation in TLE.

In conclusion, we report that a KAR-dependent synaptopathic activity induces a plasticity of I_{NaP} in DGCs in TLE. This leads to the impairment of the input-output operation of the major cortical input, the PP, in DGCs and the long-lasting disruption of their sparse firing, a crucial feature for learning and memory ^{50,52,54}.

Accepted

FIGURE LEGENDS

Figure 1. Long-lasting increase of PP-DGC firing rate triggered by rMF-CT in epileptic rats.

(A) Recording configuration and neurolucida reconstructed DGC filled with biocytin. PP: perforant path, A/C: associational/commissural pathway, rMF: recurrent mossy fibres pathway, gcl: granule cell layer. (**B**, **C**) PP-DGC firing patterns before and after A/C-CT in control (B) or after rMF-CT and subsequent TTX application (20 nM) in epileptic condition (C). Scale bars, 20 mV, 500 ms. (**D**) DGC firing rate during CT (CT-DGC firing). (**E**) Time course of the PP-DGC average firing rate before and after CT in control and epileptic conditions. (**F**) PP-DGC firing rate, plotted as a population data, before and after A/C-CT in control (left) or after rMF-CT and after subsequent TTX application (20 nM) (right) in epileptic rats; Wilcoxon. For this and following figures and tables, action potentials are truncated, small dashes represent electrical stimulations, mean values \pm SEM, * p < 0.05; ** p < 0.01; *** p < 0.001; n.s., non-significant.

Figure 2. DGCs from control and epileptic rats display similar short- and long-term depression profiles.

(A) Representative PP-input trains of EPSCs recorded in voltage-clamp at -70 mV before (left) and after CT (right) in DGCs from control (top) and epileptic rats (bottom). Scale bars, 20 pA, 200 ms. (Insets) First EPSC of the same trains before and after CT in control (top) and epileptic (bottom) conditions. Scale bars, 20 pA, 50 ms. (B) First and last EPSC amplitude within trains of PP-input before CT; Wilcoxon (control) and paired Student's (epileptic) tests. (C) First EPSC amplitude of trains before and after CT; paired Student. (D) Ratio last/first EPSC before and after CT (paired Student); in control and epileptic conditions (Student). (E) First EPSC after CT normalized to values before CT in control and epileptic conditions, Student.

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Figure 3. KARs and Ca²⁺ but not NMDARs are crucial determinants for long-lasting increase of PP-DGC firing rate triggered by rMF-CT in epileptic DGCs.

(A) PP-DGC firing pattern and (B) PP-DGC firing rate before and after rMF-CT in epileptic condition in the presence of 5 μ M UBP310; Wilcoxon. (C, E) PP-DGC firing patterns before and after A/C-CT in control (C) or after rMF-CT in epileptic condition (E) without NMDAR antagonist (no D-APV). (D, F) PP-DGC firing rate before and after A/C-CT in control (D, paired Student) or after rMF-CT in epileptic rats without NMDAR antagonist (no D-APV) (F, Wilcoxon). (G) PP-DGC firing pattern and (H) PP-DGC firing rate before and after rMF-CT in epileptic condition in the presence of 20 mM BAPTA inside the patch pipette. Scale bars, 20 mV, 500 ms. (I) rMF-CT-DGC firing rate in the presence of 40 μ M D-APV, 5 μ M gabazine, 5 μ M CGP55845 and 5 μ M UBP310 (black, + UBP310); 5 μ M gabazine and 5 μ M CGP55845 (red, no D-APV); 40 μ M D-APV, 5 μ M gabazine, 5 μ M CGP55845 and 20 mM BAPTA (grey, + BAPTA) in epileptic conditions. (J) Time course of the PP-DGC average firing rate before and after CT in epileptic conditions. Same respective color codes than in (I).

Figure 4. Simulated train of EPSP_{KA} increases PP-DGC firing rate in control rats.

(A) (*Top*) Single α EPSP_{AMPA} and α EPSP_{KA}; scale bar, 100 ms. DGC firing patterns (*middle*) evoked by somatic injection of waveforms of current (*bottom*) simulating trains of AMPAR-EPSPs (left, α EPSP_{AMPA}) or trains of KAR-EPSPs (right, α EPSP_{KA}). Scale bars, 10 mV, 250 ms. (B) Distribution of inter-spike intervals (ISIs) with Gaussian fits (left) and plotted as cumulative probabilities (middle); rMF in epileptic (N = 2852) vs. α EPSP_{KA} (N = 4225) in control condition, p = 0.999. A/C (N = 430) vs. α EPSP_{AMPA} (N = 746) in control condition, p =0.684, Kolmogorov-Smirnov. N refers to the number of ISIs. (Right) DGC firing rate during CT in control and epileptic conditions. (C) Recording configuration. (D, E) PP-DGC firing patterns

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before and after $\alpha EPSP_{AMPA}$ -CT (D) or after $\alpha EPSP_{KA}$ -CT and subsequent TTX application (20 nM) (E) in control rats. Scale bars, 20 mV, 500 ms. (F) PP-DGC firing rate plotted as a population data before and after $\alpha EPSP_{AMPA}$ -CT (left) and after $\alpha EPSP_{KA}$ -CT and subsequent 20 nM TTX application (right); Wilcoxon. (G) Time course of the PP-DGC mean firing rate before and after $\alpha EPSP_{KA}$ -CT in control condition with or without BAPTA inside the patch pipette.

