Disentangling chemical and electrical effects of status epilepticus-induced dentate gyrus abnormalities

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ARTICLE INFO

Article history:
Received 6 June 2019
Revised 10 September 2019
Accepted 14 September 2019
Available online xxxx

Keywords:
Epilepsy
Intrahippocampal
Adult neurogenesis
Granular cell dispersion
Ectopic neurons
Hilar basal dendrites

ABSTRACT

In rodents, status epilepticus (SE) triggered by chemoconvulsants can differently affect the proliferation and fate of adult-born dentate granule cells (DGCs). It is unknown whether abnormal neurogenesis results from intracellular signaling associated with drug-receptor interaction, paroxysmal activity, or both. To test the contribution of these factors, we systematically compared the effects of kainic acid (KA)- and pilocarpine (PL)-induced SE on the morphology and localization of DGCs generated before or after SE in the ipsi- and contralateral hippocampi of mice. Hippocampal insult was induced by unilateral intrahippocampal (ihpc) administration of KA or PL. We employed conditional doublecortin-dependent expression of the green fluorescent protein (GFP) to label adult-born cells committed to neuronal lineage either one month before (mature DGCs) or seven days after (immature DGCs) SE. Unilateral ihpc administration of KA or PL led to bilateral epileptiform discharges and focal and generalized behavioral seizures. However, drastic granule cell layer (GCL) dispersion occurred only in the ipsilateral side of KA injection, but not in PL-treated animals. Granule cell layer dispersion was accompanied by a significant reduction in neurogenesis after SE in the ipsilateral side of KA-treated animals, while neurogenesis increased in the contralateral side of KA-treated animals and both hippocampi of PL-treated animals. The ratio of ectopic neurons in the ipsilateral hippocampus was higher among immature as compared to mature neurons in the KA model (32.8% vs. 10.0%, respectively), while the occurrence of ectopic neurons in PL-treated animals was lower than 3% among both mature and immature DGCs. Collectively, our results suggest that KA- and PL-induced SE leads to distinct cellular alterations in mature and immature DGCs. We also show different local and secondary effects of KA or PL in the histological organization of the adult DG, suggesting that these unique epilepsy models may be complementary to our understanding of the disease.

NEWroscience 2018.

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1. Introduction

Structural alterations of the hippocampal formation are a hallmark of temporal lobe epilepsies (TLEs) [1,2]. Cell death, astrogliosis, and synaptic reorganization of the hilar region of the dentate gyrus (DG), cornu Ammonis (CA) subfields, subiculum, and extra-hippocampal regions, including limbic cortical areas, have been extensively associated with TLE pathophysiology [3,4]. Remarkably, dentate granule cells (DGCs) are considered less vulnerable to cell death [5,6], and they are frequently described as an essential group of cells that may reinforce the excitatory loop after the initial epileptogenic insult [7]. Compelling evidence suggests that DGC dispersion [8,9], ectopic and swelled DGC bodies [10,11] and presence of basal dendrites [10,12] may all contribute to the epileptic condition [13]. Overall, DGCs are strong candidates for therapeutic approaches [7], once their role in the epileptogenesis is better understood.

Interestingly, DGCs are also one of the few types of neurons that continue to be generated in the adult rodent [14,15] and human brain [16,17]. In the subgranular zone (SGZ) of the postnatal and adult DG, progenitor cells generate neurons that migrate radially, into the granule cell layer (GCL), where they differentiate and extend dendrites and axonal processes towards the molecular layer and hilus/CA3 regions, respectively [18]. These new neurons are functionally integrated into the hippocampal circuitry within four weeks [19] and contribute to hippocampal-dependent learning, mainly in tasks requiring pattern separation [20]. The lineage progression of DG stem cells towards neurons is under the influence of local circuitries, including the mossy cells [21], and therefore, a variety of brain insults, including trauma, stroke, and status epilepticus (SE), can modulate adult neurogenesis [22,23].

