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# Behavioral and structural changes in the hippocampus of wistar epileptic rats are minimized by acupuncture associated or not with phenobarbital

[*Alterações comportamentais e estruturais do hipocampo de ratos Wistar epilépticos são minimizadas pela acupuntura em associação ou não com fenobarbital*]

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## ABSTRACT

The aim of this study was to analyze the behavior and histopathological changes in the hippocampus of epileptic Wistar rats treated with acupuncture associated or not with phenobarbital. The experiment used 44 male rats with 90 days of birth, induced to status epileptics with pilocarpine hydrochloride in a single dose of 350mg/kg, separated into treatment groups and submitted for 5 minutes to the elevated plus-maze test. Group 1 received 0.2mL of saline solution orally; Group 2 treated with acupuncture at the yintang, baihui, shishencong, jizhong, naohu, thianzu points; Group 3 received orally phenobarbital, daily dose of 20mg/kg; Group 4 treated with an association of acupuncture and oral phenobarbital; Group 5 random needling. The results obtained showed that Groups 2 (acupuncture) and 4 (acupuncture and phenobarbital) presented decreased anxiety, epileptic seizures, and neuronal death in the CA1, CA3 areas of the hippocampus when compared to animals in groups 1, 3 and 5. It is concluded that the association of phenobarbital and acupuncture points used in the experiment allowed for the control of epileptic seizures, reduction of anxiety and reduction of lesions in the subareas of the hippocampus.

Keywords: acupoints, neuronal death, neuropathology, pilocarpine

## RESUMO

*O objetivo deste estudo foi analisar o comportamento e as alterações histopatológicas no hipocampo de ratos Wistar epilépticos tratados com acupuntura associada ou não a fenobarbital. O experimento utilizou 44 ratos machos, com 90 dias de nascimento, induzidos ao* status epileticus *com cloridrato de pilocarpina, em dose única de 350mg/kg, separados em grupos de tratamento e submetidos por cinco minutos ao teste de labirinto em cruz elevado. O grupo 1 recebeu, por via oral, 0,2mL de solução salina; o grupo 2 foi tratado com acupuntura nos pontos yintang, baihui, shishencong, jizhong, naohu, thianzu; o grupo 3 recebeu, por via oral, fenobarbital, dose diária de 20mg/kg; o grupo 4 foi tratado com associação acupuntura e fenobarbital por via oral; o grupo 5 recebeu agulhamento aleatório. Os resultados obtidos demonstraram que os grupos 2 (acupuntura) e 4 (acupuntura e fenobarbital) apresentaram diminuição da ansiedade, das crises epilépticas e da morte neuronal nas áreas CA1, CA3 do hipocampo quando comparados aos animais dos grupos 1, 3 e 5. Conclui-se que a associação do fenobarbital e dos pontos de acupuntura utilizados no experimento permitiu o controle das crises epilépticas, a redução da ansiedade e a diminuição das lesões nas subáreas do hipocampo.*

*Palavras-chave: acupontos, morte neuronal, neuropatologia, pilocarpina*

## INTRODUCTION

Epilepsy is a neurological disorder characterized by chronic, unconscious, recurrent seizures generated by synchronous, spontaneous, and excitable brain hyperactivity. It is estimated that 0.5 to 1% of the world population is affected by some epileptic crisis (Pauli *et al*., 2017). Temporal Lobe Epilepsy (TLE) is the most prevalent, comprising about 40% of all cases reported in the 20th century (Fernandes, 2013).

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Temporal lobe epileptic seizures may occur due to lesions in the hippocampus, striatum, amygdala, piriform cortex, entorhinal cortex, septal area, thalamus, and substantia nigra. The hippocampal formation consists of the hippocampus, dentate gyrus, subicular complex and the entorhinal cortex, with a trilaminate internal organization composed of two main cell types: the granular cells of the dentate gyrus, containing the dentate fascia and hilum, and cells pyramids of the Ammon's Horn (CA), divided into the sectors of CA1, CA2 and CA3 (Kivisaari *et al*., 2013).

The experimental models of status epileptics induction using animals were developed to improve the understanding of the physiology of the central nervous system (CNS) and the structures involved in epileptogenesis (Covolan and Mello, 2000). The pilocarpine hydrochloride model triggers neurophysiological processes and behavioral and histopathological changes in Wistar rats that are similar to TLE affecting humans (Cavalheiro *et al*., 1994; Kandratavicius *et al*., 2014).

The control of myoclonic and behavioral crises is a challenge for medicine, as cases refractory to anticonvulsant treatments are frequent in patients with TLE, which makes it a worldwide public health problem, as epileptics have a high rate of trauma physical and emotional in addition to the predisposition to develop anxiety, depression, suicide, and sudden deaths (Fisher *et al*., 2014).

Assuming that uncontrolled epileptic seizures cause injuries in areas of the hippocampus of Wistar rats, the purpose of this study was to analyze the behavior and changes in the hippocampus of epileptic rats that underwent acupuncture treatment as a single therapy or in combination with phenobarbital. The acupoints considered in the present study were Yintang (Ext 5), Baihui (DU 20), Sishencong (Ext 1), Jizhong (DU 6), Naohu (DU17) and Thianzu (BL10).

## MATERIAL AND METHODS

The experiment was done in the vivarium of the Department of Animal Morphology and Physiology and in the Laboratory of Animal Pathology of the Department of Veterinary Medicine. We received license and approval from the Animal Use Ethics Committee (CEUA) of the Federal Rural University of Pernambuco, process number 23082.012694/2018-06.

The subjects of the experiment were 44 Rattus norvegicus Albinus, Wistar lineage, males 90 days old, kept in a 12-hour light/dark cycle, with controlled temperature (20 – 24 °C) with free access to water and commercial food.

The experiment was carried out in three stages. In the first stage, the animals were adapted to experimental handling, weighing, and then separated into five groups according to treatments. Group 1, called control, treated with saline solution; group 2 with acupuncture; group 3 with phenobarbital; group 4 with association of acupuncture and phenobarbital; and group 5 with random needle application.

