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Impending role of hippocampal neurogenesis in the development of chronic epilepsy following seizures after Kainic acid and Pentylenetetrazol treatment

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Abstract

The study aimed to investigate how seizures contribute to abnormalities in generating new cells in the hippocampus and to explore the potential influence that these newly formed cells might have on subsequent seizure activity and the development of chronic epilepsy.

The findings indicated that both pentylenetetrazol and kainic acid-induced status epilepticus resulted in neurodegeneration. There was an initial increase in cell proliferation shortly after kainic acid or pentylenetetrazol treatment. Notably, the initial seizures were associated with neurogenesis, as pentylenetetrazol-treated animals with a higher number of new neurons experienced seizures. Moreover, increased expression of neuronal nitric oxide synthase (nNOS) occurred shortly after seizures and persisted for up to 8 weeks. Elevated levels of NGF and BDNF were also observed shortly after status epilepticus or kindling.

Spontaneous recurrent motor seizures seemed to be primarily caused by neuronal loss and abnormal gliosis, as they were only observed in kainic acid-treated rats with increased gliosis. Kindled rats displayed a higher rate of cell proliferation after 8 weeks of kindling, possibly due to the hyper-excited state of neurons and the establishment of a new balance between excitatory and inhibitory neurotransmitters.

These results contribute to our understanding of the factors involved in the onset of seizures and the development of chronic epilepsy. Additionally, they may aid in the development of strategies for preventing and treating epilepsy. However, further investigations are necessary to explore the potential role of newly generated cells in epilepsy development.

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Introduction

Temporal lobe epilepsy (TLE), characterized by periodic and unpredictable occurrence of seizures, is difficult to manage and cure[1]. Although the aetiology of TLE is not completely understood, it is often observed after acute seizures like status epilepticus (SE), brain injury, tumours, meningitis, and encephalitis.

Spontaneous recurrent motor seizures (SRS), neurodegenerative changes in the Dentate Gyrus, CA1–CA3 subfields, aberrant synaptic reorganization, astrocyte activation, dispersion of the granule cell layer, and cell proliferation are the characteristic features of chronic TLE[2][3]. In animal models of chronic epilepsy and in humans with severe epilepsy, neurogenesis has been found to be significantly reduced[4][5][6][7]. Abnormal hippocampal neurogenesis has also emerged as one of the important characteristics in TLE, attracting many researchers. It is not yet well understood whether neurogenesis following seizure activity is a contributing factor or a protective mechanism against the development of epilepsy.

Several studies have been conducted to study how seizures induce abnormalities in hippocampal neurogenesis[6][8][9]. As neural stem cells give rise to both neuronal and glial progenitors, about fifty percent of newly born cells differentiate into neurons and fifteen percent into glial cells[7][10]. Neurogenesis and gliosis following seizures have not been compared in investigations before. Understanding how proliferating cells affect the occurrence of seizures or the emergence of persistent epilepsy is particularly significant[11][12][13][14]. Considering this gap, the present work was designed to study the mechanism of onset of seizures and how the seizures influence cell proliferation and differentiation. The role of various neurotransmitters and growth factors needs to be investigated in TLE. We thus used a kainic acid (KA)-induced status epilepticus model and a pentylenetetrazol (PTZ)-induced kindling model for the purpose of our study because these are thought to be characteristic models of the chronic epileptic system.

Materials and Methods

Experimental design and dose schedule

Two-month-old male Wistar rats, weighing 150±5 g, were used in the study. The rats were divided into three groups.

Control group: Twelve rats were selected for each parameter. Six rats received a single intraperitoneal (i.p.) injection of saline (0.9% NaCl) to serve as a comparison with KA-treated rats. Another group of six rats received eight i.p. injections of saline, three times a week, to compare them with PTZ-kindled rats.

KA group: The number of animals was kept the same as above. Rats in this group received a single i.p. injection of KA (10 mg/kg BW) and then were monitored for seizure behaviour for 10 hr after injection. Those rats that showed severe continuous tonic-clonic seizure (status epilepticus) behaviour for at least thirty minutes were used for further experimentation.