In experimental models of TLE, animal species, administration route and chemoconvulsant used to trigger the epileptic condition may differently affect adult DG neurogenesis and GCL organization.
For example, wide granule cell dispersion (GCD) is solely observed after intrahippocampal administration of kainic acid (KA) in mice [24,25]. In this model, cell proliferation increases immediately after SE, although most newly generated neurons do not survive long [25]. Also, in unilateral administration protocols, changes in the contralateral hippocampus are often unreported [26], despite extensive lesions in the ipsilateral hemisphere. On the other hand, in systemic protocols (i.p. or s.c.), both KA and pilocarpine (PL) were able to trigger abnormal dendritic processes in immature adult-born neurons, while mature cells are spared [27–29], suggesting that newly generated neurons could play a role in epileptogenesis [30]. However, ablation of adult-born DG cells fails to control seizures [31,32] or abnormal reorganization of DG local circuitries [33]. These seemingly conflicting results could likely be explained by the effects of chemoconvulsant drugs in other regions of the brain in systemic protocols of SE induction. In the present study, we seek to demonstrate how the local administration of KA or PL, the two most common drugs used to initiate SE, affects adult-generated immature and mature granule neurons in DG. To our knowledge, this is the first study to systematically compare the DG abnormalities induced by these drugs locally, as well as by secondary paroxysmal activity in vivo.

2. Methods

2.1. Animals

All experiments were performed in accordance with international guidelines of animal experimentation and were previously approved by the local ethics committee (CEUA-UFRN). Animals were housed in standard laboratory conditions with a light/dark cycle of 12 h each (lights on at 6 am), with free access to food and water.

2.2. Intrahippocampal kainic acid and pilocarpine injections

Status epilepticus was induced under anesthesia, according to a modified version of a previous protocol [35]. Animals were anesthetized with isoflurane (5% induction and 1.5% maintenance; 1 L/min) and placed in the stereotaxic frame. Using a glass pipette attached to a micro-injector, we injected KA (50 nL, 20 mM; Cayman Chemical), PL (700 μg/μL; Sigma-Aldrich), or vehicle solution (0.9% NaCl, phosphate-buffered saline [PBS]) at a flux of 0.5 μL/min in the right dorsal hippocampus (coordinates, in mm: AP: 2.1; ML: 1.7; DV: 1.6) and waited 5 min before removing the pipette to avoid liquid reflux. Then, the craniotomy was closed with sterile bone wax, the skin sutured, and the isoflurane anesthesia interrupted (time zero for the quantification of latencies). To attenuate intense salivation and related respiratory complication during the SE, methyl-scopolamine (1 mg/kg, i.p.) was injected 30 min before anesthetizing the animal to minimize peripheral side effects associated with SE. Seizure severity was quantified for 2 h after anesthesia discontinuation and included focal (orofacial automatisms, head nodding, and unilateral and bilateral forelimb clonus) and generalised ictal behaviors (rearing, rearing and falling, and wild-running) [36]. In a subset of animals (N = 3/group), local field potential recordings from both hippocampi were used to demonstrate the presence of bilateral epileptiform discharges during the SE. After 90 min of SE, animals received diazepam (5 mg/kg, i.p.) to alleviate seizure activity and were observed for general health in their home-cage for 6 h, where they have free access to food supplements and water. Only animals experiencing severe SE (i.e., persistent behavioral seizures scoring at least Racine III for at least 60 min without spontaneous remission after 2 h) were included in the following analysis.

2.3. Tamoxifen administration and genetic recombination in adult-born granule cells

Tamoxifen (TAM, T-5648, Sigma-Aldrich) was diluted in corn oil (10 mg/mL, Sigma-Aldrich) and administered to Dcx-CreERT2 mice through i.p. injections (100 μg/kg) for two consecutive days. To evaluate the effects of SE on mature and immature granule cells, Cre-mediated genetic recombination using TAM was induced 35 days before or 7 days after SE, respectively. The experimental timelines are represented in Figs. 1A and 3A.

2.4. Immunohistochemistry (IHC), microscopy, and cellular quantification

Animals were euthanized by intraperitoneal injection of thiopental (100 mg/kg, i.p.), and transcardially perfused with saline 0.9%, followed by 4% paraformaldehyde (PFA) solution. Brains were removed and post-fixed in PFA solution at 4 °C overnight. They were then immersed in 30% sucrose solution for cryoprotection, frozen in isopentane with dry ice and stored at −80 °C. Coronal sections (40 μm) were obtained using a cryostat and stored onto ionized slides at −20 °C.