The second step was the subcutaneous application of pilocarpine hydrochloride 4% at a dose of 350mg/kg with a hypodermic needle (25×5mm) in the dorsolateral region of the neck and shoulder. Forty-five minutes after application, all animals entered the epileptic status, remaining in crises for 3 hours, followed by clinical prostration. For this reason, the animals were hydrated with 0.9% NaCl, subcutaneously, until complete recovery. Twenty-four hours after epilepsy induction, the animals presented a normal clinical picture, without the occurrence of seizures or behavioral changes, remaining so for 16 days. On the sixteenth day, the spontaneous chronic epileptic seizures started with the presence of irritability, aggressiveness and generalized and focal myoclonic seizures. The third stage, characterized by the appearance of recurrent, spontaneous chronic epileptic seizures and the beginning of treatment protocols for 90 days (Table 1).

The rats' anxiety behavior was analyzed through the application of the elevated plus-maze test (EPM). For data analysis in the EPM test, the percentage of entries in the open and the closed arms was calculated. The quantification of seizures was performed by visual observation following the Racine 1972 scale (Itzhak and Martin, 2000).

Table 1. Treatment protocol for experimental groups

| Groups | Treatment | Dosage / Acupoints | Via | Time |
|---|---|---|---|---|
| 1 | Saline solution | 0,2 ml | Oral | Every 24 hours |
| 2 | Acupuncture | Ext 1/ Ext 5/ DU 20/ DU 6/ DU 17/ BL10 | Topic | 10 min 3 times a week |
| 3 | Phenobarbital | 20 mg/kg | Oral | Every 24 hours |
| 4 | Phenobarbital | 20 mg/kg | Oral | Every 24 hours |
| | Acupuncture | Ext 1/ Ext 5/ DU 20/ DU 6/ DU 17/ BL10 | Topic | 10 min 3 times a week |
| 5 | Random needling | ********** | Topic | 10 min 3 times a week |

Source: Research data.

The acupuncture technique used in the experiment was dry needling, characterized by the stimulation of acupuncture points through the application of filiform needles of size 0.18 × 8 mm at a depth of 0.1 mm for 10 minutes, three times a week. The acupoints used were the yintang (Ex. 5), an extra point located on the forehead between the region corresponding to the eyebrows, with an energetic therapeutic indication to calm and enhance the other points used. Shishencong (Ext. 1), an extra stitch that brings together four cross-positioned stitches located on top of the head with the function of calming the mind, treating restlessness, sleep disturbances, dizziness, forgetfulness, epilepsy, hemiplegia, memory loss, anxiety, and depression. Baihui (DU 20), an extraordinary governing meridian point (Du Mai) with the energetic function of balancing emotions, clearing the mind, and promoting brain functions, located on the top of the head between the lines joining the apex of the ear. Jizhong (DU 6), an extraordinary point on the Du Mai meridian, indicated for the treatment of convulsions and relief from prostration, located in the midline of the back in the 11th rib region. Naohu (DU 17), an extraordinary point indicated for calming convulsive states, reducing fever, and improving visual acuity, located in the posterior midline of the upper margin of the occipital bulge, also belongs to the Du Mai meridian. Tianzhu (BL 10), a bladder meridian point that has the function of energetically nourishing the brain, indicated for the treatment of epilepsy and Parkinson's disease, located 1.3 cm lateral to the midline, at the height of the spinous process of the 1st cervical (C1), close to the trapezius insertion (Figure 1).

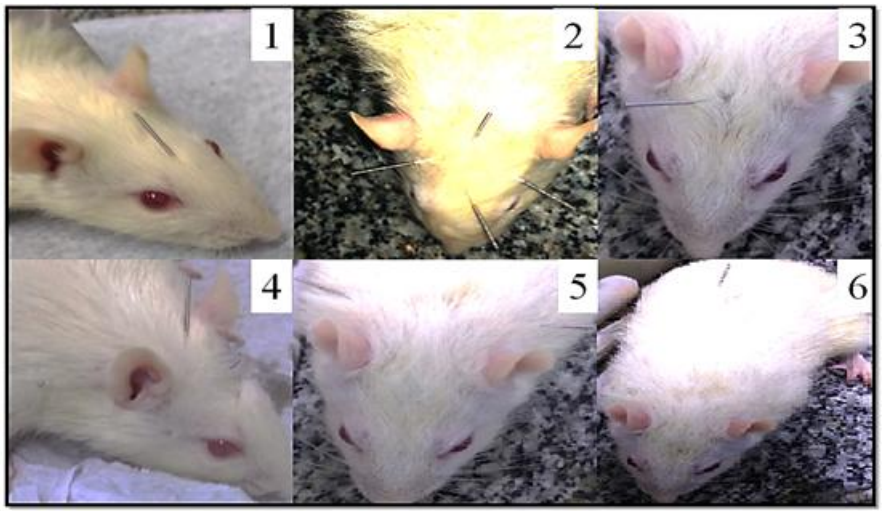


Figure 1. Image of acupoints used according to location and placement order: 1-Yintang (Ext 5); 2- Sishencong (Ext 1); 3- Baihui (DU 20); 4- Naohu (DU 17); 5- Tianzhu (BL10); 6- Jizhong (DU 6).

After the end of the experimental treatments, the animals were weighed and anesthetized intramuscularly with an association of ketamine (50 mg / kg) and xylazine (20 mg / kg) in the same syringe (Massoni, 2011). They were sacrificed by cardiac puncture, and the brain collected for histological analysis. Brains fixed in 4% formaldehyde in a sodium phosphate buffer solution (pH 7.2 and 0.01 ml) for 48 hours and processed with a routine protocol for embedding in paraffin (blocking).