Figure 5. Simulated synaptic barrages comprising half of $\alpha EPSP_{KA}$ increase PP-DGC firing rate in control rats.

(A) 10 different trains of current waveforms composed of 50% α EPSP_{AMPA} and 50% EPSP_{KA} randomly distributed and corresponding firing patterns used as CT in control DGCs. Scale bars, 20 mV, 500 ms. (B) PP-DGC firing pattern before (top) and after (bottom) α EPSP_{AMPA/KA}-CT; scale bars, 20 mV, 500 ms. (C) PP-DGC firing rate before and after CT; Wilcoxon. (D) Correlation between PP-DGC firing rate after CT and DGC firing rate during CT; this plot summarizes data obtained in control (A/C-, α EPSP_{AMPA}-, α EPSP_{KA}- and α EPSP_{AMPA/KA}-CT) and epileptic (rMF-CT and rMF-CT + UBP310) conditions.

Figure 6. Increase of I_{NaP} after rMF-CT in epileptic DGCs.

(A) Current profiles induced by incremental voltage pulses before, after rMF-CT and after subsequent application of 1 μ M TTX in epileptic DGCs. Scale bars, 100 pA, 100 ms. (Inset) rMF-CT DGC firing pattern. Scale bars, 10 mV, 200 ms. (B) Subtracted TTX-sensitive I_{NaP} recorded at -40 mV before and after CT. Scale bars, 30 pA, 50 ms. (C) I/V curves for one representative DGC before, after rMF-CT and after TTX. (D) Average I/V curves of I_{NaP} before and after rMF-CT. (E) Increase of I_{NaP} after rMF-CT in epileptic DGCs, Wilcoxon tests. (F)

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Average time course of I_{NaP} (normalized to baseline) at Vh = -40 mV before and after rMF-CT in epileptic DGCs; the dashed line indicates the averaged baseline before CT.

Figure 7. Increase of subthreshold depolarization and intrinsic excitability after rMF-CT in epileptic DGCs.

(A) Subthreshold membrane depolarization evoked by a 60 pA pulse before and after CT in control and in epileptic DGCs. Scale bars, 5 mV, 100 ms. (B) Subthreshold area (from dashed lines in (A) to signals) change after CT in control or epileptic conditions and after subsequent application of 20 nM TTX (paired Student's tests). (C) DGC firing evoked by a 100 pA pulse before, after A/C-CT in control (top), rMF-CT in epileptic (middle) or α EPSP_{KA}-CT in control (bottom) conditions and after subsequent application of 20 nM TTX. Scale bars, 20 mV, 100 ms. (D) Average *f/I* plots before, after CT and after subsequent TTX application in control (left) and epileptic (right) DGCs. (Inset) Corresponding averaged linear fits, same units than the main panel. (E) Average *f/I* plots before and after α EPSP_{AMPA}-CT (left) or α EPSP_{KA}-CT (right) in control DGCs. (Inset) Corresponding averaged linear fits, same units than the main panel.

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SUPPLEMENTARY TABLES

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		Before CT	RMP	Rinput	τ	AP amplitude	AP Threshold	AP H-W	f/l gain	Rheobase
			(mV)	(MΩ)	(ms)	(mV)	(mV)	(ms)	(Hz/nA)	(pA)
	tro	Mean ± SEM	-73.9 ± 0.4	250.1 ± 10.8	33.4 ± 1.5	67.8 ± 1.6	-39.4 ± 0.5	1.11 ± 0.03	120.1 ± 7.8	98.1 ± 3.9
	Co	n	53	55	55	55	55	55	43	43
	-									
	Ľ:	Mean ± SEM	-70.0 ± 0.4	228.5 ± 10.9	27.5 ± 1.3	73.2 ± 2.3	-42.4 ± 0.6	1.05 ± 0.03	137.3 ± 12.8	92.2 ± 7.0
	epi	n	31	31	31	31	31	31	22	23
_	Epil									
		Test	M-W	Student	Student	Student	Student	Student	M-W	M-W
		P value	0.0000	0.194	0.010 *	0.053	0.0004 ***	0.181	0.367	0.157

Supplementary Table S1. Basic membrane and intrinsic firing properties of DGCs before CT in control and epileptic rats. RMP: resting membrane potential; R_{input} : input resistance; τ : time constant; AP: action potential; H-W: half-width; M-W: Mann-Whitney. * P < 0.05; *** P < 0.001.