Sections were incubated overnight in PBS 10 mM, triton-X 0.5%, Normal Goat Serum (NGS, 5%), and the primary antibodies: rabbit anti-glial fibrillary acidic protein (1:1000, DAKO) and chicken anti-green fluorescent protein (1:500, Aves Labs). For microglial and neuronal labeling, we performed antigen retrieval with 10-mM citrate buffer, pH 6.0, for 30 min followed by a wash in PBS 10 mM and blocking solution (NGS, 5%). We used rabbit anti-Iba1 (1:1000, Wako) for microglia and rat anti-CTIP2 (CTIP2, 1:500, Abcam) as a neuronal marker. These primary antibodies also were diluted in PBS 10 mM, triton-X 0.5%, and NGS 5%, and incubated overnight at 4 °C. After this period, sections were washed in PBS 10 mM followed by incubation with secondary antibodies for 2 h at room temperature. We used Alexa-Fluor (1:1000, Thermo Scientific) diluted in PBS 10 mM, triton-X 0.5%, and NGS 5%. After washing, sections were stained with (DAPI; 1 μg/mL, Sigma-Aldrich), embedded with Aqua-Poly/Mount (Polysciences), and stored at 4 °C. Samples were analyzed using fluorescence and confocal microscopy (Axio Examiner and LSM-710, Zeiss).

Quantitative histological analyses were performed in serial sections sampled at 1/10 (i.e., one section every 400 μm, in the anteroposterior axis). Granule cell layer boundaries (with the molecular layer and hilus) were determined using the DAPI staining. To evaluate the influence of chemoconvulsant injection on the positioning of adult-born DG cells, all green fluorescent protein (GFP) positive (GFP+) neurons in the ipsi- and contralateral hippocampi in each section were marked using the Stereo Investigator (MBF Bioscience) and the resulting X and Y coordinates exported to Matlab (Mathworks). The spatial location of GFP+ cells in the DG was normalized and represented as the radius in semicircular plots. Therefore, ectopic cells located in the suprapyramidal (values between 0° and 90°) or infrapyramidal (90° and 180°) blades of the DG (for a graphical illustration of the spatial transformation, see Supplementary Fig. 1).

The cell body area was quantified using the ImageJ software (NIH) on images obtained with the confocal microscope. After scale calibration, Z series containing the complete cell image was selected, and we
delimited the fluorescent area of the cell soma as the region of interest, using the “Threshold tool” optimizing the contour and removing the mask for confirmation after selecting the area. Then, we used the Measure tool in the ROI Manager toolbox to calculate the total fluorescent area that corresponds to cell body sizes. The percentage of cells presenting hilar basal dendrites was also calculated. Basal dendrites consisted of thick processes emerging from the cell body, which often ended in complex tufts of short tangle branches with spines [10].

2.5. Statistical analysis

Statistical analyses were performed using Matlab (Mathworks) and Prism (GraphPad). Shapiro–Wilks and Levene tests were used to verify whether data were normally distributed and if group variances were equal, respectively. In case of rejection of these assumptions, nonparametric tests were used. Probability distributions of behavioral seizures and GFP + cell locations were compared using Kolmogorov–Smirnov (KS) two-sample test. The latency for the first seizure was compared using unpaired t-test. Treatments (VH, KA, or PL) were compared using analysis of variance (ANOVA) or Kruskal–Wallis tests, followed by unpaired t-test or Mann–Whitney test as posthoc comparisons when appropriate. Hemispheres (ipsilateral and contralateral to drug injection) were compared using paired t-test or Wilcoxon signed rank test. Data are expressed as the mean ± standard error of the mean (SEM). Significance was set at 5%, and all p-values are reported in the text.