After 24 hours of processing, 4 µm-thick coronal histopathological sections were made in a Leica ® rotary microtome, then the sections were adhered to the histological slides and placed in an oven for 12 hours to dry the slides. Slides were routinely processed and stained with hematoxylin- eosin for visualization of the hippocampus. Histopathological analyzes were performed using a Leica DM 500 microscope, where photomicrography was performed with 10X and 40X objectives for morphometric analysis of the hippocampal subareas. The statistical analysis of the data obtained in the morphometry of the injured areas in the hippocampus was obtained using the Kruskal-Wallis test followed by Dunn's Post Hoc test.

## RESULTS

The clinical evaluation demonstrated that the animals that received stimulation with the acupuncture technique (G2 and G4) did not present aggression and had fewer convulsive episodes. At the same time, G1 and G5 remained with generalized seizures and intense aggression. Animals treated with phenobarbital (G3) presented control of epileptic seizures, but they remained aggressive throughout the treatment. Animals treated with acupuncture associated with phenobarbital presented behavior control and reduced epileptic seizures (Figure 2).

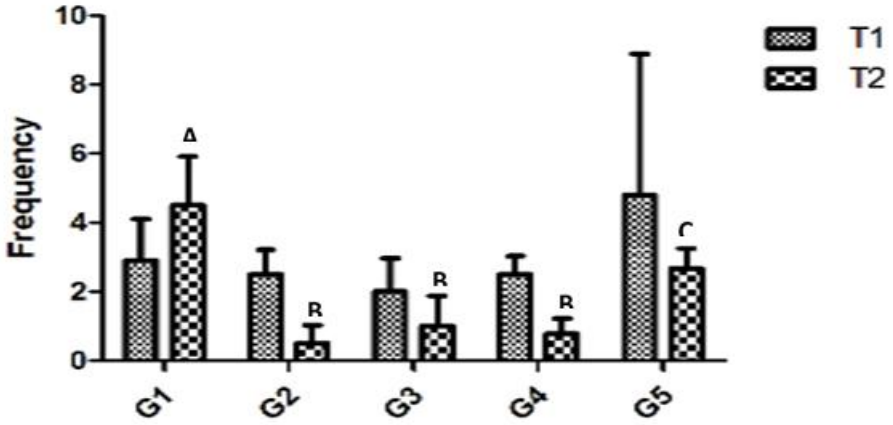


Figure 2- Mean of epileptic seizures in rats in the groups before the start of treatments (T1) and after 90 days of treatment (T2).

The EPM behavioral evaluation showed G2 and G4 exploring the open arms (OpA) for a longer time, while G1 and G5 stayed longer in the closed arm (CLA), and G3 was permanently in the open arms, but without lifting the body. These results indicate that the level of anxiety decreased in the groups treated with acupuncture and with acupuncture associated with phenobarbital. However, the animals showed active behavioral attitudes in the group only treated with acupuncture, as they explored the open arm and voluntarily lifted the body (Table 2).

Thus, it was possible to verify that both the use of acupuncture and phenobarbital were effective in reducing the level of stress and, consequently, the level of anxiety. When comparing the treatment groups to each other, the animals in the acupuncture-only group showed much lower levels of anxiety during the elevated plus-maze test.

Table 2. Values of the elevated plus-maze test of the experimental groups expressed as mean and standard deviation

| | Cruz Maze Test | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BP | | | | AP | | | | 90 days AT | | | |
| Treatments | OpA | CLA | Mean Difference | P value | OpA | CLA | Mean Difference | P value | OpA | CLA | Mean Difference | P value |
| Control | 1.40± 0.97 | 3.60± 0.97 | -2.200 | p<0,001 | 0.10± 0,32 | 4.9± 0.32 | -4.800 | p<0.001 | 0.00± 0.00 | 5.00± 0.00 | -5.000 | p<0.001 |
| Acupuncture | 0.90± 0.74 | 4.10± 0.74 | -3200 | p<0,001 | 0.40± 0.70 | 4.60± 0,7 | -4.200 | p<0.001 | 4.60± 0.70 | 0.40± 0.70 | 4.200 | p<0.001 |
| Phenobarbital | 0.80± 0.79 | 4.20± 0.79 | -3.400 | p<0,001 | 0.30± 0.48 | 4.70± 0.48 | -4.400 | p<0.001 | 2.00± 1.25 | 2.9± 1.29 | - 0.9000 | p>0.05 |
| Acupuncture +Phenobarbital | 1.10± 0.99 | 3.90± 0.99 | -2.800 | p<0,001 | 0.50± 0.53 | 4.50± 0.53 | -4.000 | p<0.001 | 4.10± 1.37 | 0.90± 1.37 | 3.200 | p<0.001 |
| Randomly | 1.01± 0.58 | 3.56± 1.27 | -3.000 | P<0.001 | 0.58± 0.45 | 4.00± 1.60 | -4.250 | p<0.001 | 1.08± 1.59 | 3.55± 1.91 | -5.000 | p<0.001 |

The histopathological study showed that the injuries observed varied in intensity and location, depending on the treatment used. Acupuncture sessions provided maintenance of the thickness of the pyramidal neuron layer in the CA1 area and a higher proportion of healthy neurons in the CA2, and phenobarbital minimized hippocampal injuries in areas CA2 and CA3. The hippocampus of G1 rats, the control group treated with saline solution, suffered a neuronal loss in areas CA1, CA2, CA3, and Dentate gyrus (GD). In the CA1, the presence of red neurons (necrosis) denotes a great death of neurons that resulted in a reduction in the thickness of the trilaminar layer of pyramidal cells. Areas CA2, CA3, and GD had dead neurons, and CA3 had vacuolization of the neuropil (Figure 3).