	Experiment	After CT	R _{input} (%)	τ (%)	AP amplitude (%)	AP Threshold (%)	AP H-W (%)
	Control	Mean ± SEM	124.1 ± 7.5	103.6 ± 11.2	91.2 ± 2.6	97.0 ± 2.3	99.7 ± 4.3
		n	9	9	9	9	9
	Epileptic	Mean ± SEM	132.8 ± 10.6	119.7 ± 10.5	89.5 ± 4.4	101.5 ± 1.6	94.7 ± 2.8
		n	7	7	7	6	7
	Epileptic	Mean ± SEM	127.0 ± 8.9	105.6 ± 6.2	90.6 ± 4.5	94.9 ± 1.6	100.9 ± 2.8
	+ UBP310	n	9	9	9	9	9
		Test	Kruskal-Wallis	ANOVA	ANOVA	Kruskal-Wallis	Kruskal-Wallis
-		P value	0.790	0.475	0.955	0.069	0.167

Supplementary Table S2. Similar evolution of basic membrane properties following CT in DGCs from control, epileptic rats and in the presence of UBP310. Percentages relative to the condition before CT.

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After CT + TTX	R _{input} (%)	τ (%)	AP amplitude (%)	AP Threshold (%)	AP H-W (%)
Mean ± SEM	100.9 ± 9.6	97.7 ± 9.5	98.0 ± 8.5	96.1 ± 4.0	100.0 ± 5.4
n	7	7	7	6	7
Test	paired Student	paired Student	paired Student	paired Student	paired Student
P value	0.943	0.734	0.539	0.377	0.818

Supplementary Table S3. Non-significant change of basic membrane properties after application of 20 nM TTX. Percentages relative to the condition after CT in the absence of TTX.

Experiment	Condition compared to before CT	f/I gain change (% ± SEM)	n	p value	Test	Difference of rheobase (pA ± SEM)	n	p value	Test
Control	After CT	-3.2 ± 11.7	15	0.237	paired Student	0 ± 8.7	15	0.561	Wilcoxon
	After CT + TTX	3.2 ± 13.6	6	0.774	paired Student	-10 ± 11.3	6	0.415	paired Student
Epileptic	After CT	68.2 ± 22.8 *	7	0.016	ANOVA (rm) +	-15.7 ± 5.3 *	7	0.039	Friedman ANOVA
	After CT + TTX	25.2 ± 21.8		0.188	Tukey HSD	8.6 ± 11.4		0.335	+ Wilcoxon
Epileptic + UBP	After CT	20.0 ± 19.7	7	0.313	paired Student	-1.3 ± 8.1	8	0.882	paired Student
αEPSP _{AMPA}	After CT	11.3 ± 24.1	7	0.671	paired Student	2.9 ± 8.1	7	0.736	paired Student
αEPSP _{KA}	After CT	63.9 ± 30.2 **	9	0.0099	ANOVA (rm) +	-28.9 ± 5.9 *	9	0.010	Friedman ANOVA
	After CT + TTX	23.2 ± 20.5		0.780	Tukey HSD	-6.7 ± 8.2		0.380	+ Wilcoxon
αΕΡSΡΑΜΡΑ/ΚΑ	After CT	68.1 ± 21.9 *	10	0.015	paired Student	-20 ± 5.2 **	10	0.004	paired Student

Supplementary Table S4. Summary of f/I gain and rheobase changes after CT and after subsequent application of 20 nM TTX. rm: repeated measures. * p < 0.05; ** p < 0.01.

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REFERENCES

- 1. Cornaggia, CM, Beghi, M, Provenzi, M, et al. Correlation between cognition and behavior in epilepsy. Epilepsia. 2006; 47 Suppl 2:34-39.
- 2. Hermann, BP, Seidenberg, M, Schoenfeld, J, et al. Neuropsychological characteristics of the syndrome of mesial temporal lobe epilepsy. Arch Neurol. 1997; 54:369-376.
- 3. Helmstaedter, C. Effects of chronic epilepsy on declarative memory systems. Prog Brain Res. 2002; 135:439-453.
- 4. Morimoto, K, Fahnestock, M, and Racine, RJ. Kindling and status epilepticus models of epilepsy: rewiring the brain. Prog Neurobiol. 2004; 73:1-60.
- Aldenkamp, AP, Arends, J, Verspeek, S, et al. The cognitive impact of epileptiform EEGdischarges; relationship with type of cognitive task. Child Neuropsychol. 2004; 10:297-305.
- 6. Majak, K and Pitkanen, A. Do seizures cause irreversible cognitive damage? Evidence from animal studies. Epilepsy Behav. 2004; 5 Suppl 1:S35-S44.
- 7. Meador, KJ. Cognitive and memory effects of the new antiepileptic drugs. Epilepsy Res.2006; 68:63-67.
- 8. Canovas, R, Leon, I, Serrano, P, et al. Spatial navigation impairment in patients with refractory temporal lobe epilepsy: evidence from a new virtual reality-based task. Epilepsy Behav. 2011; 22:364-369.