3. Results

3.1. KA- and PL-induced SE with bilateral epileptiform discharges

Unilateral intrahippocampal (ihpc) administration of KA (n = 34) or PL (n = 30) in adult C57Bl/6 mice resulted in unambiguous behavioral SE. Electrophysiological recordings performed in a subset of animals showed epileptiform discharges starting immediately after local application, evolving to long-lasting, high-amplitude, and fast-frequency activity in the hippocampus (Supplementary Fig. 2A). Bilateral ictal spikes, afterdischarges, and seizures were recorded in both hippocampi after KA (Supplementary Fig. 2B) and PL injection (Supplementary Fig. 2C). Abnormal activity recorded in the molecular and granule cell layers of the DG showed voltage phase reversal (arrows in Supplementary Fig. 2B1 and c1) suggesting local generation of these epileptiform discharges. Electrographic paroxysms continued for more than 24 h after administration of both drugs, and although it abated significantly, it did not completely disappear after the SE (data not shown). The latency for the first behavioral seizure was shorter in PL- than KA-treated animals (Supplementary Fig. 2D, unpaired t-test, p < .05) and rapidly evolved to generalized, full-blown seizures. Behaviorally, animals injected with KA first displayed facial automatisms, head and forelimb myoclonus, which slowly developed in bilateral myoclonus, rearing and posture loss. Conversely, animals receiving PL progressed quickly into rearing and falling, wild-running and repetitive and powerful jumping. The cumulative frequency distribution of focal (Supplementary Fig. 2E) and generalized (Supplementary Fig. 2F) ictal behaviors is statistically different between KA- and PL-treated animals (p < .001, KS test). Double-transgenic CDcx/GFP mice showed a similar temporal evolution of the SE. From 16 animals injected with KA, two animals died after injection, and three presented mild SE. These animals were excluded from further analysis. Pilocarpine was injected in eight animals, of which two died after surgery and the remaining 6 were included in the histological analysis. Both KA- and PL-treated animals used for further histological analysis showed spontaneous electrophysiological seizures (data not shown), but no attempt to quantify seizure frequency was made.

3.2. Increased electrical activity is not sufficient to induce GCD

Histological analysis conducted 42 days after KA or PL injection revealed extensive degeneration of the hilus of the DG and, CA3 and CA1 regions, mainly in the ipsilateral hemisphere (Supplementary Fig. 3). In the DG, GCD occurred exclusively in the ipsilateral side of KA-treated animals (Supplementary Fig. 4). The width of the GCL was about three times bigger in the ipsilateral than the contralateral side, although it was similar 15 and 42 days after KA-injection in the ipsilateral DG (177 ± 16 μm and 203 ± 10 μm, respectively; p = .18, unpaired t-test). (Supplementary Fig. 4B). In contrast, animals injected with PL presented GCD neither in the ipsilateral nor contralateral hippocampi (Supplementary Fig. 4), although GCL width increased from days 15 to 42 in both contra- and ipsilateral of PL-treated animals (Supplementary Fig. 4C). Together with our electrophysiological observations of sustained and bilateral paroxysms, these data suggest that increased electrical activity per se is not sufficient to induce GCD and that this phenomenon may be caused by a nonelectric, chemically-related effect of KA in the DG. It also suggests that the mechanism responsible for GCD rapidly evolves in the first 15 days after SE and stabilizes after it.

It has previously been shown that increased electrical activity induced by KA is associated with an augmented inflammatory response in the hippocampus [26]. To probe whether KA- and PL-induced SE could induce inflammatory responses in the ipsi- and contralateral DGs, we analyzed the morphology of microglia, the resident immune cells of the CNS, in these regions. In the brain, inflammatory response is mediated by the activated microglia, which generally respond to neuronal damage and remove the damaged cells by phagocytosis. The change in the activation status of microglial cells is reflected in their progressive morphological transformation from a highly ramified into a less ramified or ameboid cell shape. Using immunohistochemistry against IBA1, we observed that microglial cells in both KA- and PL-ipsilateral injected hippocampi displayed enlarged cell bodies and thicker processes (ameboid morphology) 3 days after SE (Supplementary Fig. 5). These morphological changes are qualitatively more pronounced in KA- than PL-injected animals. In contrast, in both hippocampi of VH-treated animals and in the contralateral hippocampi of KA- and PL-injected animals, microglial cells show multiple thin processes and small cell bodies, indicative of a resting state (Supplementary Fig. 5). These observations may indicate that a robust inflammatory response is associated with local effects of KA and PL, rather than paroxysmal activity. It also suggests that inflammation is not sufficient to induce GCD, once these two phenomena are dissociated in the PL-ipsilateral DG.

