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Research paper

Different types of *Status Epilepticus* may lead to similar hippocampal epileptogenesis processes

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ABSTRACT

About 1–2% of people worldwide suffer from epilepsy, which is characterized by unpredictable and intermittent seizure occurrence. Despite the fact that the exact origin of temporal lobe epilepsy is frequently unknown, it is frequently linked to an early triggering insult like brain damage, tumors, or *Status Epilepticus* (SE). We used an experimental approach consisting of electrical stimulation of the amygdaloid complex to induce two behaviorally and structurally distinct SE states: Type I (fully convulsive), with more severe seizure behaviors and more extensive brain damage, and Type II (partial convulsive), with less severe seizure behaviors and brain damage. Our goal was to better understand how the various types of SE impact the hippocampus leading to the development of epilepsy. Despite clear variations between the two behaviors in terms of neurodegeneration, study of neurogenesis revealed a comparable rise in the number of Ki-67 + cells and an increase in Doublecortin (DCX) in both kinds of SE.

Introduction

Over 1% of the world's population suffers from epilepsy, which is defined by irregular and unpredictable seizure occurrence (Engel, 2001). According to Pitkänen and Lukasiuk (2009), epilepsy is frequently diagnosed after an initial precipitating injury such as a brain lesion, tumor, meningitis, encephalitis, *Status Epilepticus* (SE), or febrile seizures during childhood (Engel, 1995; and ILAE Commission on Neurosurgery of Epilepsy 2004; Pitkänen and Lukasiuk, 2009). The SE, which has long been linked to the development of mesial temporal sclerosis, is one possible injury that is referred to. The SE is defined as a period of continuous seizure activity lasting at least five minutes, or repeated seizures without recovery to normal consciousness between them (Trinka et al., 2015) and it has been long implicated in the development of mesial temporal sclerosis and temporal lobe epilepsy (TLE) (Treiman, 1998).

Although SE-induced epilepsy in rodents is characterized by widespread lesions (beyond limbic system regions), it has been used to study epileptogenesis, in which different types of brain insults cause a complex cascade of neuroplastic events that ultimately result in the occurrence of spontaneous recurrent seizures (SRS) (Leite et al., 1990; Pitkänen and Lukasiuk, 2011). In fact, both human and animal models of temporal lobe epilepsy (TLE) show numerous hippocampus alterations in addition to extra-temporal structures (Sloviter, 2005; Pitkänen and Lukasiuk, 2011). In particular, partial degeneration of CA1/CA3 pyramidal neurons and hilar neurons, reductions in gamma-amino butyric acid (GABA) positive interneurons, and loss of the calbindin calcium binding protein (CBP) in dentate granule cells are among the structural changes in the hippocampus (Santos et al., 2019) reductions in gamma-amino butyric acid (GABA) positive interneurons (Shetty and Turner, 2001), loss of the calbindin calcium binding protein (CBP) in dentate granule cells (Shetty and Hattiangady, 2007), abnormal sprouting of dentate granule cell mossy fiber (Nadler et al., 1981; Mello et al., 1992, 1993), alterations in levels of expression of multiple peptides that play antiseizure roles such as neuropeptide Y (NPY), galanin, ghrelin, somatostatin and dynorphin in hippocampal circuitry (Schwarzer et al., 1995; Kovac and Walker,

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2013), among others. The axons of dentate granule cells (hippocampal mossy fibers) branch out of the dentate hilus and abnormally innervate the dentate inner molecular layer in TLE, a phenomenon known as aberrant mossy fiber sprouting (Tauck and Nadler, 1985; Sutula et al., 1992; Mello et al., 1993).

Additionally, numerous studies have demonstrated a striking increase in neurogenesis following SE or amygdala kindling (Parent et al. 1997; Scharfman, Goodman, and Sollas, 2000; Scharfman et al. 2003; Pierce et al., 2011; Parent et al. 2006). The newly formed granule cells in those SE or "kindling" models have abnormal dendritic orientation and morphology (Shapiro and Ribak, 2005; Walter et al., 2007; Santos et al., 2017); aberrant synaptic connectivity (Jessberger et al. 2007); altered number and density of dendritic spines (Santos et al. 2011; Scharfman et al. 2000). Additional research suggests that these cells lead to a reduced seizure activity threshold in the hippocampus of epileptic animals and are probably implicated with SRS (Scharfman et al., 2002; Scharfman et al. 2003; Scharfman et al., 2000; Pierce et al., 2011) Adeno-associated viral vectors expressing the human NPY gene can also be used to genetically modify animals following epileptogenesis and inject them into the hippocampus, which can reduce SRS to 40% (Noè et al., 2008; Ledri et al., 2015; Nikitidou Ledri et al., 2016).

The effects of brain damage and the changes that take place between the onset of SE and the emergence of the SRS during the latent phase are crucial in the development of epilepsy. The length and intensity of SE appear to be crucial variables that affect how epileptogenesis manifests and progresses. Thus, it is crucial to understand how epileptogenesis culminates in the emergence of SRS to analyze the neuroplasticity that occurs in limbic areas after SE. Animal models have been created over the past few decades that allow SE to be triggered without the need of chemicals by repeatedly stimulating different limbic areas electrically. Compared to the chemical models, these models have a number of benefits. First of all, they are free from the interpretative difficulties brought on by the usage of a poisonous substance and its pervasive effects on the nervous system. Second, they are capable of producing a range of SE states and behaviors (Handforth and Ackermann, 1988; Mohapel et al., 1996; Nissinen et al., 2000; Brandt et al., 2003; Tilelli et al., 2005).

Several studies have reported various types of SE based on the behavior of the animals using a model of self-sustained SE (SSSE) by electrical stimulation of the amygdaloid complex (AmyC) (Nissinen et al. 2000; Brandt et al. 2003; Tilelli et al. 2005). According to Tilelli et al. (2005), the identical protocol of electrical stimulation of the AmyC can produce at least two behaviorally distinct forms of SE, and they vary in the intensity of the behavioral components: Based on the behavioral repertoire displayed during SE, Type I and Type II can be differentiated using both the Racine/Pinel and Rovner's scales (Racine, 1972; Pinel and Rovner, 1978). The most severe behaviors (limb myoclonus, rearing, falling, and eventually running and jumping) normally found in other models of SE, referred to as Generalized SE, are present in Type I SE and are characterized by recurring stages 4 and 5. The second is classified as SE Type II or Ambulatory SE and is characterized by persistent hyperactive motor and exploratory behaviors and Racine stages 1 and 2 together with less severe seizure characteristics (facial automatisms and neck myoclonus and eventually limb myoclonus, rearing, falling). It's interesting to note that the explanation for these variations in SE phenotypic manifestation, linked to the below-described neuroanatomical particularities, rests on a nervous system trait that was first postulated at least three decades ago but has only just been explained: the short-term effects of SE have not been thoroughly examined, in particular it is unknown whether the various types of SE are associated with specific changes in the hippocampal circuitry. This is due to the fact that subtle differences in the starting point can lead to drastically different outcomes when brain circuitry scales up in complexity, resembling a chaotic-like system (Skarda and Freeman, 1987; Koch, 1999).

In the current investigation, which is a follow-up study, we assessed the histopathology in brain tissue from rats that had AmyC electrically stimulated (similar to Tilelli et al., 2005) to produce SE and found changes at acute and semi-chronic time periods. Our goal was to find out how much differing SE severity circumstances affect the hippocampus's capacity to evolve epileptogenesis processes. Our findings demonstrate that particular circuitry neurodegeneration and neurogenesis patterns are related to behavioral SE expression.