- 9. Grewe, P, Lahr, D, Kohsik, A, et al. Real-life memory and spatial navigation in patients with focal epilepsy: Ecological validity of a virtual reality supermarket task. Epilepsy Behav. 2014; 31:57-66.
- 10. Butler, CR and Zeman, AZ. Recent insights into the impairment of memory in epilepsy: transient epileptic amnesia, accelerated long-term forgetting and remote memory impairment. Brain. 2008; 131:2243-2263.
- 11. Coras, R, Pauli, E, Li, J, et al. Differential influence of hippocampal subfields to memory formation: insights from patients with temporal lobe epilepsy. Brain. 2014; 137:1945-1957.
- Kleen, JK, Scott, RC, Holmes, GL, et al. Hippocampal interictal epileptiform activity disrupts cognition in humans. Neurology. 2013; 81:18-24.
- 13. Kotloski, R, Lynch, M, Lauersdorf, S, et al. Repeated brief seizures induce progressive hippocampal neuron loss and memory deficits. Prog Brain Res. 2002; 135:95-110.
- Lenck-Santini, PP and Holmes, GL. Altered phase precession and compression of temporal sequences by place cells in epileptic rats. J Neurosci. 2008; 28:5053-5062.
- 15. Chauviere, L, Rafrafi, N, Thinus-Blanc, C, et al. Early deficits in spatial memory and theta rhythm in experimental temporal lobe epilepsy. J Neurosci. 2009; 29:5402-5410.
- 16. Kleen, JK, Scott, RC, Holmes, GL, et al. Hippocampal interictal spikes disrupt cognition in rats. Ann Neurol. 2010; 67:250-257.

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- 17. Inostroza, M, Brotons-Mas, JR, Laurent, F, et al. Specific impairment of "what-where-when" episodic-like memory in experimental models of temporal lobe epilepsy. J Neurosci. 2013; 33:17749-17762.
- Binnie, CD, Kasteleijn-Nolst Trenite, DG, Smit, AM, et al. Interactions of epileptiform EEG discharges and cognition. Epilepsy Res. 1987; 1:239-245.
- Liu, X, Muller, RU, Huang, LT, et al. Seizure-induced changes in place cell physiology: relationship to spatial memory. J Neurosci. 2003; 23:11505-11515.
- 20. Dugladze, T, Vida, I, Tort, AB, et al. Impaired hippocampal rhythmogenesis in a mouse model of mesial temporal lobe epilepsy. Proc Natl Acad Sci U S A. 2007; 104:17530-17535.
- 21. Zhou, JL, Lenck-Santini, PP, Zhao, Q, et al. Effect of interictal spikes on single-cell firing patterns in the hippocampus. Epilepsia. 2007; 48:720-731.
- 22. Zhou, JL, Lenck-Santini, PP, and Holmes, GL. Postictal single-cell firing patterns in the hippocampus. Epilepsia. 2007; 48:713-719.
- 23. Marques, CM, Caboclo, LO, da Silva, TI, et al. Cognitive decline in temporal lobe epilepsy due to unilateral hippocampal sclerosis. Epilepsy Behav. 2007; 10:477-485.
- 24. SCOVILLE, WB and MILNER, B. Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry. 1957; 20:11-21.
- 25. O'Keefe, J and Dostrovsky, J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res. 1971; 34:171-175.

John Wiley & Sons

- 26. Zola-Morgan, S, Squire, LR, and Amaral, DG. Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J Neurosci. 1986; 6:2950-2967.
- 27. BenAri, Y. Limbic Seizure and Brain-Damage Produced by Kainic Acid Mechanisms and Relevance to Human Temporal-Lobe Epilepsy. Neuroscience. 1985; 14:375-403.
- Kobayashi, M and Buckmaster, PS. Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. Journal of Neuroscience. 2003; 23:2440-2452.
- 29. Ben-Ari, Y, Crepel, V, and Represa, A. Seizures beget seizures in temporal lobe epilepsies: the boomerang effects of newly formed aberrant kainatergic synapses. Epilepsy Curr.
 2008; 8:68-72.
- 30. Thind, KK, Yamawaki, R, Phanwar, I, et al. Initial loss but later excess of GABAergic synapses with dentate granule cells in a rat model of temporal lobe epilepsy. J Comp Neurol. 2010; 518:647-667.
- 31. Scharfman, HE and Myers, CE. Hilar mossy cells of the dentate gyrus: a historical perspective. Front Neural Circuits. 2012; 6:106.
- 32. Sloviter, RS, Bumanglag, AV, Schwarcz, R, et al. Abnormal dentate gyrus network circuitry in temporal lobe epilepsy. 2012.
- 33. Myers, CE, Bermudez-Hernandez, K, and Scharfman, HE. The influence of ectopic migration of granule cells into the hilus on dentate gyrus-CA3 function. PLoS One. 2013; 8:e68208.

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- 34. Goldberg, EM and Coulter, DA. Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. Nat Rev Neurosci. 2013; 14:337-349.
- 35. Tauck, DL and Nadler, JV. Evidence of Functional Mossy Fiber Sprouting in Hippocampal-Formation of Kainic Acid-Treated Rats. Journal of Neuroscience. 1985; 5:1016-1022.
- Represa, A, Tremblay, E, and BenAri, Y. Kainate Binding-Sites in the Hippocampal Mossy Fibers - Localization and Plasticity. Neuroscience. 1987; 20:739-748.
- 37. Sutula, TP and Dudek, FE. Unmasking recurrent excitation generated by mossy fiber sprouting in the epileptic dentate gyrus: an emergent property of a complex system. Prog Brain Res. 2007; 163:541-563.
- 38. Peng, Z, Zhang, N, Wei, W, et al. A reorganized GABAergic circuit in a model of epilepsy: evidence from optogenetic labeling and stimulation of somatostatin interneurons. J Neurosci. 2013; 33:14392-14405.
- 39. Represa, A, Robain, O, Tremblay, E, et al. Hippocampal plasticity in childhood epilepsy.Neurosci Lett. 1989; 99:351-355.
- 40. Gabriel, S, Njunting, M, Pomper, JK, et al. Stimulus and potassium-induced epileptiform activity in the human dentate gyrus from patients with and without hippocampal sclerosis. J Neurosci. 2004; 24:10416-10430.