3.3. Effects of KA and PL on mature adult-born granule cell position and morphology

Through TAM-mediated Cre-recombination of cells expressing CreERT2 under the promoter of the gene Dcx (expressed by neuron-committed progenitors and immature neurons of the adult DG during the first 3–4 weeks of differentiation [38]), we could evaluate the position and morphologies of granule cells generated between 5 to 8 weeks before chemoconvulsant injections (Fig. 1A). In VH-treated animals, GFP + cells were observed throughout the GCL, both in the suprapyramidal and infrapyramidal blade of the DG. The GFP + cells displayed typical DGC morphologies and molecular signatures, as indicated by CTIP2 expression (Fig. 1B and Supplementary Fig. 6). The GFP + cells within the GCL were homogeneously distributed in the medial-lateral axis (Fig. 1B), and the GFP + punctate staining was detected mainly in the hilus (Fig. 1B, vehicle), suggesting that these cells establish synapses onto the dendrites of hilar cells. Similar staining patterns were observed in PL-treated animals and in the contralateral hippocampus of KA-treated animals. However, in the ipsilateral hippocampus of KA-treated animals, we observed a significant number of GFP + cells displaying glial morphologies and expressing the astrocyte glial fibrillary acidic protein (GFAP) (Fig. 1B, middle panel

Please cite this article as: D.M.S. Moura, I.R.P. de Sales, J.A. Brandão, et al., Disentangling chemical and electrical effects of status epilepticus-induced dentate gyrus abnormalities, Epilepsy & Behavior, https://doi.org/10.1016/j.yebeh.2019.106575
and Supplementary Fig. 6C). Despite this fact, the mean number of GFP+ neurons in the contra- and ipsilateral hemispheres was similar in all groups (total number of GFP+ neurons, VH: 278 ± 93 and 257 ± 92; KA: 223 ± 99 and 191 ± 83; PL: 147 ± 51 and 108 ± 5; chi-square = 1.08 and 0.52; d.f. = 2; p = .58 and 0.77, for contra- and ipsilateral, respectively), and most of them were located at short distances from the hilar border of the GCL (Fig. 1C, D). Surprisingly, the distribution of mature GFP+ neurons located within the GCL in KA- and PL-treated animals did not differ from controls (Fig. 1C, D), despite the considerable GCD in the ipsilateral hippocampus of KA-treated animals (Fig. 1B). However, the absolute number of ectopic GFP+ granule cells was significantly higher in ipsilateral, but not in the contralateral hemisphere of KA- or in both hippocampi of PL-treated animals (number of ectopic cells, VH: 4.3 ± 1.9 and 3.3 ± 0.9; KA: 7.3 ± 1.9 and 10.3 ± 2.3; PL: 3.0 ± 1.7 and 0.7 ± 0.3; chi-square = 2.56 and 7.44; d.f. = 2; p = .29 and 0.02, for contra- and ipsilateral, respectively). This effect was also valid when normalizing the number of ectopic neurons by the total number of GFP+ cells (Fig. 1E).

Morphological alterations of GFP+ neurons were also distinct in the KA and PL groups. In VH-treated animals, these cells showed spherical soma and a cone-shaped tree of apical dendrites (Fig. 2A). In the vast majority of cells, branching of the dendritic tree started in the transition zone between the granule cell and the inner molecular layers, while for the suprapyramidal blade of the GCL, it extended up to the hippocampal fissure (Fig. 2B). While adult-born neurons generated before KA-induced SE show a clear hypertrophy of cell soma (Fig. 2C, D) and a high proportion of cells present hilar basal dendrites (contralateral: 1.9 ± 0.9%; ipsilateral: 64.8 ± 4.4%, from 240 to 190 cells sampled from 4 animals, respectively), these alterations were not observed in PL-treated animals (Fig. 2E, F, G). The basal dendrites present in KA-group have short branches and are extremely ramified and complex (Fig. 2H). Quantification of cell body area confirmed that KA injection promoted an enlargement of cell soma in the ipsilateral hemisphere (Kruskal–Wallis test, chi-square = 5.60 and 7.20; d.f. = 2; p = .061 and 0.027, for contra- and ipsilateral, respectively; Fig. 2H). Pilocarpine-injected animals did not present significant alterations in morphology of cell soma and processes (Fig. 2H). Taken together, our observations suggest that cellular alterations in the epileptic hippocampus are most likely to be triggered by KA, and therefore, epileptiform discharges elicited by chemoconvulsants would marginally contribute to these cellular abnormalities.

