Research Report

Doublecortin-positive newly born granule cells of hippocampus have abnormal apical dendritic morphology in the pilocarpine model of temporal lobe epilepsy

Gabriel Maisonnave Arisi, Norberto Garcia-Cairasco*

Department of Physiology, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, 14049-900, Brazil

ARTICLE INFO

Article history:
Accepted 7 June 2007
Available online 5 July 2007

Keywords:
Doublecortin
Dentate gyrus
Mossy fiber sprouting
3D reconstruction
Stereology
Rat

ABSTRACT

Here, we describe dentate gyrus newly born granule cells morphology in rats’ temporal lobe epilepsy pilocarpine model. Digital reconstruction of doublecortin-positive neurons revealed that apical dendrites had the same total length and number of nodes in epileptic and control animals. Nonetheless, concentric spheres analyses revealed that apical dendrites spatial distribution was radically altered in epileptic animals. The apical dendrites had more bifurcations inside the granular cell layer and more terminations in the inner molecular layer of epileptic dentate gyrus. Branch order analyses showed that second- and third-order dendrites were shorter in epileptic animals. Apical dendrites were concentrated in regions like the inner molecular layer where granular neuron axons, named mossy fibers, sprout in epileptic animals.

The combination of altered dendritic morphology and number enhancement of the new granular neurons suggests a deleterious role of hippocampal neurogenesis in epileptogenesis. Being more numerous and with dendrites concentrated in regions where aberrant axon terminals sprout, the new granular neurons could contribute to the slow epileptogenesis at hippocampal circuits commonly observed in temporal lobe epilepsy.

© 2007 Elsevier B.V. All rights reserved.

1. Introduction

The role of hippocampal neurogenesis in physiological and pathological conditions has been intensely investigated in recent years (for a review, see Ming and Song, 2005). The hippocampus participates in learning and memory but also in the most common human epileptic syndrome, the temporal lobe epilepsy (TLE; Sutula et al., 1989). Plastic alterations such as aberrant zinc-enriched synaptic terminals are one of the hippocampal sclerosis hallmarks in TLE patients. These aberrant terminals are present in the inner molecular layer and hilus of dentate gyrus (DG) resulting from the sprouting of granule cells axons, named mossy fibers (Tauck and Nadler, 1985; Sutula et al., 1998).

Along with the plastic change of mossy fibers sprouting, an increase in hippocampal neurogenesis rate was reported in epilepsy experimental models using immunohistochemical staining for bromodeoxyuridine (BrdU; Parent et al., 1997;
In recent studies, the use of an endogenous neurogenesis marker such as doublecortin (DCX), a microtubule-associated phosphoprotein expressed by migrating and differentiating neurons, allowed the study of new granular neuron dendritic arborization (Rao and Shetty, 2004; Couillard-Després et al., 2005). Alterations in the apical dendritic tree of adult-generated granular neurons were observed in aging, employing DCX immunostaining (Rao et al., 2005), and with voluntary exercise, employing Golgi-Cox staining (Redila and Christie, 2006). Adult-generated granular neurons exhibit transient basal dendrites, an immature characteristic of these neurons; basal dendrites are more frequent in epileptic hippocampus (Ribak et al., 2000; Spigelman et al., 1998).

Together, these results show that plastic changes occur in the hippocampus and newly born granule cells are susceptible to morphological changes in physiological conditions.

The behavioral and histopathological characteristics of TLE can be modeled in experimental animals with systemic injection of pilocarpine, a cholinergic muscarinic agonist (Turski et al., 1989; Cavalheiro et al., 1991; Mello et al., 1992; Cavazos et al., 2004). Pilocarpine induces status epilepticus (SE) and, after a latent period, spontaneous recurrent limbic seizures in the animals (Cavalheiro et al., 1991; Mello et al., 1992).

Since the newly generated neurons differentiate into functional granule cells integrated in the DG (van Praag et al., 2002), we wanted to investigate plastic changes in DCX-positive (DCX+) granular neurons morphology in the TLE pilocarpine model with three-dimensional digital reconstruction techniques. Dendrites length, nodes and spatial distribution were quantified and analyzed in control and epileptic animals 30 days after treatment with pilocarpine.