Experimental procedures

Our findings are the result of new histochemical and immunohistochemical analysis for FluoroJade-C (FJC), Neu-N, Ki-67, Prox-1, Doublecortin (DCX) and Neuropeptide Y (NPY) in the tissues of the animals presented in a previously published study from our group (Tilelli et al., 2005) where previous results and detailed methods can be viewed. All procedures were approved by the University of São Paulo Institutional care and experiments with laboratory animals were conducted in accordance with the rules of the Brazilian Society for Neuroscience and Behavior, which are based on the Society for Neuroscience guidelines for animal experimentation and were approved by Ribeirão Preto School of Medicine Commission on Ethics on Animal Experimentation (CETEA; protocol # 195/2005).

Briefly, adult male Wistar rats (total 36; n = 4, per group) were stereotaxically implanted, under anesthesia (ketamine, 70 mg/kg, Bayer S.A., and xylazine, 7 mg/kg, Agender LTDA), with Teflon-coated bipolar stainless-steel electrodes (A-M Systems, Inc). One stimulation/recording bipolar electrode was implanted in the AmyC (3.6 mm posterior, 5.0 mm lateral and 6.5 mm ventral to bregma) the surface of the brain. To record the spread of electrographic seizure activity to the ipsilateral hippocampus, a bipolar electrode was implanted into the hilus (distance between tips 0.8 mm; coordinates of the lower tip: 4.1 mm posterior, 2.6 mm lateral and 3.7 mm ventral to bregma). Animals received 30 min of biphasic square waves electrical stimulation, 300 μA amplitude, 60 Hz frequency, 100 ms duration, every 500 miliseconds leading to development of SSSE. After 1.5 h of SSSE all animals received 5 mg/kg Diazepam i.p. Three hours, 24 h or 15 days after the SE, animals were perfused with Millonig's buffer followed by PFA 4%, had their brains removed, cryoprotected in sucrose 30%, sliced in 40 μm slices using a cryostate and kept at − 20ºC in antifreeze solution until 24 h prior to the histology procedures, when they were washed overnight in 100 mM PBS.

For further details on animals housing, stereotaxic surgery, SE induction, euthanasia, tissue processing, behavioral recording and analyses, please refer to Tilelli et al. (2005).

FluoroJade C

The FJC staining was used to identify neurons in degeneration (Schmued et al., (1997) slightly modified by Castro et al. (2011) and performed as follows: brain sections (5 from each animal) were immersed in 100% ethanol for 3 min, followed by 1 min in 70% ethanol and 1 min in distilled water. Slides were then transferred to a solution of 0.06% potassium permanganate for 15 min and gently shaken on a rotating platform. Sections were then rinsed three times for 1 min each in distilled water and transferred to the FJC staining solution with gentle shaking for 30 min. The 0.0001% working solution of FJC was prepared by adding 1 ml of stock FJC solution (0.01%; Histo-chem INC) to 99 ml of 0.1% acetic acid in distilled water. After staining, the sections were rinsed three times each for 1 min in distilled water and were cover-slipped with Fluoromount G (SouthernBiotech – USA). Severity of neuronal damage in a section was semi-quantitatively assessed by a grading system similar to that previously described by Nissinen et al. (2000), and applied in Tilelli et al. (2005), as follows: score 0, no obvious damage; score 1, small lesions involving 20–50% of neurons; score 2, lesions involving more than 50% of neurons.

Immunohistochemistry

Immunohistochemical assays were done in free-floating tissue (5 slices from each animal). For single labeling, sections were quenched with 3% peroxidase in 10% methanol for 30 min before blocking solution and overnight incubation with primary antibodies Rabbit anti-NPY (1:750, Peninsula, USA) or Goat anti-DCX marker of immature neurons (1:500, Santa Cruz Biotechnology INC.; CA, USA) The sections were then incubated with the secondary antibody: biotinylated Goat anti-rabbit (1:500; Vector Laboratories) or Donkey anti-Goat (1:500; Chemicon) for 1 h. Finally, sections were incubated in avidin-biotin-peroxidase complex (Elite ABC Kit, Vector Laboratories) for 1 h and developed with diaminobenzidine reaction (DAB Peroxidase (HRP) Substrate Kit, Vector Laboratories).

For the immunofluorescence, 5 slices from each animal were washed with 0.1 M phosphate buffer saline (0.1 M PBS), pH 7,4, for 30 min. Slices were then transferred to a 0.75% glycine in PBS for 10 min and then incubated for 1 h in blocking solution with 2% of bovine serum albumin (Sigma-BSA) and 0.2% Triton X-100 (Sigma) in PBS. The tissue was incubated with primary antibodies overnight at 4 °C in blocking solution. The following primary antibodies were used: Mouse anti-NeuN (1:700, MAB 377, Chemicon International), marker of mature neurons, Rabbit anti-Ki-67, marker of cell proliferation at dentate granule cells (both at 1:2000, gently donated by Prof. PhD. Alysson Muotri, University of California, San Diego). The slices were then rinsed in blocking solution and incubated for 1–2 h at room temperature with secondary antibodies diluted in blocking solution. The following secondary antibodies were used: goat anti-rabbit Alexa Fluor 594; goat anti-mouse Alexa Fluor 488 (1:1500 from Invitrogen). After the antibodies incubation the slices were rinsed in PBS and cover-slipped with