**Fig. 1.** Mature granule cells responses upon intrahippocampal administration of chemoconvulsants. (A) Timeline of experimental procedures (TAM: tamoxifen administration; VH: vehicle; KA: kainic acid; PL: pilocarpine; IHC: perfusion and immunohistochemistry). (B) Mosaic reconstruction of representative confocal images of coronal sections of the ipsilateral DG in control (vehicle, top) and epileptic animals (middle and bottom). GFP (green) and DAPI (red) staining in cDcx/GFP mice 50 days after TAM and 15 days after ihpc injection. Scale bar is 200 μm in all images. (C) Semicircular diagrams representing the distribution of all granule cells (green dots) in the DG in both hemispheres. The number of cells counted is informed in the bottom-right of each semicircular diagram. (D) Spatial distribution of mature granule cells within DG subregions (ML: molecular layer; GCL: granule cell layer; and hilus). Note that granule cells are denser in the hilar border of the GCL in all groups. (VH: n = 4; KA: n = 4; and PL: n = 3). (E) Percentage of ectopic cells in both hemispheres (£ p = .057, Mann–Whitney test). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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3.4. Effects of KA and PL on immature adult-born granule cell position and morphology

To evaluate the effects of SE in the position and morphology of immature granular cells, we induced the TAM-mediated recombination 7 days after KA or PL injection and analyzed them after 35 days (Fig. 3A). According to the lineage progression for adult generation of new granular neurons, type 2b progenitors start expressing Dcx and continually express it until they terminally differentiate into mature neuron, which usually lasts for 3–4 weeks [38]. Therefore, by performing recombination 7 days after chemoconvulsant injection, we intended to label cells that were in the process of differentiation during SE and type 2b progenitors that will generate progeny after SE. In the control group, most GFP+ cells coexpressed the granule cell marker CTIP2, but only a few cells integrated BrdU (data not shown), suggesting that most recombined cells 1 week after SE are nonproliferating immature neuroblasts. In the contralateral side of KA injection, as well as in both hippocampi of PL-injected animals, we observed a significant increase in the number of GFP + neurons 35 days after TAM injection (Fig. 3B, C). Interestingly, however, the total number of GFP + neurons in the KA-ipsilateral side was severely reduced while it increased in PL-treated animals (total number of GFP + neurons, VH: 105 ± 36, KA: 23 ± 6, PL: 772 ± 52; Kruskal–Wallis chi-square = 8.73, d.f. = 2; p = .013). A significant proportion of GFP + cells in KA-ipsilateral displayed glial morphologies and expressed the astrocytic marker GFAP (Supplementary Fig. 6C), while the majority of GFP + cells in the PL group was presented a neuronal phenotype (Supplementary Fig. 6B). Interestingly, in the contralateral hemisphere, both treatments (KA and PL) increased the number of neurons in comparison to VH-treated animals (total number of GFP + neurons, VH: 81 ± 10, KA: 282 ± 87, PL: 538 ± 121; Kruskal–Wallis chi-square = 7.50, d.f. = 2; p = .024).

In the control group, GFP + neurons were mostly located in the inner portion of the GCL and virtually no ectopic cell was observed (Fig. 3B, E). Conversely, a high number of ectopic GFP + cells were observed in the ipsilateral hilus of KA- but not PL-treated animals (Fig. 3B, D, E). In both KA- and PL-treated animals, GFP + neurons within the ipsilateral, but not contralateral, GCL showed a more dispersed distribution as compared to the typical pattern of distribution near the hilar border observed in control animals (Fig. 3D).

Although the number of GFP + neurons was drastically reduced in the injected hemisphere in KA group, morphological analyses of these cells revealed striking abnormalities in soma shape and dendritic branching patterns (Fig. 4). Indeed, we observed a significant proportion of GFP + neurons with basal dendrites in the ipsilateral side.

Fig. 2. Basal dendrites and area of the cell body of mature granule cells. (A–F) Confocal photomicrographs of mature GCs in representative animals treated with vehicle (A,B), kainic acid (C,D), and pilocarpine (E,F). Inset in D (D′) highlights the complex arborization of basal dendrites together with dendritic spines. Scale bar is 20 μm in all images. (G) Proportion of granule cells presenting basal dendrites (*p < .05, Mann-Whitney test). N.D.: not detected. (H) Average cell body area in sampled GCs (*p < .05, Mann-Whitney test; n = 20 cells/hemisphere/animal).
of KA-treated animals (16.7 ± 11.8%, from 43 cells sampled from 5 animals). Contrarily, morphology of GFP+ neurons in PL-treated animals was mostly unaltered as compared to controls (Fig. 4A,E). Quantification of the cell body area of GFP+ neurons within the GCL revealed hypertrophy of the soma after SE (Kruskal–Wallis test, chi-square = 6.27 and 8.12; d.f. = 2; p = .044 and p = .017, for contra- and ipsilateral, respectively; Fig. 4H). Similar to that observed for mature neurons, cell body area in the ipsilateral hemisphere of KA-treated animal was almost twice the size of their contralateral cells. Surprisingly, cell body area was slightly increased in PL-treated animals in the contralateral hippocampus (Mann–Whitney U test, p = .057 and p = .035, for VH and KA groups, respectively; Fig. 4H).