41. de Lanerolle, NC, Lee, TS, and Spencer, DD. Histopathology of Human Epilepsy. 2012.

John Wiley & Sons

- 42. Peret, A, Christie, LA, Ouedraogo, DW, et al. Contribution of Aberrant GluK2-Containing Kainate Receptors to Chronic Seizures in Temporal Lobe Epilepsy. Cell Rep. 2014; 8:347-354.
- 43. Epsztein, J, Represa, A, Jorquera, I, et al. Recurrent mossy fibers establish aberrant kainate receptor-operated synapses on granule cells from epileptic rats. J Neurosci. 2005; 25:8229-8239.
- 44. Artinian, J, Peret, A, Marti, G, et al. Synaptic kainate receptors in interplay with INaP shift the sparse firing of dentate granule cells to a sustained rhythmic mode in temporal lobe epilepsy. J Neurosci. 2011; 31:10811-10818.
- 45. Heinemann, U, Beck, H, Dreier, JP, et al. The dentate gyrus as a regulated gate for the propagation of epileptiform activity. Epilepsy Res Suppl. 1992; 7:273-280.
- 46. Hsu, D. The dentate gyrus as a filter or gate: a look back and a look ahead. Prog Brain Res. 2007; 163:601-613.
- 47. Bragin, A, Jando, G, Nadasdy, Z, et al. Gamma (40-100 Hz) oscillation in the hippocampus of the behaving rat. J Neurosci. 1995; 15:47-60.
- 48. Chrobak, JJ and Buzsaki, G. Gamma oscillations in the entorhinal cortex of the freely behaving rat. J Neurosci. 1998; 18:388-398.
- 49. Pernia-Andrade, AJ and Jonas, P. Theta-gamma-modulated synaptic currents in hippocampal granule cells in vivo define a mechanism for network oscillations. Neuron. 2014; 81:140-152.

John Wiley & Sons

- 50. Jung, MW and Mcnaughton, BL. Spatial Selectivity of Unit-Activity in the Hippocampal Granular Layer. Hippocampus. 1993; 3:165-182.
- 51. Chawla, MK, Guzowski, JF, Ramirez-Amaya, V, et al. Sparse, environmentally selective expression of Arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience. Hippocampus. 2005; 15:579-586.
- 52. Leutgeb, JK, Leutgeb, S, Moser, MB, et al. Pattern separation in the dentate gyrus and CA3 of the hippocampus. Science. 2007; 315:961-966.
- 53. Acsady, L and Kali, S. Models, structure, function: the transformation of cortical signals in the dentate gyrus. Prog Brain Res. 2007; 163:577-599.
- 54. Treves, A, Tashiro, A, Witter, MP, et al. What is the mammalian dentate gyrus good for? Neuroscience. 2008; 154:1155-1172.
- 55. Epsztein, J, Sola, E, Represa, A, et al. A selective interplay between aberrant EPSPKA and INaP reduces spike timing precision in dentate granule cells of epileptic rats. Cereb Cortex. 2010; 20:898-911.
- 56. Vervaeke, K, Hu, H, Graham, LJ, et al. Contrasting effects of the persistent Na+ currenton neuronal excitability and spike timing. Neuron. 2006; 49:257-270.
- 57. Mahon, S and Charpier, S. Bidirectional plasticity of intrinsic excitability controls sensory inputs efficiency in layer 5 barrel cortex neurons in vivo. J Neurosci. 2012; 32:11377-11389.

John Wiley & Sons

- 58. Pinheiro, PS, Lanore, F, Veran, J, et al. Selective block of postsynaptic kainate receptors reveals their function at hippocampal mossy fiber synapses. Cereb Cortex. 2013; 23:323-331.
- 59. Daoudal, G and Debanne, D. Long-term plasticity of intrinsic excitability: learning rules and mechanisms. Learn Mem. 2003; 10:456-465.
- 60. Taddese, A and Bean, BP. Subthreshold sodium current from rapidly inactivating sodium channels drives spontaneous firing of tuberomammillary neurons. Neuron. 2002; 33:587-600.
- 61. Crill, WE. Persistent sodium current in mammalian central neurons. Annu Rev Physiol. 1996; 58:349-362.
- 62. Noebels, JL, Avoli, M, Rogawski, M, et al. "Jasper's Basic Mechanisms of the Epilepsies" Workshop. Epilepsia. 2010; 51 Suppl 5:1-5.
- 63. Poolos, NP and Johnston, D. Dendritic ion channelopathy in acquired epilepsy. Epilepsia.2012; 53 Suppl 9:32-40.
- 64. Beck, H and Yaari, Y. Plasticity of intrinsic neuronal properties in CNS disorders. Nat Rev Neurosci. 2008; 9:357-369.
- 65. Noam, Y, Bernard, C, and Baram, TZ. Towards an integrated view of HCN channel role in epilepsy. Curr Opin Neurobiol. 2011; 21:873-879.
- 66. Lerche, H, Shah, M, Beck, H, et al. Ion channels in genetic and acquired forms of epilepsy. J Physiol. 2013; 591:753-764.