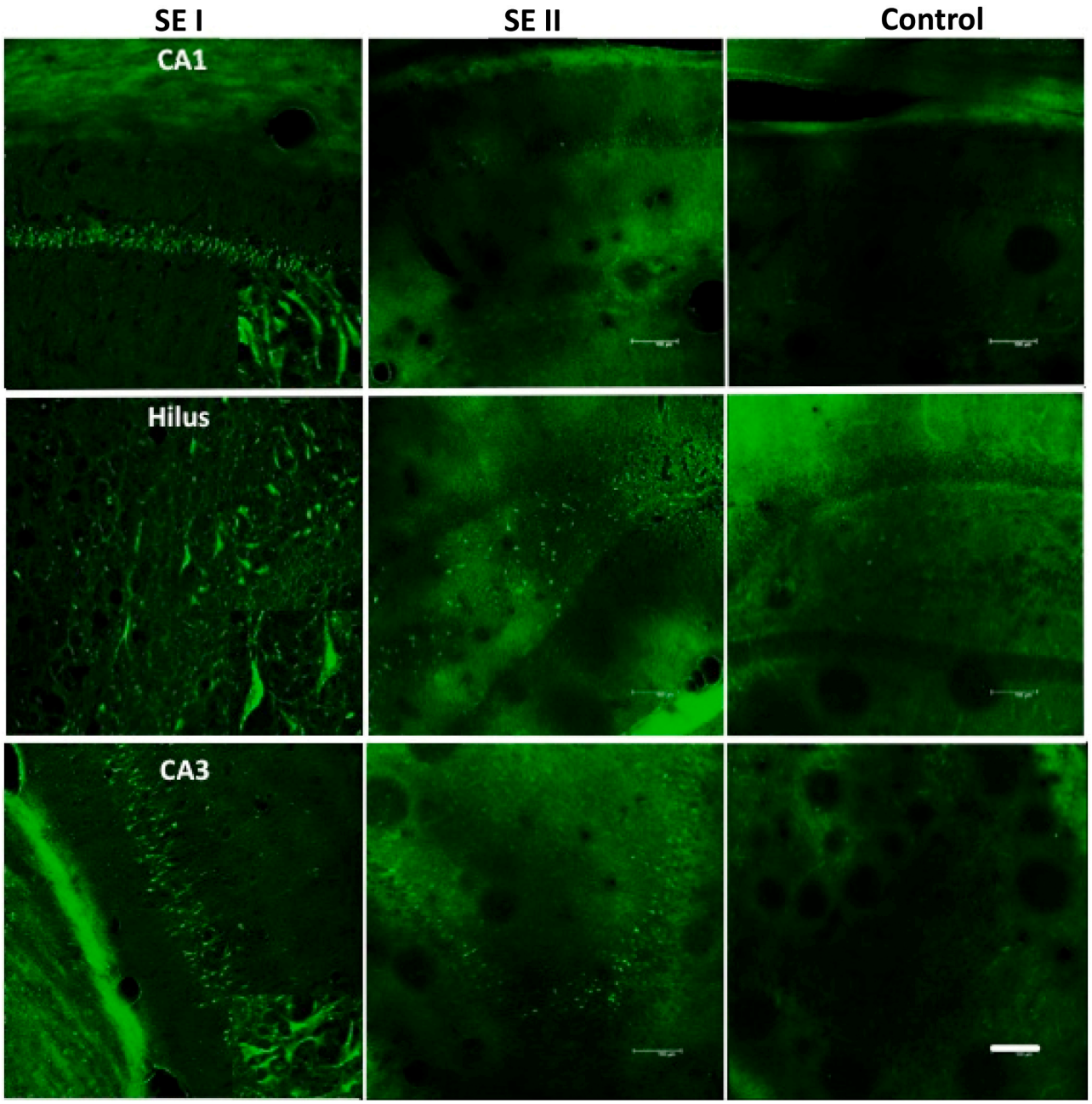

Fig. 1. Hippocampal neurodegeneration after different SE. Confocal maximum projection of Fluorojade C staining labeling degenerating neurons (FJC+ in green) in the hippocampus of rats. SE Type I induces more severe damage and more FJC+ cells are observed in both brain's hemispheres. SE Type II induces less severe damage, with few cells detected at the ipsilateral side of amygdala stimulation. Scale Bar: 100 μm.

Fluoromount G.

Statistics and data analysis

Statistical comparisons were performed with GraphPad Prism 5 (GraphPad Software, Inc.; CA, USA), and significance was accepted with a p value ≤ 0.05. Comparisons between two groups were determined using a *t*-test. Multiple group comparisons were made using One-Way analysis of variance (ANOVA) and *post hoc* test of Bonfferoni and Tukey were used to identify further factors that differed significantly. Specific statistical test and p-values are described in the results.

Results

The Severity of seizures during SE can be correlated with the amount of neurodegeneration

By using FJC staining at 3 and 24 h after SE, we examined the potential for neurodegeneration in numerous brain regions, including the hippocampus. No SE or control group showed any labeling in the hippocampus at the three-hours mark, demonstrating that cell death or neurodegeneration do not take place or can be seen by FJC staining at this early stage following SE. On the other hand, animals with SE Type I had considerably more tagged cells dispersed bilaterally in the hippocampus hilus, CA3 and CA1, at 24 h following SE. In the hilus, CA3 and CA1 of the hippocampus, ipsilateral to the stimulation site, we were able to see very little staining in Type II rats (Fig. 1). To map the existence of neurodegeneration, we also conducted a semiquantitative study of various temporal lobe structures (Table 1). In our animals, a similar pattern to that seen in Nissinen et al. (2000) was observed, with Type I SE showing bilateral cell damage and Type II SE showing unilateral and sparser cell damage.

We employed the mitotic marker Ki-67 in rats 3 and 24 h after SE stimulation to study the impact of various forms of SE on neurogenic subgranular zone cell proliferation. With no statistically significant differences between the two types of SE ($p = 0.075$), we observed that 3 h after SE, both Type I and Type II animals already displayed a significant increase in Ki-67 immunostaining (Fig. 2) when compared to the controls. This suggests that SE induced/activated cell proliferation in both groups of animals. Additionally, both types I and II demonstrated a significantly higher level of Ki-67 immunostaining in the hippocampus

Table 1
Neuronal cell damage in different regions of the temporal lobe.

Brain Structures	Sub-regions	FJC$^+$	
Amygdaloid Complex		SE Type I	SE Type II
	Central Nucleus (CeA)	XX	X
	Medial Nucleus (MeA)	XX	X
	Basolateral Nucleus (BLA)	XX	XX
Hippocampal formation			
	Dentate Gyrus	φ	φ
	Hilus	XX	X
	CA3	XX	X
	CA2	φ	φ
	CA1	XX	φ
	Subiculum	XX	φ
Piriform Cortex			
	Layer II	XX	φ
	Layer III	XX	X
Enthorinal Cortex			
	Layer II	XX	X
	Layer III	XX	X
	Deep Layers	XX	X
Neocortex			
	Barrel Field	X	X
	S2	X	φ
	IG-CG1	XX	φ

Different types of SE cause similar increase in cell proliferation and neurogenesis

of animals perfused 24 h after SE compared to control animals (P 0.001). In the subgranular zone (SGZ) of the dentate gyrus, the Ki-67 tagged cells showed up as clusters, as was previously observed using the pilocarpine SE model (Parent et al. 1997). The number of Ki-67 + cells in the hippocampus did not differ between the two sides at each time point, which is an interesting finding and suggests that both Type I and Type II animals saw a similar increase in cell proliferation in the hippocampus ($P = 0.085$).

The process of neurogenesis is multi-staged and strictly controlled. Newly formed neurons undergo various maturation stages after exiting the cell cycle before becoming fully developed granule neurons (Zhao et al., 2006). We used DCX immunohistochemistry to mark the neuroblasts produced in SGZ, in order to examine the role of SE in the differentiation and maturation of developing neurons. Similar outcomes were observed 14 days after SE with a rise in the number of immature newborn neurons expressing DCX. When compared to the control group, we observed a substantial increase in the number of new neurons in the SGZ/GCL of the dentate gyrus in both types of SE groups after 14 days after SE stimulation (p 0001, Fig. 3). when compared to the control group. It's interesting to note that there was no difference in the number of newborn DCX+ cells between the two SE types ($P = 0084$), indicating that both SE types have a similar effect on the rise in neurogenesis.

NPY as a putative protective endogenous peptide

In animal models of epilepsy, NPY is well-known to have a significant antiseizure function (Dubé, 2007; Kang et al., 2000; Noè et al., 2008). We carried out IHC for NPY in the hippocampus to determine whether it would aid in explaining the differences seen in rats exposed to the identical stimulation protocol. Qualitative data indicate that animals in Type I appear to have an asymmetry in NPY+ cells in the hilus of DG, with fewer cells in the hippocampus on the side opposite to the stimulation site and lower overall NPY density, even though there were insufficient slices to conduct cell counting and statistical analysis. However, Type II demonstrated symmetrical NPY expression, and NPY+ interneurons were seen in both experimental groups.in greater amount when qualitatively compared to controls (Fig. 4).