4. Discussion

Here, we show that an intrahippocampal injection of either KA or PL is sufficient to induce bilateral electrical spikes and afterdischarges, as well as behavioral seizures in mice, compatible with SE. Chronically, these animals also develop spontaneous seizures, but gross and fine histological alterations are clearly distinct in KA- and PL-injected animals, suggesting that the hyperexcitability observed in these models are likely caused by distinct mechanisms.

Granule cell dispersion is a phenomenon where granule cells acquire a less compact configuration in the DG, likely due to the down-regulation of REELIN expression [24]. Previous evidence in the literature have suggested that increased neuronal excitation, reduced inhibition, or both could be factors triggering GCD [39]. Our results do not support this view. Actually, we show that GCD is restricted to the hippocampus receiving 1 nmol of KA, despite the increased neuronal excitation observed in the contralateral side. Similarly, increased electrical activity elicited by 3.4 μmol of PL is unable to induce GCD in both the ipsi- and contralateral hippocampi. Other models using systemic administration of PL also failed to replicate the GCD observed in KA models [4,40]. These observations suggest that KA has some additional local effect that contributes to GCD [41].

According to this notion, we observed strong microglia activation in the hippocampus injected with KA, but not in the contralateral side. This observation is in agreement with previous findings describing that intrahippocampal injection of 1 nmol KA (same concentration used in our study), but not 0.037 nmol KA, increases the expression of inflammatory mediators, such as interleukin 1β (IL-1β), IL-6, tumor necrosis...
factor αα (TNF-αα), and transforming growth factor ββ (TGF- ββ), and leads to GCD [26]. Microglia activation is also present in the PL-injected hippocampus, although to a lesser extent. Thus, it is plausible to speculate that both increased electrical activity and strong inflammatory response are required to downregulate REELIN expression and promote GCD. Alternatively, high concentrations of KA may be toxic for REELIN-producing neurons (and likely other neuronal classes), leading to the degeneration of this population, which in turn would promote the Neuroinflamma response observed in this model. Future experiments are required to establish causal relations among these different phenomena.

Another important question is related to the functional consequences of GCD for epilepsy and spontaneous seizure. Although weak correlation between aberrant mossy fiber sprouting and GCD has been reported [4], no correlation has been found between the granular cell density and seizure activity [3,42]. In this respect, other effects than the reduction of GCD such as increased signaling of proinflammatory cytokine and mTORC1 activation could explain the increased seizure susceptibility after SE-induced epileptogenesis [43]. In the human resected hippocampus, dispersion of the GCL increases from 100 μm in controls to 182 μm (average for all epilepsy-related specimens), an increase of 82% [8] after many years of the epileptic condition. In the present study, GCD reached more than 300% in just 35 days, which corroborates the hypothesis that GCD in KA-treated animals is a much more severe phenomenon than its counterpart in humans.

It has also been reported that systemic [44] and intrahippocampal [24,35] administration of KA increases proliferation in the SGZ of the dentate gyrus [25]. However, neurogenesis is reduced in the ipsilateral SGZ when KA is locally administered [10,24,45]. Our results corroborate these findings and suggest that KA may locally affect neuronal and glial specification. In fact, while the total number of GFP+ cells is increased in both hippocampi after KA injection, only in the contralateral side does this increase in cell number reflect an increase in neurogenesis, whereas neuronal differentiation and/or survival are dramatically hampered in the ipsilateral side of KA-treated animals. Intriguingly, most GFP+ cells in this side seem to adopt an astroglial fate. Future work should help to elucidate whether KA may be directly or indirectly modulating the neuronal lineage progression in the adult hippocampus. We also show an increased number of GFP+ neurons in both ipsi- and contralateral hippocampi of PL-treated animals, raising the hypothesis that cell proliferation and/or survival in the DG are positively regulated by electrical activity or by direct activation of M1 muscarinic receptor