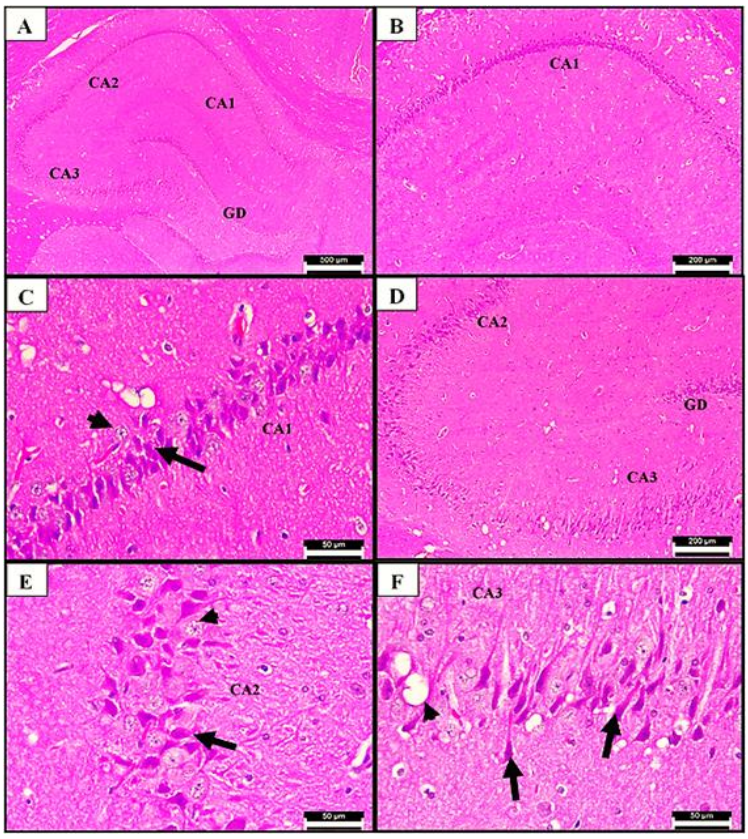


Figure 3. Coronal section of epileptic Wistar rat brain (hippocampus) treated with saline solution. H&E - **A-** Areas CA1, CA2, CA3 and Dentate gyrus (GD) **B** –Note thickness reduction in Area CA1 due to neuronal death. **C-** Note in the CA1 area a whole neuron (arrowhead) and several red neurons (arrow). **D** - Areas CA2 and CA3 and GD with the presence of dead neurons. **E-** Area CA2. Note whole neuron (arrowhead) and neuron in the process of death (arrow). **F** - Area CA3. Note dead neurons (arrow) and vacuolization of the neuropile (arrowhead).

In Group 2, the hippocampus of rats treated with acupuncture presented a more preserved hippocampal areas (CA1, CA2, CA3, and GD), with CA1 maintaining the thickness of the pyramidal neuron layer and the absence of red neurons. In CA2, it was observed the presence of several healthy neurons, with red neurons in the CA3 area (Figure 4).