Fig. 1 – Three-dimensional reconstruction of soma and dendritic arborizations. Doublecortin immunostaining revealed the presence of newly born granule cells in control (A) and epileptic (D) dentate gyrus. Three-dimensional reconstruction was performed in 20 neurons from each group as represented in the overlay (B and E) depicting the soma (pink), apical dendrites (blue) and basal dendrites (green). Sholl’s concentric spheres analysis (C and F) was performed to quantify the number of dendritic nodes, endings, intersections, and total length inside each sphere. The first sphere has the radius equal to the granular cell layer (GCL) thickness followed by spheres with increments of 50 μm in their radii. SGZ, subgranular zone; IML, inner molecular layer; MML, median molecular layer; OML, outer molecular layer. Scale bar=100 μm.
2. Results

2.1. Pilocarpine treatment and spontaneous recurrent seizures

Of the twenty seven animals treated with pilocarpine, seventeen (63%) developed SE. Ten of these animals died in the acute phase of treatment. The video monitoring of the remaining seven animals recorded a mean of 8.4 limbic seizures with a minimum of 2 and a maximum of 15 seizures observed in each animal for the entire observation period. The control animals were seizure-free. The most frequent seizure class observed was the animal rearing and falling (Racine, 1972); a mean value of 3.3 was calculated for all seizures observed.

2.2. Doublecortin immunostaining and stereological results

Immunocytochemical staining revealed the presence of DCX+ neurons in the granule cell layer and the subgranular zone (GCL/SGZ) of the hippocampal DG of control (Fig. 1A; n=7 rats) and epileptic animals (Fig. 1D; n=7 rats). DCX+ cells were absent in the suprapyramidal, or enclosed, blade of the DG in episodic animals (Fig. 1D; n=7 rats). DCX+ cells were absent in the supraparapyramidal, or enclosed, blade of the DG in the 600-μm septal portion of the hippocampus, in other areas the DCX+ neurons were evenly distributed with clusters occurring only in DG crests in more caudal slices. Some ectopically located DCX+ neurons were located near the CA3 pyramidal neurons in epileptic animals.

Quantification of the absolute number of DCX+ neurons in the GCL/SGZ by the optical fractionator method revealed the presence of 8987±751 cells (mean±SEM; n=7 rats) per DG of control animal and 19,337±1782 cells (mean±SEM; n=7 rats) per DG of pilocarpine-treated animal (p<0.001). So the pilocarpine-treated animals have roughly double the number of DCX+ neurons than the control animals, 1 month after SE.

2.3. DCX+ granular neuron dendritic morphology

The neuronal reconstruction generated data about the morphology of DCX+ neurons (Figs. 1B and E). The comparison between the morphological characteristics of DCX+ neurons of control (Fig. 1B) and pilocarpine-treated animals (Fig. 1E) depicted in Table 1 revealed a remarkable similarity of somatic control (Fig. 1B) and pilocarpine-treated animals (Fig. 1E) which resulted in more intersections crossing into the molecular layer (Fig. 2B). Interestingly, most of these dendrites ended in the first 50 μm of the molecular layer, i.e., in the inner molecular layer (Fig. 2C). As a consequence of this pattern of ramification and termination, the summed dendritic length present in the GCL and in the first 50 μm of the molecular layer (GCL+50) were increased in pilocarpine-treated animals and the contrary occurred in the following 50 μm of the molecular layer (GCL+100; Fig. 2D).

2.3.2. Branch order analyses

The comparison between median dendritic lengths for each branch order revealed that second-, third- and sixth-order dendrites were shorter in DCX+ granular neurons from epileptic animals (Fig. 3). Second-order dendrites had 35.55 μm in control animals against 16.6 μm in epileptic animals (p<0.01), third-order dendrites had 40.7 μm in control animals against 21.65 μm in epileptic animals (p<0.01), and sixth-order dendrites had 40.4 μm in control animals against 19.45 μm in epileptic animals (all median values; p<0.01).

2.4. neo-Timm staining

Autometallographic silver Timm staining revealed the distribution of zinc-enriched synaptic terminals in the hippocampus (Fig. 4). As expected, the control animals showed no staining in the DG molecular layer (n=5) and the pilocarpine-treated animals presented staining in the inner molecular layer (n=7). The sprouting score (Cavazos et al., 1991) was 0 for control animals, with no granules between the tips and crest of the DG and varied between 0 to 3, with prominent number of granules in a continuous pattern with occasional patches of granules, and 4, with prominent number of granules forming a confluent laminar band, in pilocarpine-treated animals. The mean thickness of the silver-stained sprouting band was 48±4 μm.