Discussion

Neurodegeneration

In the present study we conducted more detailed histological analysis in hippocampus from animals with two different types of SSSE induced by electrical stimulation of basolateral amygdala. In Tilleli et al. (2005) the authors suggested the patterns of neurodegeneration indirectly, based on lack of Neu-N immunoreactivity (Mullen et al., 1992). In the present study the previous findings were confirmed by means of FJC histochemistry, demonstrating that distinct patterns of cell death in the hippocampus, among other plastic events, are associated with distinct patterns of seizure behavior during SE. In Type I animals (more severe behavioral seizures) we observed significantly bilateral higher number of FJC positive neurons in the hilus of the dentate gyrus, CA1 and CA3, with relative preservation of the granule cells within the granule cell dentate gyrus and CA2 than in Type II animals (less severe behavioral seizures). Type II animals had FJC positive cells only in the ipsilateral side of the stimulation.

Neuronal death is one of the most conspicuous findings in hippocampal sclerosis (Cavazos et al., 1994; Gorter et al., 2003; Walker, 2015). Previous studies in rodents have documented that a low number of induced-clonic seizures is sufficient to cause hippocampal neuronal death (Wasterlain et al., 1993; Fujikawa et al., 2000a; Fujikawa, 2003) and the ILAE proposed in 2013 that not only the number of degenerating cells but the pattern of neurodegeneration should be taken into account for better understanding the subtypes of epileptic syndromes (Blümcke et al., 2013). Chemically-induced seizure models (systemic and

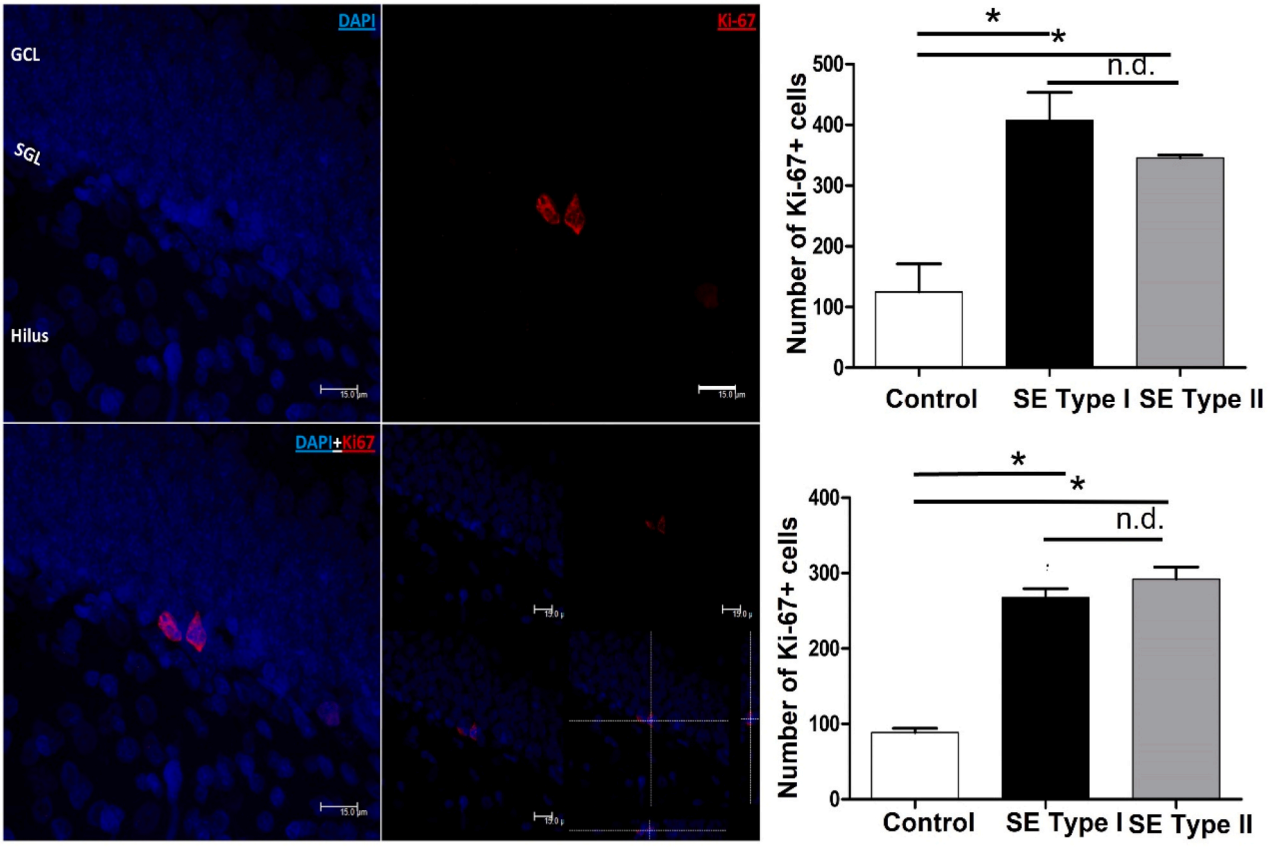

Fig. 2. Increase of Cell Proliferation at SGZ. Confocal maximum projection of immunolabeling for Ki-67 (in red) and nuclei staining with DAPI (in blue) in the rat hippocampus. Note the nuclei double staining with Ki-67 and DAPI in cells in the process of proliferation at hippocampus SGZ. The graph shows quantification of groups of Ki-67 + cells in SGZ of the hippocampus at 3 h (superior) and 24 h (inferior) after SE. *p < 0.05. Scale Bar: 15 μm.

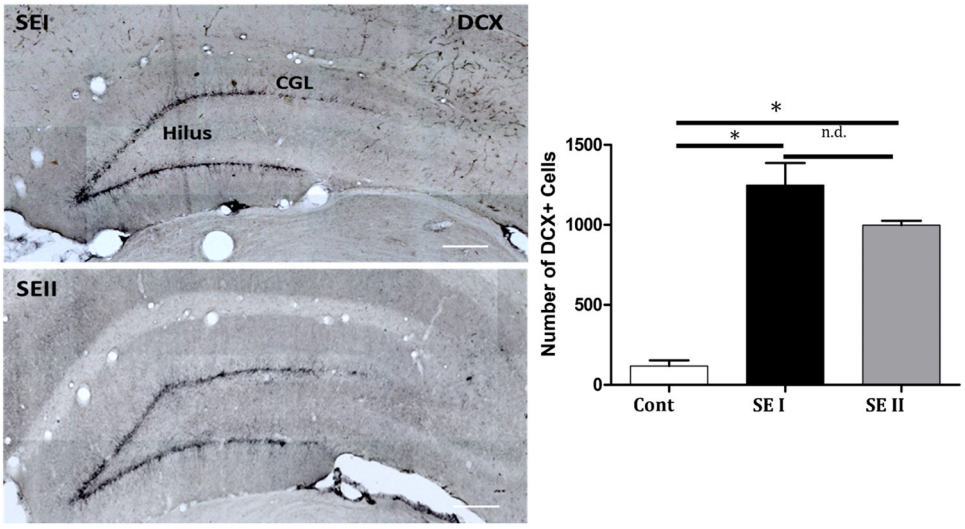

Fig. 3. Increased neurogenesis in DG. Photomicrograph of immunolabeling neuroblast expressing doublecortin (DCX) immature neurons in granule cell dentate gyrus 14 days after SE. Increase of immature neurons in DG in Type I SE and Type II SE. Graph show number of DCX-labeled neurons per animal in the DG at 14 days after SE. * **p < 0.001. Scale Bar: 100 μm.

intra-hippocampal administration of pilocarpine) have shown neuronal loss in several other areas of the brain such as secondary auditory cortex, endopiriform nucleus, piriform cortex, entorhinal cortex, several amygdaloid complex areas (central nucleus, cortical nucleus, amygdalo-hippocampal transition area) and several thalamic areas, highlighted by FJC staining, in the 24 h time-point (Castro et al., 2011). We observed similar neurodegeneration pattern in Type I SE rats in the same areas.