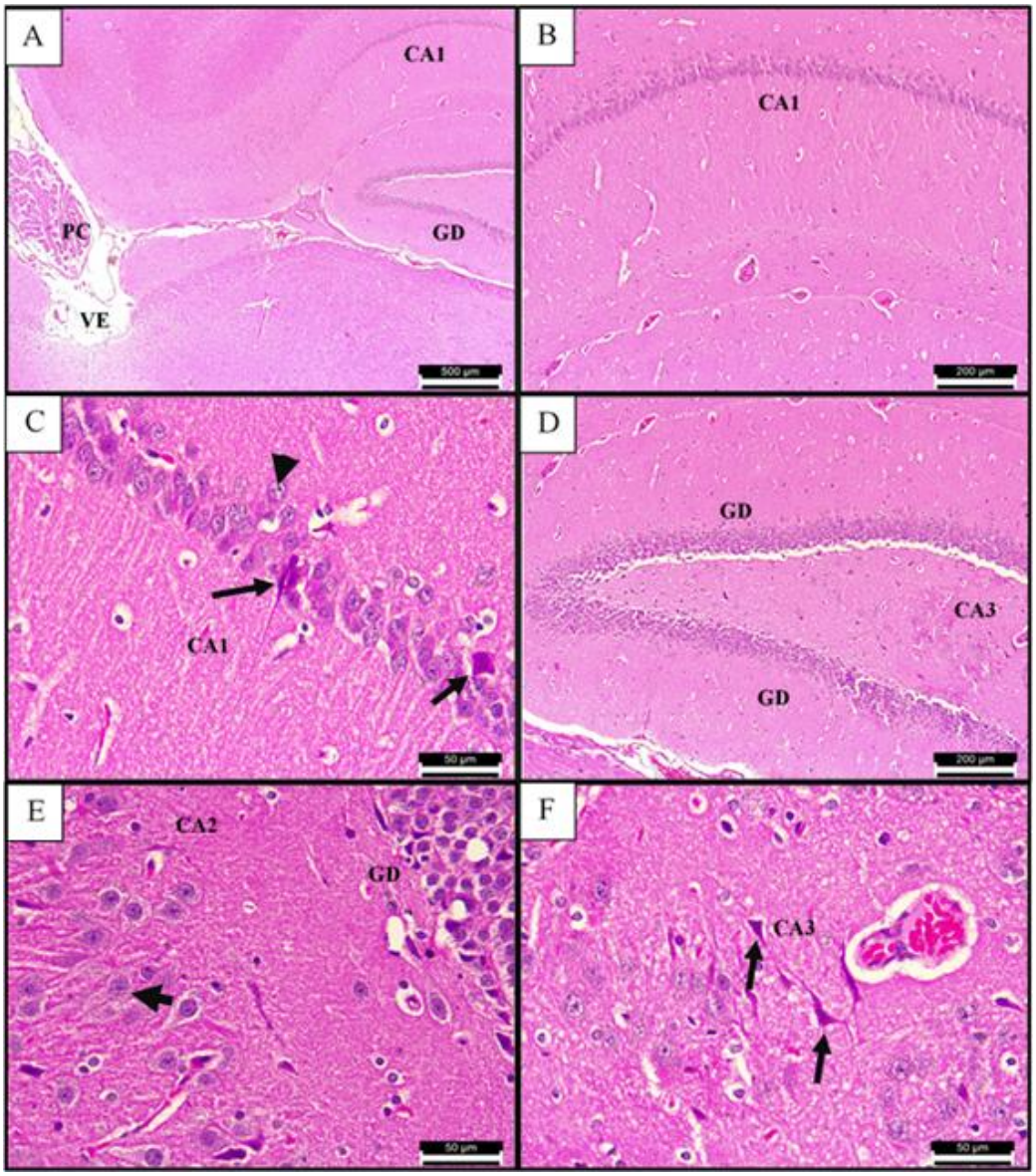


Figure 4. Coronal section of epileptic Wistar rat brain (hippocampus) treated with acupuncture. H & E- **A** - Brain ventricle containing choroid plexus (CP). Hippocampus - Note the area CA1 and Gyrus Dentate. **B** - Hippocampus - Area CA1- Note the maintenance of the thickness of the neuron layer, absence of red neurons. **C**- Detail of neurons in the CA1 Area of the Hippocampus. Note dead neurons (arrow) and healthy neurons (arrowhead). **D**- Dentate gyrus area and hippocampus CA3 area. **E** - Area CA2 to notice intact neurons (short arrow). Dentate gyrus (GD) **F.** Detail of the CA3 area - red neurons (arrow).

Few red neurons observed in the CA2 and CA3 areas of hippocampus of the G3 animals. Most neurons were viable in these areas, with neurons in the process of death and vacuolization of the neuropile being noticed in the CA2 area. In the CA3 area, many viable neurons were observed (Figure 5).

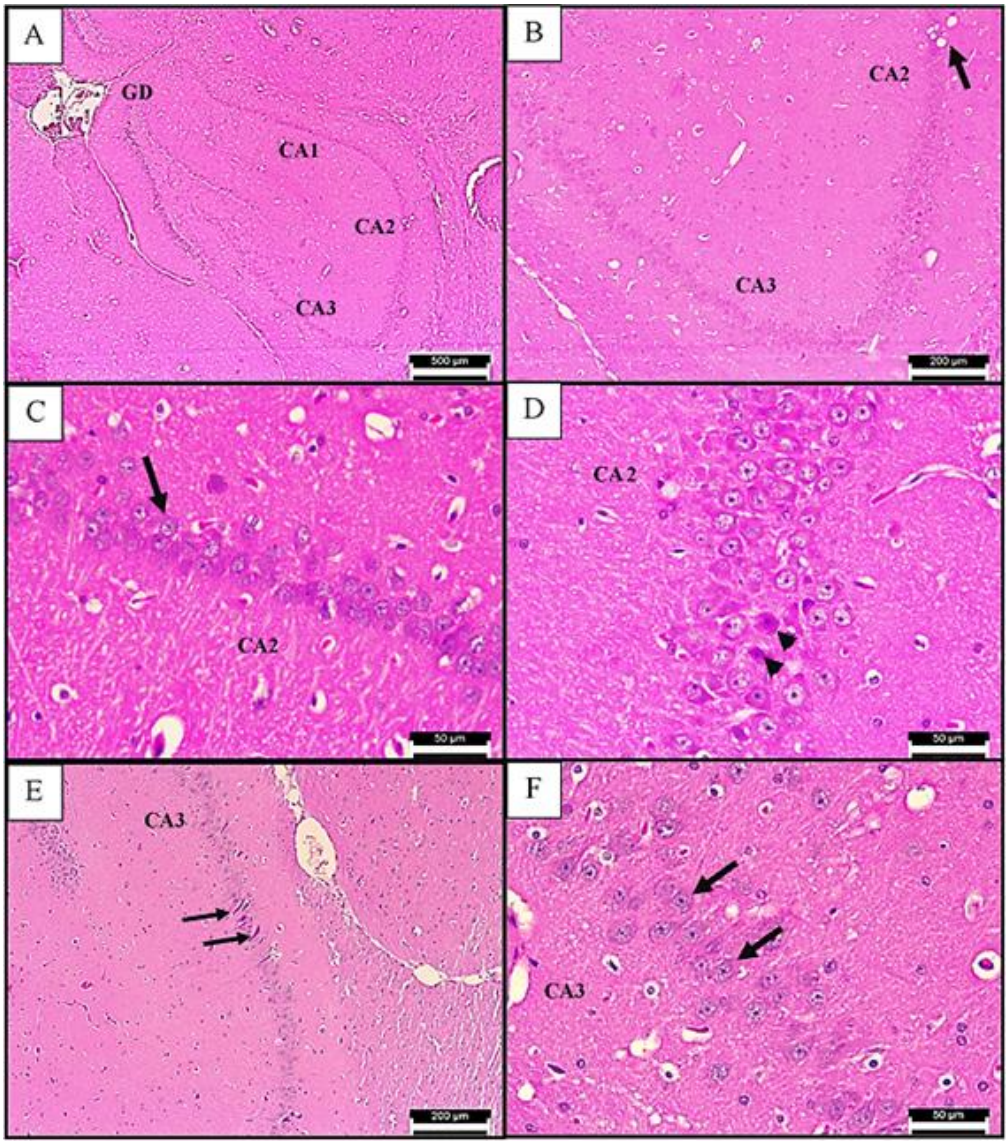


Figure 5. Coronal section of epileptic Wistar rat brain (hippocampus) treated with phenobarbital. H & E- **A** - Hippocampus - Areas CA1, CA2, CA3 and Giro Denteado. **B** - Area CA2 and CA3 with few red neurons and most viable neurons. Note vacuolation of neuropila (arrow). **C** - Area CA2 - viable neuron (arrow). **D**- Area CA2 neuron in process of death (arrowhead). **E** - Area CA3 - Note dead neurons (arrow). **F** - Area CA3 presence of intact neurons (arrow).