John Wiley & Sons

- 67. Bender, RA, Soleymani, SV, Brewster, AL, et al. Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. J Neurosci. 2003; 23:6826-6836.
- 68. Young, CC, Stegen, M, Bernard, R, et al. Upregulation of inward rectifier K+ (Kir2) channels in dentate gyrus granule cells in temporal lobe epilepsy. J Physiol. 2009; 587:4213-4233.
- 69. Surges, R, Kukley, M, Brewster, A, et al. Hyperpolarization-activated cation current Ih of dentate gyrus granule cells is upregulated in human and rat temporal lobe epilepsy. Biochem Biophys Res Commun. 2012; 420:156-160.
- 70. Stegen, M, Kirchheim, F, Hanuschkin, A, et al. Adaptive intrinsic plasticity in human dentate gyrus granule cells during temporal lobe epilepsy. Cereb Cortex. 2012; 22:2087-2101.
- 71. Nagerl, UV, Mody, I, Jeub, M, et al. Surviving granule cells of the sclerotic human hippocampus have reduced Ca(2+) influx because of a loss of calbindin-D(28k) in temporal lobe epilepsy. J Neurosci. 2000; 20:1831-1836.
- 72. Kumar, SS and Buckmaster, PS. Hyperexcitability, interneurons, and loss of GABAergic synapses in entorhinal cortex in a model of temporal lobe epilepsy. J Neurosci. 2006; 26:4613-4623.
- 73. Zhang, W and Linden, DJ. The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nat Rev Neurosci. 2003; 4:885-900.

36

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- 74. Turrigiano, G, Abbott, LF, and Marder, E. Activity-dependent changes in the intrinsic properties of cultured neurons. Science. 1994; 264:974-977.
- 75. Ganguly, K, Kiss, L, and Poo, M. Enhancement of presynaptic neuronal excitability by correlated presynaptic and postsynaptic spiking. Nat Neurosci. 2000; 3:1018-1026.
- 76. Xu, J, Kang, N, Jiang, L, et al. Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. J Neurosci. 2005; 25:1750-1760.
- 77. Sheffield, ME, Best, TK, Mensh, BD, et al. Slow integration leads to persistent action potential firing in distal axons of coupled interneurons. Nat Neurosci. 2011; 14:200-207.
- 78. Fan, Y, Fricker, D, Brager, DH, et al. Activity-dependent decrease of excitability in rat hippocampal neurons through increases in I(h). Nat Neurosci. 2005; 8:1542-1551.
- 79. Campanac, E, Gasselin, C, Baude, A, et al. Enhanced intrinsic excitability in basket cells maintains excitatory-inhibitory balance in hippocampal circuits. Neuron. 2013; 77:712-722.
- 80. Melyan, Z, Wheal, HV, and Lancaster, B. Metabotropic-mediated kainate receptor regulation of IsAHP and excitability in pyramidal cells. Neuron. 2002; 34:107-114.
- 81. Ruiz, A, Sachidhanandam, S, Utvik, JK, et al. Distinct subunits in heteromeric kainate receptors mediate ionotropic and metabotropic function at hippocampal mossy fiber synapses. J Neurosci. 2005; 25:11710-11718.

82. Fisahn, A, Heinemann, SF, and Mcbain, CJ. The kainate receptor subunit GluR6 mediates metabotropic regulation of the slow and medium AHP currents in mouse hippocampal neurones. J Physiol. 2005; 562:199-203.

- 83. Contractor, A, Mulle, C, and Swanson, GT. Kainate receptors coming of age: milestones of two decades of research. Trends Neurosci. 2011; 34:154-163.
- 84. Lerma, J and Marques, JM. Kainate receptors in health and disease. Neuron. 2013; 80:292-311.
- 85. Nikitin, ES, Vavoulis, DV, Kemenes, I, et al. Persistent sodium current is a nonsynaptic substrate for long-term associative memory. Curr Biol. 2008; 18:1221-1226.
- 86. Aizenman, CD and Linden, DJ. Rapid, synaptically driven increases in the intrinsic excitability of cerebellar deep nuclear neurons. Nat Neurosci. 2000; 3:109-111.
- 87. Chen, L, Bohanick, JD, Nishihara, M, et al. Dopamine D1/5 receptor-mediated long-term potentiation of intrinsic excitability in rat prefrontal cortical neurons: Ca2+-dependent intracellular signaling. J Neurophysiol. 2007; 97:2448-2464.
- 88. Hyun, JH, Eom, K, Lee, KH, et al. Activity-dependent downregulation of D-type K+
 channel subunit Kv1.2 in rat hippocampal CA3 pyramidal neurons. J Physiol. 2013; 591:5525-5540.
- 89. Schmidt-Hieber, C, Jonas, P, and Bischofberger, J. Subthreshold dendritic signal processing and coincidence detection in dentate gyrus granule cells. J Neurosci. 2007; 27:8430-8441.