![Fig. 4. Basal dendrites and cell body area of immature granule cells.](image-url)

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Besides these differences in neuronal output rates in the adult DG, KA and PL also had different effects in the positioning of mature and immature granule cells. While KA-injected animals show higher number of ectopic neurons both in the contra- and ipsilateral hippocampi, the frequencies of these cells are similar between VH- and PL-injected animals. Notably, nearly 50% of neurons generated after KA injection are misplaced, whereas only about 10% of neurons generated before KA injection are ectopically located in the ipsilateral hippocampus, indicating that mature neurons are less affected by KA-induced electrical activity/inflammation, despite severe GCD.

Our results also indicate that KA, but not PL, has a drastic effect on granule cell morphology, leading to the formation of hilar basal dendrites and swelling of cell soma. These alterations were observed both in granule cells generated prior to or after KA injection and are restricted to the ipsilateral KA side. Granule cells with enlarged cell body of neurons and aberrant basal dendrites are also found in animal models knocking out Phosphatase and Tensin Homolog on Chromosome Ten (PTEN) [45]. This molecule is involved in regulating neuronal biology, and its mutation is present in patients with epilepsy and autism disorders [46]. Loss of PTEN increased excitatory afferent input in the granule cells and provided morphological changes, as seen in our KA-injected animals, suggesting that this pathway might be affected and could increase the propensity of the hippocampal network to seize. Although a more detailed analysis is necessary to confirm this hypothesis.

Collectively, our results provide compelling evidence that ihpc administration of KA and PL lead to different histological alterations in the dentate gyrus, despite the fact that both chemoconvulsants induce sustained epileptiform discharges in both hippocampi and spontaneous seizures. Understanding the precise cellular mechanisms triggering hyperexcitability within the dentate gyrus circuitry in these two different models may shed light on the understanding of different presentations of mesial TLE observed in humans [47]. Most importantly, it may help to understand why some treatments work well for some patients but not for others, and therefore, contribute to the design of new therapeutic approaches.

5. Conclusions

The results presented herein reveal that KA and PL induce different cellular and morphological abnormalities in the DG, even though both chemoconvulsants trigger comparable behavioral SE, increased electrical activity within both hippocampi and chronic spontaneous seizures. Kainic acid, but not PL injection, leads to severe GCD and a significant increase in the proportion of ectopic neurons, DGCs with enlarged body size and hilar basal dendrites. These alterations were more frequent in immature than mature neurons, suggesting that as adult-born cells integrate in the DG circuitry, it becomes less likely to change its phenotype. Collectively, our data indicate that KA and PL have different effects on the cellular organization of the DG after SE and, therefore, must be interpreted as independent and complementary models of TLE (for example, with and without hippocampal sclerosis). Further investigation will unveil the relevance of this phenomenon to the occurrence of recurrent seizures and associated comorbidities.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yebeh.2019.106575.

References


We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Acknowledgments

The authors would like to thank Dr. Magdalena Götz for kindly providing the Dcx-CreERT2 and CAG-CAT-GFP transgenic animals, Rebecca Diniz for confocal microscopy support and Josy Pontes, Mariana Campelo Medeiros, Alessandra Nascimento for animal care. We also would like to thank Wilfredo Blanco, Cecilia Hedin-Pereira, Diego Laplagne, Katarina Leão, Eduardo Sequerra, Adriano Tort, and Dráulio Barros de Araújo for insightful comments on an earlier version of this manuscript. This work was supported by CNPq (466959/2014-1 to MRC and 480875/2012-0 to CMQ), CAPES (23038.010291/2013-45 to MRC), Financiadora de Estudos e Projetos (FINEP) 01.17.0009.00 (to CMQ and MRC). D.M. was a recipient of PNP/ CAPES scholarship.

Declaration of competing interest

None.

Ethical publication statement

Please cite this article as: D.M.S. Moura, I.R.P. de Sales, J.A. Brandão, et al., Disentangling chemical and electrical effects of status epilepticus-induced dentate gyrus abnormalities, Epilepsy & Behavior, https://doi.org/10.1016/j.yebeh.2019.106575.