We discovered that behaviorally severe SE leads to greater neurodegeneration. According to our theory, the intensity of SE can be utilized to estimate how much neurodegeneration will occur 24 h after SE. More neurons and brain regions are activated and more degeneration is

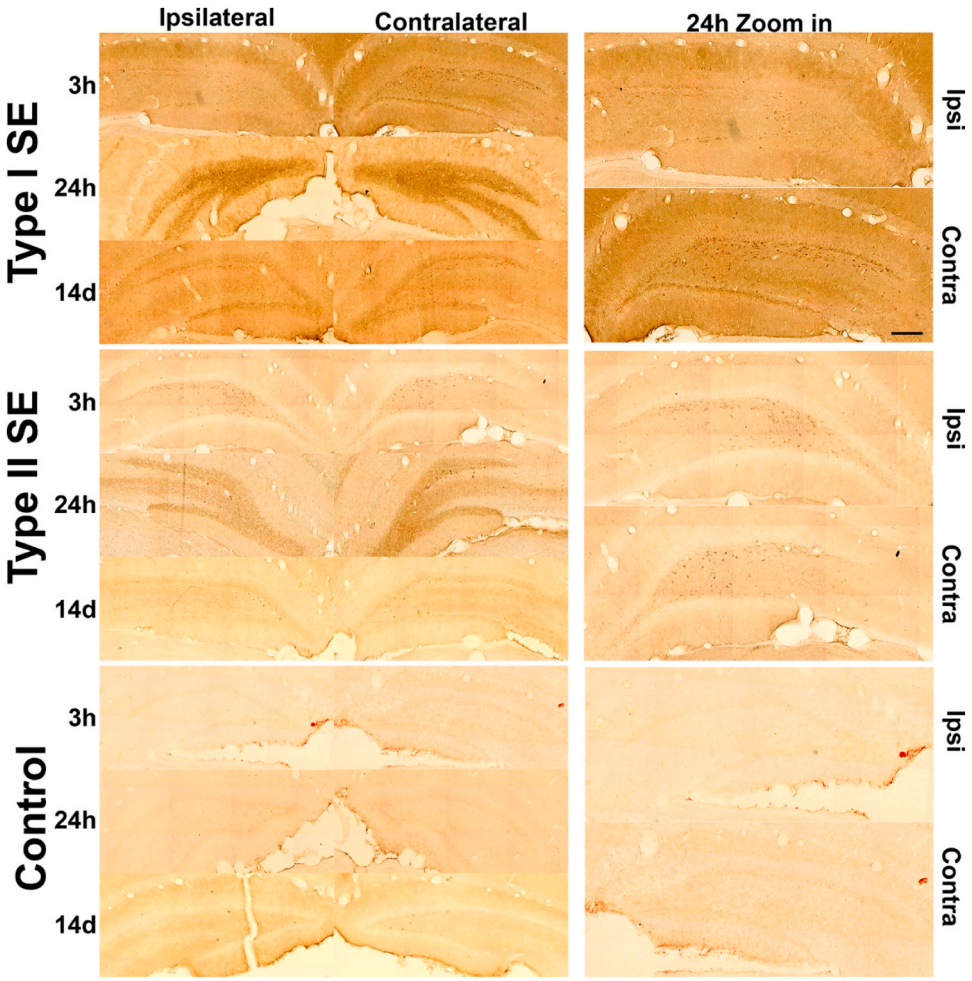

Fig. 4. NPY expression in DG. Photomicrograph of immunolabeling interneurons expressing NPY into dentate gyrus 14 days after SE. A qualitative examination suggests that the side of the hippocampus opposing the stimulation has less NPY+ neurons. Scale Bar: 100 μm.

induced by more severe behavioral patterns. Olney et al. (1983) while evaluating the neurotoxic effects of glutamate analogs, showed that administration of kainic acid caused neuronal death in limbic structures. The mechanisms by which kainic acid, as well as other excitatory chemical agents, cause neuronal death can be partially explained by the "excitotoxic hypothesis", which has provided a plausible explanation regarding the pathological induction of neuronal death in a wide variety of neurological diseases. According to this, the excessive activation of NMDA receptor on postsynaptic membranes induced by large glutamate release during SE results in an excessive influx of calcium into the postsynaptic neurons and in the presynaptic terminals as well (Wasterlain et al., 1993; Fujikawa et al., 2000b; Fujikawa, 2003). The inability of the cell to bind the excessive intracellular calcium triggers the activation of various processes including necrosis and/or apoptosis and consequent neuronal death (Wasterlain et al. 1993; Fujikawa, 2003; Fujikawa, 1996). The hypothesis is further strengthened by the findings that administration of NMDA receptor antagonists has a neuroprotective effect in animals even after the onset of SE (Fujikawa, Daniels, and Kim, 1994; Fujikawa, 1995).

Cell Proliferation and Neurogenesis

Our study is the first to demonstrate increase in hippocampal neurogenesis during epileptogenesis in a model of SE induced by electrical stimulation of the amygdala, in agreement with findings in others animal models of TLE (Parent et al. 1997; Parent et al. 1998; Bengzon et al. 1997; Nakagawa et al. 2000; Romcy-Pereira & Garcia-Cairasco, 2003; Jessberger et al. 2005).

We found an expressive increase in cell proliferation as soon as 3 h after SE. Several studies demonstrated that in acute and chronic models of limbic seizures neurogenesis in the dentate gyrus is stimulated (Parent et al. 1997; Bengzon et al. 1997; Romcy-Pereira & Garcia-Cairasco, 2003). In adult rodent models of TLE induced by kainic acid or pilocarpine, cell proliferation in the dentate gyrus is increased 2–5 fold after a latent period of several days (Hattiangady et al., 2004). It has been demonstrated that in other experimental models of epilepsy, such as electrical stimulation of the amygdala (Parent et al., 1998), electrical stimulation of hippocampus (Bengzon et al., 1997), audiogenic kindling (Romcy-Pereira and Garcia-Cairasco, 2003), and KA-induced (Jessberger et al., 2005; Shapiro et al., 2005), repeated seizures stimulate neurogenesis in the hippocampal granular cell layer (GCL). Some studies have shown the Type 2 progenitor cells are more susceptible to stimuli like voluntary exercise, extended exposure to antidepressant drugs, and are linked to increase of cell proliferation in seizures (Hsieh and Eisch, 2010).

To analyze the differentiation of new cells into neurons we quantified the number of immature neurons at 14 days after SE. We found expressive increase of DCX positive cells in subgranular zone and GCL, suggesting a robust differentiation of new neurons in the dentate gyrus. Interestingly, we have found no difference in the number of DCX cells among the animals with different severity of SE. However, Ki-67 expression was not different bilaterally, what could indicate that although it is known that proliferation of new neurons in hippocampus is an activity-dependent event, there is no direct correlation between the

level of cell death observed in FJC and the stimulation for neurogenesis. The presence of migration and integration of ectopic cells were found in other studies, such as the pioneering work of Parent et al. (1997). Using the DCX immuno-labeling, Arisi & Garcia-Cairasco (2007) also found ectopic cell migration to polymorphic layer of the dentate gyrus in epileptic animals after pilocarpine-induced SE. Granule neurons in the hilus make abnormal connections and exhibit electrophysiological characteristics similar to those described for mature dentate gyrus neurons (Scharfman et al., 2000).