John Wiley & Sons

- Krueppel, R, Remy, S, and Beck, H. Dendritic integration in hippocampal dentate granule cells. Neuron. 2011; 71:512-528.
- 91. Sambandan, S, Sauer, JF, Vida, I, et al. Associative plasticity at excitatory synapses facilitates recruitment of fast-spiking interneurons in the dentate gyrus. J Neurosci. 2010; 30:11826-11837.
- 92. Mateos-Aparicio, P, Murphy, R, and Storm, JF. Complementary functions of SK and Kv7/M potassium channels in excitability control and synaptic integration in rat hippocampal dentate granule cells. J Physiol. 2013.
- 93. Neunuebel, JP and Knierim, JJ. Spatial firing correlates of physiologically distinct cell types of the rat dentate gyrus. J Neurosci. 2012; 32:3848-3858.
- 94. Jinde, S, Zsiros, V, Jiang, Z, et al. Hilar mossy cell degeneration causes transient dentate granule cell hyperexcitability and impaired pattern separation. Neuron. 2012; 76:1189-1200.
- 95. Zhang, W and Buckmaster, PS. Dysfunction of the dentate basket cell circuit in a rat model of temporal lobe epilepsy. J Neurosci. 2009; 29:7846-7856.
- 96. Zhang, W, Yamawaki, R, Wen, X, et al. Surviving hilar somatostatin interneurons enlarge, sprout axons, and form new synapses with granule cells in a mouse model of temporal lobe epilepsy. J Neurosci. 2009; 29:14247-14256.
- 97. Yim, MY, Hanuschkin, A, and Wolfart, J. Intrinsic rescaling of granule cells restores pattern separation ability of a dentate gyrus network model during epileptic hyperexcitability. Hippocampus. 2014.

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Figure 1. Long-lasting increase of PP-DGC firing rate triggered by rMF-CT in epileptic rats. (A) Recording configuration and neurolucida reconstructed DGC filled with biocytin. PP: perforant path, A/C: associational/commissural pathway, rMF: recurrent mossy fibres pathway, gcl: granule cell layer. (B, C) PP-DGC firing patterns before and after A/C-CT in control (B) or after rMF-CT and subsequent TTX application (20 nM) in epileptic condition (C). Scale bars, 20 mV, 500 ms. (D) DGC firing rate during CT (CT-DGC firing). (E) Time course of the PP-DGC average firing rate before and after CT in control (left) or after rMF-CT and after subsequent TTX application (20 nM) (right) in epileptic rats; Wilcoxon. For this and following figures and tables, action potentials are truncated, small dashes represent electrical stimulations, mean values ± SEM, * p < 0.05; ** p < 0.01; *** p < 0.001; n.s., non-significant.

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Figure 2. DGCs from control and epileptic rats display similar short- and long-term depression profiles. (A) Representative PP-input trains of EPSCs recorded in voltage-clamp at -70 mV before (left) and after CT (right) in DGCs from control (top) and epileptic rats (bottom). Scale bars, 20 pA, 200 ms. (Insets) First EPSC of the same trains before and after CT in control (top) and epileptic (bottom) conditions. Scale bars, 20 pA, 50 ms. (B) First and last EPSC amplitude within trains of PP-input before CT; Wilcoxon (control) and paired Student's (epileptic) tests. (C) First EPSC amplitude of trains before and after CT; paired Student. (D) Ratio last/first EPSC before and after CT (paired Student); in control and epileptic conditions (Student). (E) First EPSC after CT normalized to values before CT in control and epileptic conditions, Student.

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Artinian et al., Fig.3 B A Epileptic - UBP310 PP-input (ZH) PP-input rMF-input СТ (After CT) (Before CT) rate (firing r B Pp-0 Before After СТ СТ D C Control - No D-APV PP-input PP-input A/C-input firing rate (Hz) (After CT) (Before CT) CI DOC -dd 0 Before After CT СТ E Epileptic - No D-APV F rMF-input firing rate (Hz) PP-input PP-input (After CT) (Before CT) CT DGC C Before After CT CT G Epileptic - BAPTA Н PP-input rMF-input PP-input firing rate (Hz) (Before CT) (After CT) CT PP-DGC Before After CT CT J Т 6 Epileptic + UBP310 Epileptic - D-APV CT-DGC firing rate (Hz) PP-DGC firing rate (Hz) Epileptic + BAPTA 6 2 BP31U APAPAPTA 0 UBP310 10 20 30 10 Time (min)

Figure 3. KARs and Ca2+ but not NMDARs are crucial determinants for long-lasting increase of PP-DGC firing rate triggered by rMF-CT in epileptic DGCs.