3. Discussion

Here, we demonstrate that pilocarpine-induced seizures altered the newly born granule cell dendritic morphology. The epileptic animals had DCX+ apical dendrites with more

### Table 1 – DCX+ neurons morphological measurements (n=20 neurons per group)

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Soma perimeter (μm)</th>
<th>Soma area (μm²)</th>
<th>Dendritic nodes (n)</th>
<th>Dendritic endings (n)</th>
<th>Apical dendritic length (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>32.9±1.1</td>
<td>73.8±4.8</td>
<td>6.1±0.6</td>
<td>7.3±0.6</td>
<td>542.1±54.0</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>32.0±0.8</td>
<td>68.8±2.7</td>
<td>7.6±0.6</td>
<td>8.9±0.6</td>
<td>515.3±39.2</td>
</tr>
</tbody>
</table>
bifurcations inside the granular cell layer and more endings in the inner molecular layer. The new granule cell dendrites were concentrated at mossy fiber sprouting sites like the hilus and the inner molecular layer. We confirm with doublecortin immunostaining previous results obtained with BrdU immunostaining that neurogenesis is enhanced in epileptic animals ([Parent et al., 1997; Bengzon et al., 1997; Scott et al., 1998; Nakagawa et al., 2000]), with the finding of double the number of DCX+ neurons in epileptic animals than in controls. We also confirm that granular neuron basal dendrites are more common in epileptic DG ([Spigelman et al., 1998; Buckmaster and Dudek, 1999; Ribak et al., 2000, 2004; Dashtipour et al., 2002; Shapiro et al., 2005]).

The study of adult-generated granular cell dendritic morphology was made possible by the labeling of DCX, an endogenous neurogenesis marker ([Des Portes et al., 1998; Gleeson et al., 1998]). DCX is expressed in the developing CNS by migrating and differentiating neurons. It was demonstrated

Fig. 2 – Numeric results of Sholl’s concentric spheres analysis of apical dendritic arborization of the DCX+ granular neurons in controls (gray) and epileptic animals (black). In panel A, number of dendritic bifurcations (nodes) inside each sphere. In panel B, number of dendrites that intersect each sphere. In panel C, number of dendritic endings inside each sphere. In panel D, summed dendritic length inside each sphere. *p<0.05, **p<0.001.

Fig. 3 – Dendrite branch order length measurements (median values) revealed that second-, third- and sixth-order dendrites were shorter in epileptic (black) animals than in control (gray) animals. **p<0.01, Mann–Whitney.
recently, in studies combining DCX and BrdU stainings, that DCX immunostaining is a reliable tool for adult neurogenesis assessment (Rao and Shetty, 2004; Couillard-Després et al., 2005).

3.1. Enhanced proliferation in epileptic animals

Neurogenesis increase in epilepsy models was first observed using BrdU immunostaining after pilocarpine-induced SE (Parent et al., 1997). Augmentation of neurogenesis was also observed in epilepsy models of electrical kindling and kainic acid in the same year also with BrdU immunostaining (Bengzon et al., 1997). Since these seminal papers, neurogenesis enhancement role in epileptogenesis is under investigation. Radley and Jacobs (2003), using a 5-HT_1A receptor antagonist treatment in epileptic animals, proposed a serotonergic mechanism by which seizures could enhance neurogenesis. This enhancement was blocked and the SRS was more rare, but without statistical difference between experimental groups. On the other hand, inhibition of the inflammatory pathway enzyme cyclooxygenase-2 suppressed the neurogenesis enhancement and diminished SRS frequency and duration (Jung et al., 2006). These studies, like ours, were made in short-term epilepsy models; in a chronic epilepsy study, Hattiangady et al. (2004) observed a neurogenesis decrease in relation to control levels 5 months after kainic acid treatment. In a study with kainic acid injected directly into the hippocampus Heinrich et al. (2006) showed that neurogenesis was not enhanced and was not responsible for the granule cell dispersion observed in some TLE animal models and patients.

These results suggest that the enhanced neurogenesis could be a transitory inflammatory response of the nervous tissue mediated by many chemical pathways. They also suggest a deleterious role in epileptogenesis for the new neurons generated after the initial insult. In the epileptogenesis latent phase an increased production of new granular neurons in an altered development milieu could contribute to the epileptic circuit formation. In the chronic phase a decreased production of granular neurons could contribute to memory and learning deficits observed in epilepsy.