NPY plasticity

The exact role of NPY and the receptors involved in its "antiseizure" action are not fully known. Although only qualitatively, we currently have shown that animals exposed to the same initial insult appear to differentially express NPY+ cells after the occurrence of SSSE according to behaviorally distinct classes of seizures. We observed in the most severe type of SE (Type I) a clear asymmetry in the expression of NPY with more hilar positive cells contralateral to the site of stimulation. In Type II animals the expression is symmetric and in both groups the NPY plasticity is activity-dependent and there are more cells in experimental animals, in comparison to the control group, in accordance with the literature (Tu et al., 2005, 2006; Howell et al., 2007; Cardoso et al., 2010).

One plausible speculation is that the brain could compensate hyperexcitability in epilepsy by favoring multiple endogenous antiseizure systems by means, among others, of up/downregulation of neurotransmitters and/or receptors (Dragunow, 1986; Guo et al., 2014; Wallace, 2003). In that context, one promising endogenous substance that can be used as a target for humans' treatment is the NPY. Richichi et al. (2004) have demonstrated that the injection of neurotropic recombinant adeno-associated viral particles carrying the human gene for NPY into the hippocampal formation of rats leads to long-lasting expression of NPY with decrease in occurrence of acute induced epileptic seizures and SE, as well as slowing down the acquisition of kindling. Interestingly, some years later the same group showed that those injections successfully decrease spontaneous seizures in a chronic rat model of TLE (Noè et al., 2008). Additionally, it was demonstrated that NPY suppresses excitatory synaptic transmission in principal neurons in human epileptic hippocampal slices (Ledri et al., 2015) and it seems likely that the effects of NPY is most prominent via its receptor type 2 and those same authors suggest that the overexpression of both the ligand and the receptor may be the best option for individualized fine-tuning treatment for pharmacoresistant TLE patients (Nikitidou Ledri et al., 2016).

Considering that the nervous system is highly plastic, just as an action produces a reaction, after an initial insult both epileptogenesis as well as antiepileptogenic mechanisms take place. As it usually happens in a complex system, the same signal (SE, neurodegeneration) can trigger adaptations (neurogenesis) in different ways, in this case towards excitation and inhibition, therefore, antiepileptogenesis can be viewed as an alternative "interpretation" of the initial insult, initiating compensatory responses such as NPY-associated plasticity in order to bring back homeostasis to the circuits.

Conclusion

Briefly, the current study showed that a similar initial insult (electrical stimulation of the AmyC) in a rat model of TLE led to different types of SE due to different behavioral pattern and cell death brain areas. More severe seizure behaviors present with generalized (bilateral) brain degeneration, while less severe seizure behaviors are related to unilateral brain neuronal death. On the other hand, both types of SE drive similar increase of cell proliferation and neurogenesis in both side of the hippocampus as we have shown, although qualitatively, the NPY expression in the hilus of hippocampal formation demonstrate no differences. Small differences in the position of the stimulation electrode or individual nuances can be responsible for the different outcomes. Usually, the animals that do not respond as expected are removed from the experimental groups, however by doing that we could be missing important data that would help us understanding how epileptogenesis processes transform a normal brain into an epileptic one.

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CRediT authorship contribution statement

Victor Rodrigues Santos: Conceptualization, Formal analysis, Investigation, Histolocial analysis, Methodology, Resources, Writing – original draft. **Cristiane Q. Tilelli:** Conceptualization, animal surgery, formal analysis, writing. **Artur Fernandes:** Formal Analysis of NPY cells. **Olagide W. de Castro**: Formal Analysis, Neurodegeneration Analysis, Writing. **Flávio Del Vecchio**: Animal Surgery. **Norberto Garia Cairasco**: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing – original draft.

References

Arisi, G.M., Garcia-Cairasco, N., 2007. Doublecortin-positive newly born granule cells of hippocampus have abnormal apical dendritic morphology in the pilocarpine model of temporal lobe epilepsy. Brain Res 5, 126–134.

Bengzon, J., Kokaia, Z., Elmér, E., Nanobashvili, A., Kokaia, M., Lindvall, O., 1997. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. Proc. Natl. Acad. Sci. USA 94, 10432–10437 (Available from). ⟨http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=23380&tool=pmcentrez&rendertype=abstract⟩.

Blümcke, I., Thom, M., Aronica, E., Armstrong, D.D., Bartolomei, F., Bernasconi, A., Bernasconi, N., Bien, C.G., Cendes, F., Coras, R., Cross, J.H., Jacques, T.S., Kahane, P., Mathern, G.W., Miyata, H., Moshé, S.L., Oz, B., Özkara, Ç., Perucca, E., Sisodiya, S., Wiebe, S., Spreafico, R., 2013. International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: a task Force report from the ILAE commission on diagnostic methods. Epilepsia 54, 1315–1329.

Brandt, C., Glien, M., Potschka, H., Volk, H., Löscher, W., 2003. Epileptogenesis and neuropathology after different types of status epilepticus induced by prolonged electrical stimulation of the basolateral amygdala in rats. Epilepsy Res 55, 83–103 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/12948619⟩.

Brandt, C., Potschka, H., Löscher, W., Ebert, U., 2003. N-methyl-d-aspartate receptor blockade after status epilepticus protects against limbic brain damage but not against epilepsy in the kainate model of temporal lobe epilepsy. Neuroscience 118, 727–740.

Cardoso, A., Freitas-da-Costa, P., Carvalho, L.S., Lukoyanov, N.V., 2010. Seizure-induced changes in neuropeptide Y-containing cortical neurons: potential role for seizure threshold and epileptogenesis. Epilepsy Behav. 19, 559–567.

Castro, O.W., Furtado, M.A., Tilelli, C.Q., Fernandes, A., Pajolla, G.P., Garcia-Cairasco, N., 2011. Comparative neuroanatomical and temporal characterization of FluoroJade-positive neurodegeneration after status epilepticus induced by systemic and intrahippocampal pilocarpine in Wistar rats. Brain Res 1374, 43–55 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/21147076⟩.

Cavazos, J.E., Das, I., Sutula, T.P., 1994. Neuronal loss induced in limbic pathways by kindling: evidence for induction of hippocampal sclerosis by repeated brief seizures. J. Neurosci. 14, 3106–3121.

Dragunow, M., 1986. Endogenous anticonvulsant substances. Neurosci. Biobehav Rev. 10, 229–244.

Dubé, C., 2007. Neuropeptide Y: Potential role in recurrent developmental seizures. Peptides 28, 441–446.

Engel, J., 1995. Concepts of epilepsy. Epilepsia 36, S23–S29.

Engel, J., 2001. Classification of epileptic disorders. Epilepsia 42, 316.

Fujikawa, D.G., 1995. Neuroprotective effect of ketamine administered after status epilepticus onset. Epilepsia 36, 186–195 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/7821277⟩.

Fujikawa, D.G., 1996. The temporal evolution of neuronal damage from pilocarpine-induced status epilepticus. Brain Res 725, 11–22 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/8828581⟩.