The association of acupuncture and phenobarbital (G4) provided maintenance of the thickness of the neuron layer in the CA1, CA2, and CA3 area with a large presence of intact neurons. However, it was noticed dead neurons in the CA2 and GD area; and in the CA3 area, the presence of red and neurons in the process of cell death (Figure 6).

The epileptic rats submitted to needling (Group 5) had intense neuronal death in the areas CA1, CA2, CA3, and GD. The reduction in the thickness of the neuron layer in the CA1 area is compatible with numerous red neurons, denoting a process of necrosis. The CA3 area contained numerous dead neurons (Figure 7).

According to the morphometric assessment of the hippocampal areas, neuronal death was more frequent in the untreated groups and the subareas CA1, CA2, and CA3, with the dentate gyrus

region having the lowest percentage of neuronal death when compared to all other groups.

The percentage of neuron death in subarea CA1 was significantly different ($p < 0.05$) between G1 and G2, G3 and G4; and between G2 and G5. In subarea CA2, there was a significant difference in the frequency and intensity of dead neurons ($p < 0.05$) between G1, G2 and G4. Neuronal death in G4 was less intense than in G1 and G5. The presence of dead neurons in the CA3 subarea showed a significant difference ($p < 0.05$) between G1 and G2, G3 and G4. The dentate gyrus region also showed significant differences ($p < 0.05$) between G1 and G4 (Figure 8).

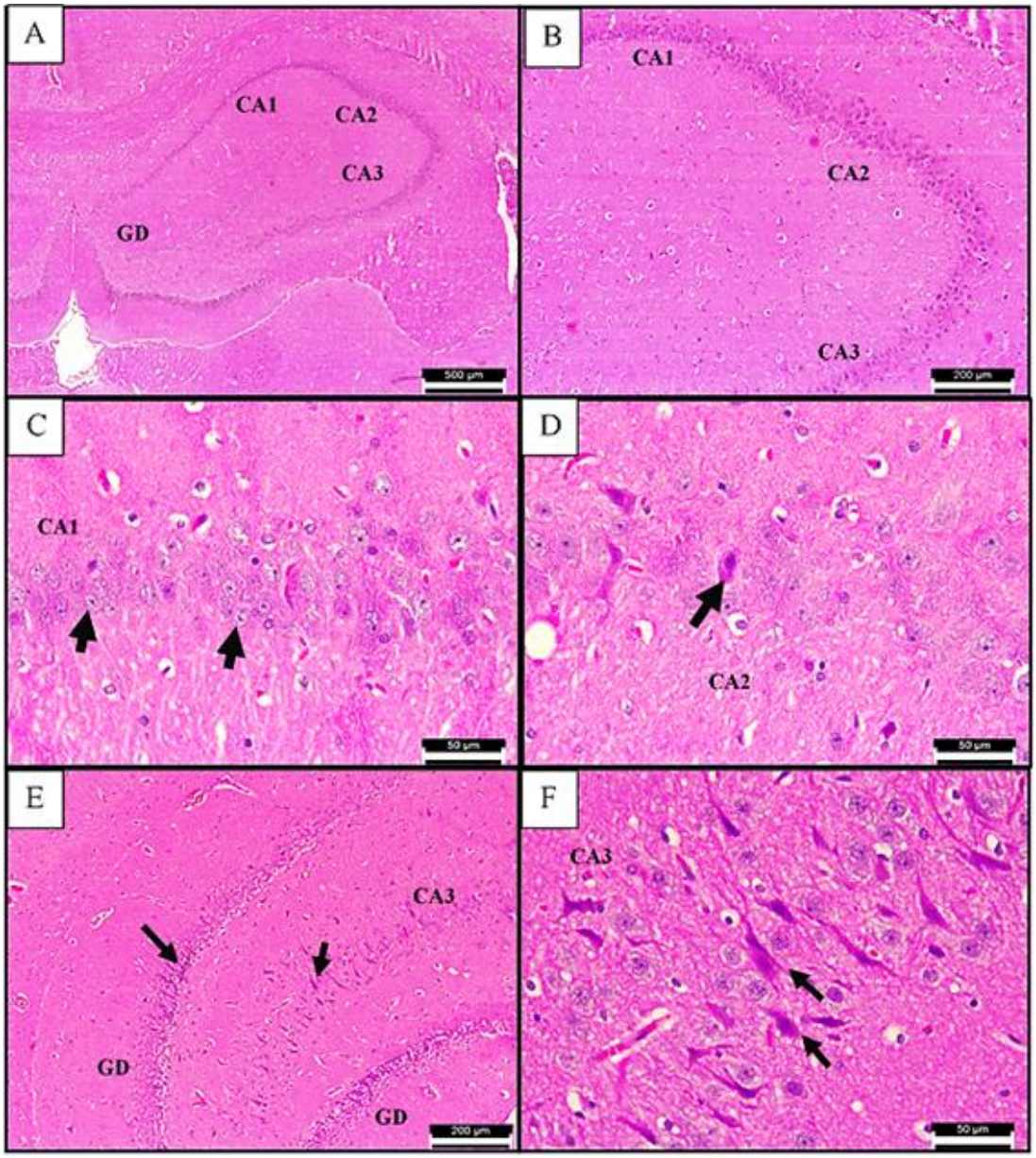


Figure 6. Coronal section of brain (hippocampus) of induced Wistar rat epileptics treated with association of acupuncture with phenobarbital. H & E- **A** - Hippocampus - Areas CA1, CA2, CA3 and GD (Jagged Gyrus). **B** - Area CA1, CA2 and CA3 note the maintenance of the thickness of the neuron layer. **C**- Detail of neurons in the CA1 area of the Hippocampus; healthy neurons (arrowhead). **D** - Area CA2, notice red neurons (arrow). **E** - Note red neurons in the GD (Arrow) and in the CA3 area of the hippocampus (short arrow). **F** - In detail, observe neurons in the process of cell death in the CA3 area of the hippocampus (arrow).