3.2. Apical dendritic tree abnormalities and mossy fiber sprouting

Detailed apical dendritic morphology of mature granular neurons was described with Golgi impregnation (Desmond and Levy, 1982) and horseradish peroxidase intracellular labeling (Claiborne et al., 1990). Only with the advent of DCX staining it was made possible to study the dendritic arborization of adult-generated granular neurons (Hattiangady et al., 2004; Ribak et al., 2004; Rao and Shetty, 2004; Rao et al., 2005). The first digital reconstruction with a detailed morphological description of DCX+ granular neurons was made by Rao and Shetty (2004). In aging animals, it was demonstrated that DCX+ granular neurons had smaller apical dendritic trees with less bifurcations and endings than in young animals (Rao et al., 2005). Recently it was demonstrated that exercise also modifies the dendritic morphology of newly born granular neurons (Redila and Christie, 2006). In a recent study, Heinrich et al. (2006) stated that DCX-positive dendrites appeared shorter and strongly reduced in number after kainic acid treatment.

We found similar total apical dendritic length in controls, with 542.1 ± 54 μm, and epileptic animals, with 515.3 ± 39.2 μm. These values, in turn, are very similar to the 518.3 ± 43.9 μm observed by Rao and Shetty (2004). Interestingly, apical dendritic tree has a total length of 2800 μm in mature granular neurons in the rat DG-free blade (Claiborne et al., 1990). This suggests that adult-generated granular neurons have much smaller dendritic arborizations than the granular neurons generated during development.

In our study, the apical dendrites spatial distribution was radically altered in epileptic animals with more bifurcations inside the granular cell layer and more endings in the inner molecular layer. Dendrites of DCX+ neurons were concentrated inside the granular and inner molecular layer and were rarer in the middle and outer molecular layers. This distribution could be explained by the much shorter second- and third-order dendrite branches of DCX+ granular neurons of epileptic animals. It seems that during growth the dendrites ramified and terminated closer to the soma and, since the...
total dendritic length was maintained, did not reach the middle and outer molecular layer.

Neurotrophins, such as brain-derived neurotrophic factor (BDNF), could cause the dendritic growth observed in the inner molecular layer. Accumulation of BDNF mRNA at granular proximal dendrites was demonstrated after pilocarpine treatment (Tongiorgi et al., 2004). Increased expression of BDNF by gene transfer promoted the formation of basal dendrites and the branching of apical dendrites close to the soma (Danzer et al., 2002). Interestingly these morphological alterations closely resemble the ones observed in our study, suggesting a BDNF stimulus to its occurrence.

Another important characteristic of the aberrant dendritic morphology of the new granular neurons is that new dendrites concentrate in zones where mossy fiber sprouting occurred. The inner molecular layer, or its first 50 μm, and the granular layer were occupied by aberrant mossy fiber terminals as shown here and in many other studies (Tauck and Nadler, 1985; Sutula et al., 1998).

3.3. Basal dendrites

The vast majority of dentate gyrus mature granular neurons only have apical dendrites. Immature granular cells have also basal dendrites in a bipolar conformation that, during development, is gradually transformed into the mature form (Seress, 1992; Spigelman et al., 1998). However, basal dendrites are retained in 10% of primate mature granular cells and are a rare finding on rodent normal DG (Seress and Frotscher, 1990). Even between primates there are differences: frequency of granular neurons basal dendrites in humans is twofold of that in rhesus monkeys (Seress and Mrzljak, 1987). The basal dendrites of mature neurons could be restricted to the hilus, referred as hilar basal dendrites, or extend into the molecular layer, referred as recurrent basal dendrites (Dashtipour et al., 2002).

A common finding in epilepsy is that basal dendrites frequency increases in dentate granular neurons. These dendrites are present in 30% of epileptic patients' granular neurons and between 5% (Ribak et al., 2000) and 12% (Buckmaster and Dudek, 1999) of experimental animals' granular neurons. In normal animals of our study, hilar basal dendrites were observed in 30% of reconstructed DCX+ neurons, exactly the same proportion observed by Rao and Shetty (2004) but less than the 55% registered by Ribak et al. (2004). The basal dendrite lengths of control and epileptic groups in our study were very similar to those observed by Shapiro et al. (2005). In epileptic animals, hilar basal dendrites were present in 85% of reconstructed DCX+ neurons. Therefore, the same increase in basal dendrites frequency, observed in the overall granular cell population in epilepsy, is also observed in new neurons.