Fujikawa, D.G., 2003. Neuronal death in mesial temporal sclerosis: separating morphology from mechanism. Epilepsia 1607–1608 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/14636340⟩.

Fujikawa, D.G., Daniels, A.H., Kim, J.S., 1994. The competitive NMDA receptor antagonist CGP 40116 protects against status epilepticus-induced neuronal damage. Epilepsy Res 17, 207–219 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/7912191⟩.

Fujikawa, D.G., Itabashi, H.H., Wu, A., Shinmei, S.S., 2000a. Status epilepticus-induced neuronal loss in humans without systemic complications or epilepsy. Epilepsia 41, 981–991 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/10961625⟩.

Fujikawa, D.G., Shinmei, S.S., Cai, B., 2000b. Seizure-induced neuronal necrosis: implications for programmed cell death mechanisms. Epilepsia 41 (Suppl 6), S9–S13 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/10999512⟩.

Gorter, J.A., Gonçalves Pereira, P.M., van Vliet, E.A., Aronica, E., Lopes da Silva, F.H., Lucassen, P.J., 2003. Neuronal cell death in a rat model for mesial temporal lobe epilepsy is induced by the initial status epilepticus and not by later repeated spontaneous seizures. Epilepsia 44, 647–658.

Guo, H., Zhang, H., Kuang, Y., Wang, C., Jing, X., Gu, J., Gao, G., 2014. Electrical stimulation of the substantia nigra pars reticulata (SNr) suppresses chemically induced neocortical seizures in rats. J. Mol. Neurosci. 53, 546–552.

Handforth, A., Ackermann, R.F., 1988. Functional [14C]2-deoxyglucose mapping of progressive states of status epilepticus induced by amygdala stimulation in rat. Brain Res 460, 94–102.

Hattiangady, B., Rao, M.S., Shetty, A.K., 2004. Chronic temporal lobe epilepsy is associated with severely declined dentate neurogenesis in the adult hippocampus. Neurobiol. Dis. 17, 473–490 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/15571983⟩.

Howell, O.W., Silva, S., Scharfman, H.E., Sosunov, A.A., Zaben, M., Shatya, A., McKhann, G., Herzog, H., Laskowski, A., Gray, W.P., 2007. Neuropeptide Y is important for basal and seizure-induced precursor cell proliferation in the hippocampus. Neurobiol. Dis. 26, 174–188 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/17317195⟩.

Hsieh, J., Eisch, A.J., 2010. Epigenetics, hippocampal neurogenesis, and neuropsychiatric disorders: unraveling the genome to understand the mind. Neurobiol. Dis. 39, 73–84 (Available from). ⟨http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2874108&tool=pmcentrez&rendertype=abstract⟩.

Jessberger, S., Römer, B., Babu, H., Kempermann, G., 2005. Seizures induce proliferation and dispersion of doublecortin-positive hippocampal progenitor cells. Exp. Neurol. 196, 342–351 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/16168988⟩.

Jessberger, S., Zhao, C., Toni, N., Clemenson, G.D., Li, Y., Gage, F.H., 2007. Seizure-associated, aberrant neurogenesis in adult rats characterized with retrovirus-mediated cell labeling. J. Soc. Neurosci. 27, 9400–9407 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/17728453⟩.

Kang, T.C., Park, S.H., Park, S.K., Lee, J.C., Jo, S.M., Do, S.G., Suh, J.G., Oh, Y.S., Lee, J.Y., Won, M.H., 2000. The temporal and spatial expressions of neuropeptide Y induced by seizure in the hippocampal complex of gerbil. Brain Res 870, 179–184.

Koch, C., 1999. Complexity and the nervous system. Science 80- (284), 96–98.

Kovac, S., Walker, M.C., 2013. Neuropeptides in epilepsy. Neuropeptides 47, 467–475.

Ledri, M., Sorensen, A.T., Madsen, M.G., Christiansen, S.H., Ledri, L.N., Cifra, A., Bengzon, J., Lindberg, E., Pinborg, L.H., Jespersen, B., Gotzsche, C.R., Woldbye, D.P. D., Andersson, M., Kokaia, M., 2015. Differential effect of neuropeptides on excitatory synaptic transmission in human epileptic hippocampus. J. Neurosci. 35, 9622–9631.

Leite, J.P., Nakamura, E.M., Lemos, T., Masur, J., Cavalheiro, E.A., 1990. Learning impairment in chronic epileptic rats following pilocarpine-induced status epilepticus. Braz. J. Med Biol. Res 23, 681–683.

Mello, L.E., Cavalheiro, E.A., Tan, A.M., Kupfer, W.R., Pretorius, J.K., Babb, T.L., Finch, D.M., 1993. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. Epilepsia 34, 985–995.

Mello, L.E., Cavalheiro, E.A., Tan, A.M., Pretorius, J.K., Babb, T.L., Finch, D.M., 1992. Granule cell dispersion in relation to mossy fiber sprouting, hippocampal cell loss, silent period and seizure frequency in the pilocarpine model of epilepsy. Epilepsy Res Suppl. Discuss. 59–60.

Mohapel, P., Dufresne, C., Kelly, M.E., McIntyre, D.C., 1996. Differential sensitivity of various temporal lobe structures in the rat to kindling and status epilepticus induction. Epilepsy Res 23, 179–187 (Available from). ⟨http://linkinghub.elsevier.com/retrieve/pii/0920121195000844⟩.

Mullen, R.J., Buck, C.R., Smith, A.M., 1992. NeuN, a neuronal specific nuclear protein in vertebrates. Development 116, 201–211.

Nadler, J.V., Perry, B.W., Gentry, C., Cotman, C.W., 1981. Fate of the hippocampal mossy fiber projection after destruction of its postsynaptic targets with intraventricular kainic acid. J. Comp. Neurol. 196, 549–569.

Nakagawa, E., Aimi, Y., Yasuhara, O., Tooyama, I., Shimada, M., McGeer, P.L., Kimura, H., 2000. Enhancement of progenitor cell division in the dentate gyrus triggered by initial limbic seizures in rat models of epilepsy. Epilepsia 41, 10–18 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/10643917⟩.

Nikitidou Ledri, L., Melin, E., Christiansen, S.H., Gøtzsche, C.R., Cifra, A., Woldbye, D.P. D., Kokaia, M., 2016. Translational approach for gene therapy in epilepsy: Model system and unilateral overexpression of neuropeptide Y and Y2 receptors. Neurobiol. Dis. 86, 52–61.

Nissinen, J., Halonen, T., Koivisto, E., Pitka, A., 2000. A new model of chronic temporal lobe epilepsy induced by electrical stimulation of the amygdala in rat. Epilepsy Res 38, 177–205.

Noè, F., Pool, A.-H., Nissinen, J., Gobbi, M., Bland, R., Rizzi, M., Balducci, C., Ferraguti, F., Sperk, G., During, M.J., Pitkänen, A., Vezzani, A., 2008. Neuropeptide Y gene therapy decreases chronic spontaneous seizures in a rat model of temporal lobe epilepsy. Brain A J. Neurol. [Internet] 131, 1506–1515 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/18477594⟩.

Olney, J.W., deGubareff, T., Sloviter, R.S., 1983. "Epileptic" brain damage in rats induced by sustained electrical stimulation of the perforant path. II. Ultrastructural analysis of acute hippocampal pathology. Brain Res Bull. [Internet] 10, 699–712 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/6871738⟩.