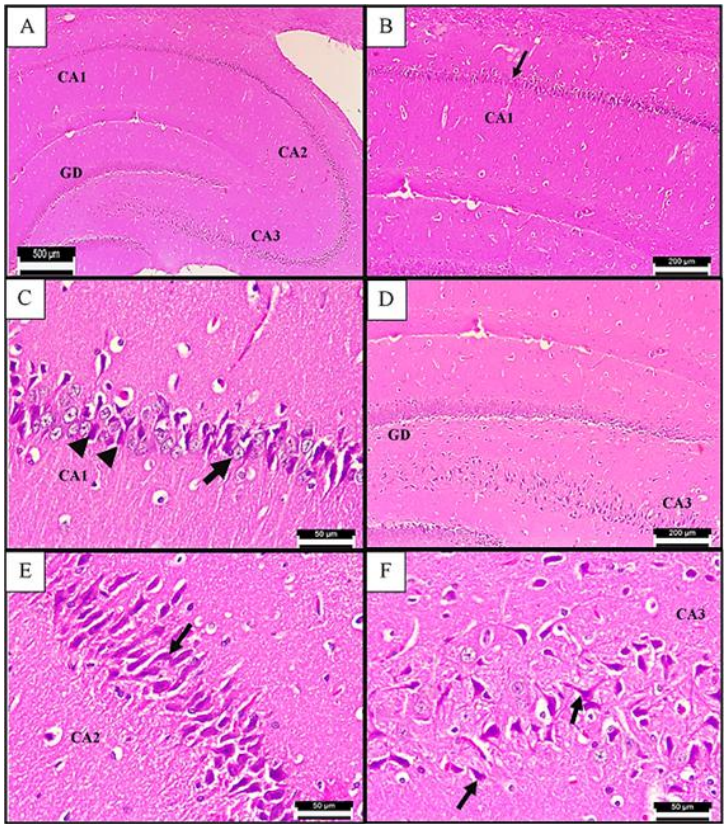


Figure 7. Coronal section of epileptic Wistar rat brain (hippocampus) subjected to random needling. H & E- A - Hippocampus - Areas CA1, CA2, CA3 and GD (Jagged Gyrus). B - Area CA1 observe a reduction in the thickness of the neuron layer (arrow). C - Detail of neurons in the CA1 Area of the Hippocampus. Note healthy neurons (arrow) and red neurons (arrowhead; necrosis). D - Area CA3 and GD. E - Detail of area CA2. Notice dead neuron (arrow). F - Detail of area CA3. Note dead neurons (arrow).

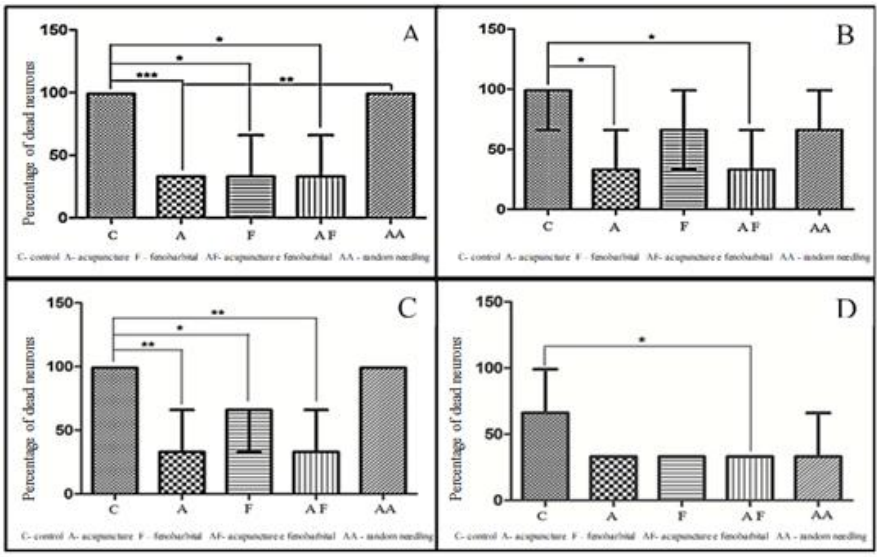


Figure 8. Frequency and intensity of dead neurons in hippocampal subfields of groups of epileptic Wistar rats in relation to acupuncture (A), phenobarbital (F), saline (C) and random needle (AA) treatments. Graph A- Showing in the hippocampal region CA1 statistically significant difference between the treatment groups in the proportion of dead neurons in terms of frequency and intensity, where * for $p < 0.05$ ; ** for $p < 0.01$ and *** for $p < 0.001$. Graph B- CA2 subarea region showing significant difference where *P<0.05 between control and acupuncture and control and acupuncture associated with phenobarbital. Graph C- Demonstrates significant differences in the CA3 region for *p<0.05 between the control and phenobarbital groups and **p<0.01 between the control and acupuncture groups, and control and acupuncture associated with phenobarbital. Graph D- Region of the dentate gyrus the significant difference of *p<0.05 between the control and acupuncture groups associated with phenobarbital.

## DISCUSSION

The results obtained in this study showed that all animals developed temporal lobe epilepsy (ELT) after induction to status epileptics, causing injuries in areas of the hippocampus, with neuronal death. Similar results described in experiments performed by Cavalheiro *et al*. (2006).

Pizzutto *et al*. (2009) described that the behavioral tests for simulating the natural behavior of challenges inherent to the species are a good instrument to define the level of anxiety of studied species. Attarian *et al*. (2003) reported that the behavior of active avoidance and the exploitation or immobility that rat's exhibit is natural in rodents, as part of the instinct for preservation. In the present study, acupuncture treatment of animals in epilepticus provided normal behavior due to the reduction in the level of anxiety. On the other hand, the immobility time observed in rats in status epilepticus, without treatment, is related to anxiety, depression, and the lack of performance in this species and humans (Krishnakumar *et al*., 2009).