The hilus is also a site where mossy fiber sprouting was observed (Sutula et al., 1998; Wenzel et al., 2000). Basal dendrites of the new granular neurons could receive synaptic input from the hilar aberrant mossy fiber terminals (Spigelman et al., 1998; Ribak et al., 2000). Formation of synapses between mossy fibers sprouted terminals and vacant DCX+ dendrites, in hilus, granular and inner molecular layers, is only suggested by our results, but is a likely event.

In conclusion, hippocampal neurogenesis was enhanced after an epileptic insult and the new granular neurons had abnormal dendritic morphology. Apical dendrites were more ramified inside the granular cell layer and their endings concentrated in the inner molecular layer. Basal dendrites were longer and more frequent. With this altered morphology the new granular neuron dendrites were concentrated in regions where mossy fiber sprouting occurred. The formation of synapses between the aberrant mossy fiber terminals and the new dendrites could contribute to hippocampal epileptogenesis.

4. Experimental procedures

4.1. Animals and housing

A total of 34 Wistar male rats were used in this study, weighing 250–300 g and aging 6–7 weeks in the beginning of experiments. The animals were maintained in a 12-h light–dark cycle (06:00–18:00) with food and water ad libitum. The experimental procedure was approved by the Commission on Animal Experimentation Ethics of the University of São Paulo Medical School at Ribeirão Preto (protocol #243/2005).

4.2. Seizure induction

Twenty seven animals were treated with methyl-scopolamine (1 mg/kg, i.p.; all drugs provided by Sigma, St. Louis, USA, unless otherwise stated) 30 min before pilocarpine hydrochloride to minimize its peripheral effects. Pilocarpine was injected in these animals in a single dose of 320 mg/kg i.p. SE beginning was considered when animals suffered a class 3 motor seizure in Racine’s scale of limbic seizures (Racine, 1972), characterized by forelimb clonus, followed by sustained tonic-clonic seizures that lasted for more than 5 min. All animals were treated with 10 mg/kg diazepam i.p. (União Química, São Paulo, Brazil) 90 min after SE onset. The seven control animals were injected with methyl-scopolamine, saline 0.9% i.p. instead of pilocarpine, and diazepam. Starting 3 days after SE, animals were video monitored 8 h/day (9:00–17:00) for 20 days with a VHS for observation of spontaneous recurrent seizures (SRS).

4.3. Tissue processing

Thirty days after SE the animals were deeply anesthetized with nembutal (80 mg/kg) and transcardially perfused first with saline solution for 1 min and then with 375 ml of a buffered fixative solution for 30 min (4% formaldehyde and 0.1% glutaraldehyde in 0.1 M PBS, pH 7.4). All solutions were pumped with a rotatory pump (Masterflex 7558-10, Cole-Parmer, Vernon Hills, USA) with a 12.5 ml/min flux rate. Skulls were removed and fixed for 2 h more. Tissue was immersed in the fixative solution for 1 h; the brains were removed and fixed for 2 h more. Tissue was cryoprotected in 30% sucrose solution in phosphate buffer overnight and frozen in dry ice-refrigerated iso-pentane alcohol. An exhaustive series of 60-μm coronal slices containing the entire hippocampus was made in a cryostat (Microm HM 505, San Marcos, CA, USA) between the coordinates –1.7 and –6 mm from bregma (Paxinos and Watson, 1998). With a random start between slices 1 and 20 from the series, every 17th slice was selected for immunostaining, totaling four sections per animal.
4.4. Doublecortin immunohistochemistry

Immunostaining was performed with free-floating slices. Slices were washed with phosphate-buffered saline (0.1 M PBS, pH 7.4) then immersed in a 3% hydrogen peroxide and 20% methanol in PBS solution, washed in PBS and incubated for 48 h at 4 °C with the α-doublecortin antibody (1 μg/ml; Santa Cruz Biotechnology, cat. no. sc-8067) diluted in a blocking solution of 3% nonfat donkey serum in PBS. Slices were washed in PBS and in 0.05% Tween 20 in PBS for 15 min before incubation with donkey α-goat biotinylated antibody for 1 h at RT (Chemicon, cat. no. AP180B, Temecula, CA, USA) diluted in the blocking solution. Slices were washed in PBS before incubation with ABC Elite kit for 1 h (Vector Labs, Burlingame, CA, USA), washed again in PBS and stained with diaminobenzidine (DAB) or DAB–Nickel solution through peroxidase reaction.