Parent, J.M., Elliott, R.C., Pleasure, S.J., Barbaro, N.M., Lowenstein, D.H., 2006. Aberrant seizure-induced neurogenesis in experimental temporal lobe epilepsy. Ann. Neurol. 59, 81–91 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/16261566⟩.

Parent, J.M., Janumpalli, S., McNamara, J.O., Lowenstein, D.H., 1998. Increased dentate granule cell neurogenesis following amygdala kindling in the adult rat. Neurosci. Lett. 247, 9–12.

Parent, J.M., Yu, T.W., Leibowitz, R.T., Geschwind, D.H., Sloviter, R.S., Lowenstein, D. H., 1997. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. N. Y. 17, 3727–3738.

Pierce, J.P., McCloskey, D.P., Scharfman, H.E., 2011. Morphometry of hilar ectopic granule cells in the rat. J. Comp. Neurol. [Internet] 519, 1196–1218 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/21344409⟩.

Pinel, J., Rovner, L., 1978. Experimental epileptogenesis: kindling-induced epilepsy in rats. Exp. Neurol. 202, 190–202.

Pitkänen, A., Lukasiuk, K., 2009. Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. Epilepsy Behav. EB 14 (Suppl 1), 16–25.

Pitkänen, A., Lukasiuk, K., 2011. Mechanisms of epileptogenesis and potential treatment targets. Lancet Neurol. [Internet] 10, 173–186 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/21256455⟩.

Racine, R.J., 1972. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electro Clin. Neurophysiol. 32, 281–294 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/4110397⟩.

Romcy-Pereira, R.N., Garcia-Cairasco, N., 2003. Hippocampal cell proliferation and epileptogenesis after audiogenic kindling are not accompanied by mossy fiber sprouting or Fluoro-Jade staining. Neuroscience 119, 533–546 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/12770566⟩.

Santos, V.R., de Castro, O.W., Pun, R.Y.K., Hester, M.S., Murphy, B.L., Loepke, A.W., Garcia-Cairasco, N., Danzer, S.C., 2011. Contributions of mature granule cells to structural plasticity in temporal lobe epilepsy. Neuroscience 197, 348–357 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/21963349⟩.

Santos, V.R., Melo, I. santana, Pacheco, A.L.D., Castro, O.W., De, 2019. Life and death in the hippocampus: what's bad (Available from). Epilepsy Behaviour, 106595. https://doi.org/10.1016/j.yebeh.2019.106595.

Scharfman, H.E., Goodman, J.H., Sollas, A.L., 2000. Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: functional implications of seizure-induced neurogenesis. J. Neurosci. 20, 6144–6158.

Scharfman, H.E., Sollas, A.E., Berger, R.E., Goodman, J.H., Pierce, J.P., 2003. Perforant path activation of ectopic granule cells that are born after pilocarpine-induced seizures. Neuroscience 121, 1017–1029.

Schmued, L.C., Albertson, C., Slikker, W., 1997. Fluoro-Jade: a novel fluorochrome for the sensitive and reliable histochemical localization of neuronal degeneration. Brain Res 751, 37–46.

Schwarzer, C., Williamson, J.M., Lothman, E.W., Vezzani, A., Sperk, G., 1995. Somatostatin, neuropeptide Y, neurokinin B and cholecystokinin immunoreactivity in two chronic models of temporal lobe epilepsy. Neuroscience 69, 831–845.

Shapiro, L.A., Korn, M.J., Ribak, C.E., 2005. Newly generated dentate granule cells from epileptic rats exhibit elongated hilar basal dendrites that align along GFAP-immunolabeled processes. Neuroscience 136, 823–831 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/16344154⟩.

Shapiro, L.A., Ribak, C.E., 2005. Integration of newly born dentate granule cells into adult brains: hypotheses based on normal and epileptic rodents. Brain Res Brain Res Rev. [Internet] 48, 43–56 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/15708627⟩.

Shetty, A.K., Hattiangady, B., 2007. Restoration of calbindin after fetal hippocampal CA3 cell grafting into the injured hippocampus in a rat model of temporal lobe epilepsy. Hippocampus 17, 943–956.

Shetty, A.K., Turner, D.A., 2001. Glutamic acid decarboxylase-67-positive hippocampal interneurons undergo a permanent reduction in number following kainic acid-induced degeneration of ca3 pyramidal neurons. Exp. Neurol. 169, 276–297.

Skarda, C.A., Freeman, W.J., 1987. How brains make chaos in order to make sense of the world. Behav. Brain Sci. 10, 161.

Sloviter, R.S., 2005. The neurobiology of temporal lobe epilepsy: too much information, not enough knowledge. C. R. Biol. 328, 143–153.

Sutula, T.P., Golarai, G., Cavazos, J., 1992. Assessing the functional significance of mossy fiber sprouting. Epilepsy Res Suppl. 7, 251–259.

Tauck, D.L., Nadler, J.V., 1985. Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. J. Neurosci. J. Soc. Neurosci. 5, 1016–1022.

Tilelli, C.Q., Del Vecchio, F., Fernandes, A., Garcia-Cairasco, N., 2005. Different types of status epilepticus lead to different levels of brain damage in rats. Epilepsy Behav. 7, 401–410 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/16140590⟩.

Treiman, D.M., 1998. Clinical trials for status epilepticus. Adv. Neurol. 76, 173–178.

Trinka, E., Cock, H., Hesdorffer, D., Rossetti, A.O., Scheffer, I.E., Shinnar, S., Shorvon, S., Lowenstein, D.H., 2015. A definition and classification of status epilepticus–report of the ILAE task force on classification of status epilepticus. Epilepsia 56, 1515–1523.

Tu, B., Jiao, Y., Herzog, H., Nadler, J.V., 2006. Neuropeptide Y regulates recurrent mossy fiber synaptic transmission less effectively in mice than in rats: Correlation with Y2 receptor plasticity. Neuroscience 143, 1085–1094 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/17027162⟩.

Tu, B., Timofeeva, O., Jiao, Y., Nadler, J.V., 2005. Spontaneous release of neuropeptide Y tonically inhibits recurrent mossy fiber synaptic transmission in epileptic brain. J. Neurosci. J. Soc. Neurosci. 25, 1718–1729 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/15716408⟩.

Walker, M.C., 2015. Hippocampal sclerosis: causes and prevention. Semin Neurol. 35, 193–200.

Wallace, M.J., 2003. The endogenous cannabinoid system regulates seizure frequency and duration in a model of temporal lobe epilepsy. J. Pharm. Exp. Ther. 307, 129–137.

Walter, C., Murphy, B.L., Pun, R.Y.K., Spieles-Engemann, A.L., Danzer, S.C., 2007. Pilocarpine-induced seizures cause selective time-dependent changes to adult-generated hippocampal dentate granule cells. J. Neurosci. 27, 7541–7552.

Wasterlain, C.G., Fujikawa, D.G., Penix, L., Sankar, R., 1993. Pathophysiological mechanisms of brain damage from status epilepticus. Epilepsia [Internet] 34 (Suppl 1), S37–S53 (Available from). ⟨http://www.ncbi.nlm.nih.gov/pubmed/8385002⟩.

Zhao, C.M., Teng, E.M., Summers, R.G., Ming, G.L., Gage, F.H., Jr, M., Jr RGS, G.L., 2006. Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. J. Neurosci. 26, 3–11.