Models of ELT reproduce most of the clinical and neuropathological characteristics in humans (for example, loss of cells in the hippocampus, sprouting of mossy fibers, and dispersion of cells in the dentate gyrus) (Ben-Ari *et al*., 1981; Turski *et al*., 1983). In some of these models, neuronal loss is not restricted to the sclerotic hippocampus and occurs in the parahippocampal cortical regions, thalamus, end pyriform cortex, and amygdala (Cardoso *et al*., 2011; Inostroza *et al*., 2011). At least some of these areas, such as the amygdala, are involved in cognitive deficits and emotional dysfunctions (Phelps and Ledoux, 2005; Balleine and Killcross, 2006). However, according to Inostroza *et al*. (2012), altered emotional behaviors are not inherent to the epileptic condition in the experimental ELT; instead, they probably reflect changes in anxiety levels related to HPA axis-dependent deregulation.

According to Houser (1990) and El Bahh *et al*. (1999), hippocampal sclerosis and granular cells' dispersion occur in refractory epileptic seizures. Meldrum and Bruton (1992) observed that in ELT cases, the occurrence of hippocampal sclerosis was more intense in the pyramidal neurons of the CA1, CA3 areas, as was observed in the results found in this experimental study.

Houser (1990) and El Bahh *et al*. (1999) related neuronal death to the presence of excess intracellular calcium due to uninterrupted neuronal excitation caused by seizures. This corroborates the results observed in the hippocampus of untreated animals (G1 and G5) affected by uncontrollable epileptic seizures, resulting in intense neuronal death in the hippocampus, as described by Cavalheiro *et al*. (2006).

Fisher *et al*. (1998) described that untreated seizures could lead to an increase in necrotic areas in the hippocampus, as observed in the hippocampal subarea CA3 of the animals belonging to the control group (G1) and random needling (G5). Meldrum and Bruton (1992), Fisher *et al*. (1998), and Covolan and Mello (2000) stated that temporal lobe epilepsy leads to hippocampal sclerosis with the death of pyramidal neurons in the CA1, CA3 areas, and neurons of the dentate hilum, being more resistant to neuronal damage, the pyramidal neurons of the CA2 area and granular teeth (GD).

Turski *et al*. (1983) described that the subareas CA1, CA2, and dentate gyrus (DG) present intense neuronal destruction in epileptic Wistar rats without seizure control. It suggests that seizure control directly influences the intensity of neuronal death, as observed in the hippocampus of rats that did not receive treatment, which remained with uninterrupted neuronal excitation. According to studies described by Houser (1990) and El Bahh *et al*. (1999), this pattern can be justified due to hyperactive excitability and hyperactive synchronicity in the action potential (AP) in the synaptic clefts.

The quick removal of glutamate from the synapse by its transporters is necessary for normal excitatory neurotransmission and the prevention of induced glutamate toxicity (Yernool *et al*., 2004). The mechanism of so-called excitotoxicity is related to ischemic diseases, chronic neurodegenerative diseases, and acute neurodegenerative diseases, such as epilepsy, hypoxia, anoxia, and head trauma (Grewer and Rauen, 2005).

Siesjo *et al*. (1986) correlated the CA3 area injuries with the release of excitatory amino acids that interact with calcium channels, promoting the influx of this ion and altering intracellular homeostasis. The rats that were treated with phenobarbital contained fewer CA3 injuries in the hippocampus. Phenobarbital acts on rats' nervous system, potentiating synaptic inhibition mediated by GABA and blocking calcium channels in neurons exercising control of myoclonic epileptic seizures (Ahir and Pratten, 2014).

According to Mello *et al*. (1992), neurons in the subarea CA2 and GD are relatively more resistant to neuronal damage. However, in the experiment, it was noted that only animals that received acupuncture treatment as a single therapy or in association with phenobarbital had a lower intensity of neuronal death in CA2 and DG. The animals that received only acupuncture treatment had 37.83% of neurons killed in the neuronal population analysis in the regions CA1, CA2, and CA3, with the lowest percentage of neuron death among all the sub-areas of the groups studied.

The injuries found in the brain of rats treated with acupuncture were less intense, suggesting that the excessive release of neurotransmitters was minimized by the treatment, as described by Lin *et al*. (2014). According to these authors, stimulation of acupoints can activate the body's natural neuroprotective mechanisms that lead to neuronal homeostasis formation.

The acupuncture sessions with the Yintang, Baihui, Sishencong, Jizhong, Naohu, and Thianzu points provide control of the intensity and frequency of epileptic seizures, reflecting the maintenance of the pyramidal neuron layer thickness in the CA1 area and a higher proportion of healthy neurons in the CA2. In addition, crisis control with phenobarbital minimized hippocampal injuries in areas CA2 and CA3.

Wen *et al*. (2010) described that acupuncture influences the release, synthesis, reuptake, and degradation of central neurotransmitters and modulators. This includes monoamines, acetylcholine, amino acids, and prostaglandin, somatostatin, and neurotrophic factors in the central nervous system.

Fang *et al*. (2009) stated that acupuncture can stimulate dermal nerve terminals, activating the release of endogenous opioid peptides, neurotransmitters (e.g., serotonin, dopamine, acetylcholine neurotransmitters), gamma-aminobutyric acid (GABA), glycine, and taurine. It also attenuates the activity of norepinephrine and excitatory amino acids, including glutamate and aspartic acid, explaining the observed control of crises and neuronal integrity in the hippocampus of rats treated with acupuncture in this experiment.

## CONCLUSION

Given the results obtained in the behavioral, histopathological, and morphometric study of the hippocampus of epileptic Wistar rats, it concluded that the stimulation of yintang, baihui, shishencong, jizhong, naohu, thianzu acupoints were effective as a single therapy or in association with phenobarbital in controlling Epileptic seizures, anxiety, depression, and neuronal preservation in the experimental model of Temporal Lobe Epilepsy.

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