4.5. Stereological analyses

Slides containing the immunostained sections were positioned in a Olympus BX60 microscope with a motorized stage (Ludl 2002, Hawthorne, NY, USA) and a CCD camera (Optronics DEI-750, Goleta, CA, USA) connected to a computer running the Stereo Investigator software (Microbrightfield, Williston, VT, USA). The optical fractionator method (West et al., 1991) was employed to estimate the total number of DCX+ granular neurons in the hippocampus. The granular cell layer (GCL) and subgranular zone (SGZ) of the dentate gyrus were delineated with the tracing function of the Stereo Investigator software using a 10× magnification objective (0.4 NA). A preliminary population estimate was performed to estimate the desired number of counting sites per slice. The counting parameters were set to a counting frame of 40×40 μm distributed in a randomly positioned lattice of 150×150 μm. Since a mean thickness of 20 μm of mounted tissue was measured, guard zones of 5 μm at the top and bottom of slice were employed with a dissector depth of 10 μm. The DCX+ neuronal counting was performed in each of the counting frames within the disector depth with an oil immersion 100× magnification objective lens (1.35 NA).

4.6. Dendritic morphology analyses

For morphological analyses of the DCX+ dendritic trees the Neurolucida software (Microbrightfield) was used in the same microscopy setup employed for the stereological studies described above. Forty neurons from the DG-free blade (infrapyramidal) were traced in their entirety, twenty neurons from the control group of animals (n = 7) and twenty neurons from the pilocarpine-treated animals (n = 7). Cells were chosen for reconstruction using the following criteria: clear somatic boundaries, presence of a developed apical dendritic tree and absence of severed dendrites.

The coordinate files generated by the three-dimensional reconstruction were analyzed in the Neuroexplorer component of the Neurolucida software, generating data of morphological measurements like cell body perimeter and area, dendritic length and number of nodes and endings. The length for each dendrite branch (indicated by bifurcations) was measured for each order away from the cell body (Robinson and Kolb, 1999).

The distance from the DCX+ soma to the transition between the GCL and the inner molecular layer was measured for each of the reconstructed cells. The concentric spheres analysis of Sholl (1953) was performed to measure the branching pattern of the dendritic growth away from the soma. The first sphere has the radius equal to the GCL thickness measured for each DCX+ neuron followed by spheres with increments of 50 μm in their radii.

4.7. neo-Timm autometallography

Neo-Timm staining was performed to identify ionic zinc stored in synaptic vesicles at glutamaticergic terminals according to Danscher et al. (2004). Briefly, sections were immersed in 0.1% sodium sulfide in 50 mM PBS solution for 48 h at 4 °C before being immersed in developer solution for about 1.5 h in darkness. The developing solution consisted of 80 ml of 50% filtered arabic gum, 15 ml of 2 M sodium citrate buffer (pH 3.5), 20 ml of 0.38 M hydroquinone, and 0.5 ml of 1 M silver nitrate solution. After removal from the developer, sections were washed in tap water, and rinsed in distilled water. After washing, sections were dehydrated in sequential ethanol, immersed in xylene and coverslipped with Canadian balsam.

The scoring method for measuring mossy fiber sprouting with neo-Timm histochemistry of Cavazos et al. (1991) was used to quantify silver granule precipitation in the inner molecular layer of the hippocampal DG. The scoring varies from 0: no granules between the tips and crest of the DG, to 5: confluent dense laminar band of granules in the supragranular region that extends into the inner molecular layer. Two blind and independent observers evaluated the mossy fiber sprouting in two slices per animal at positions −2.5 and −5 mm from bregma. When present, the sprouting band thickness was measured with the Stereo Investigator software.

4.8. Statistical analyses

The experimental groups were compared with Student’s t-test for data with normal distributions and Mann–Whitney Wilcoxon rank sum test for non-normal distribution data, values were considered statistically different between groups if p<0.05. Values are expressed as mean ±SEM.

Acknowledgments

This work was financially supported by CAPES, FAPESP, PRONEX, and CNPq. The abstract form of this work was awarded the Aristides Leão Prize in basic research by the Brazilian League Against Epilepsy (supported by Novartis). We thank Maira Licia Foresti and Maria Luiza C. Dal-Cól for helping with the animals care. G.M.A. holds a CAPES PhD Fellowship (Brazil).

REFERENCES

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 9), 51–60.