(A) PP-DGC firing pattern and (B) PP-DGC firing rate before and after rMF-CT in epileptic condition in the presence of 5 µM UBP310; Wilcoxon. (C, E) PP-DGC firing patterns before and after A/C-CT in control (C) or after rMF-CT in epileptic condition (E) without NMDAR antagonist (no D-APV). (D, F) PP-DGC firing rate before and after A/C-CT in control (D, paired Student) or after rMF-CT in epileptic rats without NMDAR antagonist (no D-APV) (F, Wilcoxon). (G) PP-DGC firing pattern and (H) PP-DGC firing rate before and after rMF-CT in epileptic condition in the presence of 20 mM BAPTA inside the patch pipette. Scale bars, 20 mV,
 500 ms. (I) rMF-CT-DGC firing rate in the presence of 40 µM D-APV, 5 µM gabazine, 5 µM CGP55845 and 5 µM UBP310 (black, + UBP310); 5 µM gabazine and 5 µM CGP55845 (red, no D-APV); 40 µM D-APV, 5 µM gabazine, 5 µM CGP55845 and 20 mM BAPTA (grey, + BAPTA) in epileptic conditions. (J) Time course of the PP-DGC average firing rate before and after CT in epileptic conditions. Same respective color codes than in (I).

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Figure 4. Simulated train of EPSPKA increases PP-DGC firing rate in control rats. (A) (Top) Single aEPSPAMPA and aEPSPKA; scale bar, 100 ms. DGC firing patterns (middle) evoked by somatic injection of waveforms of current (bottom) simulating trains of AMPAR-EPSPs (left, aEPSPAMPA) or trains of KAR-EPSPs (right, aEPSPKA). Scale bars, 10 mV, 250 ms. (B) Distribution of inter-spike intervals (ISIs) with Gaussian fits (left) and plotted as cumulative probabilities (middle); rMF in epileptic (N = 2852) vs. aEPSPKA (N = 4225) in control condition, p = 0.999. A/C (N = 430) vs. aEPSPAMPA (N = 746) in control condition, p = 0.684, Kolmogorov-Smirnov. N refers to the number of ISIs. (Right) DGC firing rate during CT in control and epileptic conditions. (C) Recording configuration. (D, E) PP-DGC firing patterns before and after aEPSPAMPA-CT (D) or after aEPSPKA-CT and subsequent TTX application (20 nM) (E) in control rats. Scale bars, 20 mV, 500 ms. (F) PP-DGC firing rate plotted as a population data before and after aEPSPAMPA-CT (left) and after aEPSPKA-CT and subsequent 20 nM TTX application (right); Wilcoxon. (G) Time course of the PP-DGC mean firing rate before and after aEPSPKA-CT in control condition with or without BAPTA inside the patch pipette.

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(A) 10 different trains of current waveforms composed of 50% aEPSPAMPA and 50% EPSPKA randomly distributed and corresponding firing patterns used as CT in control DGCs. Scale bars, 20 mV, 500 ms. (B) PP-DGC firing pattern before (top) and after (bottom) aEPSPAMPA/KA-CT; scale bars, 20 mV, 500 ms. (C) PP-DGC firing rate before and after CT; Wilcoxon. (D) Correlation between PP-DGC firing rate after CT and DGC firing rate during CT; this plot summarizes data obtained in control (A/C-, aEPSPAMPA-, aEPSPKA- and aEPSPAMPA/KA-CT) and epileptic (rMF-CT and rMF-CT + UBP310) conditions.

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(A) Current profiles induced by incremental voltage pulses before, after rMF-CT and after subsequent application of 1 µM TTX in epileptic DGCs. Scale bars, 100 pA, 100 ms. (Inset) rMF-CT DGC firing pattern. Scale bars, 10 mV, 200 ms. (B) Subtracted TTX-sensitive INAP recorded at -40 mV before and after CT.
 Scale bars, 30 pA, 50 ms. (C) I/V curves for one representative DGC before, after rMF-CT and after TTX. (D) Average I/V curves of INAP before and after rMF-CT. (E) Increase of INAP after rMF-CT in epileptic DGCs, Wilcoxon tests. (F) Average time course of INAP (normalized to baseline) at Vh = -40 mV before and after rMF-CT in epileptic DGCs; the dashed line indicates the averaged baseline before CT.

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Figure 7. Increase of subthreshold depolarization and intrinsic excitability after rMF-CT in epileptic DGCs.
 (A) Subthreshold membrane depolarization evoked by a 60 pA pulse before and after CT in control and in epileptic DGCs. Scale bars, 5 mV, 100 ms. (B) Subthreshold area (from dashed lines in (A) to signals) change after CT in control or epileptic conditions and after subsequent application of 20 nM TTX (paired Student's tests). (C) DGC firing evoked by a 100 pA pulse before, after A/C-CT in control (top), rMF-CT in epileptic (middle) or aEPSPKA-CT in control (bottom) conditions and after subsequent application of 20 nM TTX. Scale bars, 20 mV, 100 ms. (D) Average f/I plots before, after CT and after subsequent TTX application in control (left) and epileptic (right) DGCs. (Inset) Corresponding averaged linear fits, same units than the main panel. (E) Average f/I plots before and after aEPSPAMPA-CT (left) or aEPSPKA-CT (right) in control DGCs. (Inset) Corresponding averaged linear fits, same units than the main panel.

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