PTZ group: Number of animals was kept the same as above. Rats received repeated i.p. injections of PTZ (35 mg/kg BW), three times a week. The dosage was determined in the study by Szyndler et al[15]. After each injection, the rats were placed individually in cages and were observed for 30 min. The intensity of convulsions was recorded on a five-point scale: 0, no response; 1, ear and facial twitching; 2, myoclonic jerks without rearing; 3, myoclonic jerks, rearing; 4, turn over into side position, clonic–tonic seizures; 5, turn over into back position, generalized tonic–clonic convulsions.

Rats considered kindled exhibited stage 5 of seizures on two consecutive trials. Typically, the criterion for kindled seizures was 5-9 injections of PTZ. All rats considered as kindled presented stage 5 of seizures after the last two PTZ injections.

After completing the drug treatment, rats from the KA and PTZ groups were divided into two sets. The first set of rats from each group was perfused 48 hr after SE or kindling and examined for various parameters as described in the next paragraph. The second set of rats from each group was kept for eight weeks and then examined for similar parameters. The rats from the second set in the PTZ and KA groups were regularly monitored for seizure behaviour for at least one hour per day up to 8 weeks.

Parameters studied

After SE or kindling, neurodegenerative changes were studied by Nissl's staining, Fluorojade B staining, and the TUNEL assay. Cell proliferation and differentiation were examined immunohistochemically using anti-BrdU or GFAP (glial marker) or Calbindin (neuronal marker) antibodies. Additionally, changes in GABAergic neurons, nitrergic neurons[16], BDNF, and NGF expression were also investigated.

Cell count: Immunoperoxidase stained sections were analysed using bright field microscopy. Photographs were captured on a BX 51 microscope (Olympus, Japan) equipped with a magnifier digital camera and a computer-assisted image analysis system (Image Pro Express, Media Cybernetics, Incorporation). To ensure anatomically matched sections and consistency in counts between animals, three serial sections were examined at each -3.3, -4.3, and -5.8 coordinates[17],

as measured from bregma. Three coordinates along the rostral-caudal axis were examined to exclude any regional peculiarities that might bias results. Cells were counted in the CA1, CA2, CA3, hilar, and DG subfields with a 40X objective lens in both the right and left lobes of the hippocampus.

Statistical analysis

Results were expressed as the average number (mean±S.E.M) of BrdU-positive cells per section. Differences between means were determined by one-way analysis of variance (ANOVA) followed by Tukey's posthoc test for comparisons among all pairs of groups. The analysis was conducted using Prism software (v.6, GraphPad, La Jolla, CA, U.S.A).

Ethical considerations

Protocols were pre-approved by M.L.S. University Institutional Animal Ethics Committee (Reg. No. 973/ac/06/CPCSEA). Experiments on animals were conducted and analyzed by experimenters who were blinded to the treatment groups.

Results

Rats from KA-treated groups showed SRS, while SRS was absent in PTZ-treated group rats.

Nissl staining, Fluorojade B staining, and TUNEL assay: A large number of healthy cells with stained nuclei and nucleoli were observed in the control brain. Degenerating cells (shrunken and darkly stained cell bodies) were detected after 48 hr, primarily in the CA3, DG, and hilar regions in KA-treated brains. Dispersion of cells and distorted morphology of the DG was also observed. After 8 weeks of KA treatment, a few degenerating cells were observed compared to 48 hr KA treatment. In the PTZ-treated brain, a comparatively low number of degenerating cells was observed, and there was no change in DG morphology.

A significant increase in Fluorojade B-positive neurons was observed within the Dentate Gyrus compared to the control group, suggesting acute neuronal degeneration specific to this region in response to the treatment. Although the Dentate Gyrus appeared to be more susceptible to the treatment, with a significant number of degenerating neurons, signs of mild activation of glial cells indicating a localized neuroinflammatory response were also observed.

After an 8-week recovery period following kainic acid and PTZ treatment, partial recovery within the dentate gyrus was observed. The number of Fluorojade B-positive neurons appeared to decrease compared to the 48-hour time point, indicating that some neurons may have regenerated or repaired over time. Despite evidence of partial recovery, a notable number of degenerating neurons persisted in the dentate gyrus after 8 weeks, indicating lasting damage. Glial cell activation, while still detectable, had decreased in intensity compared to the 48-hour time point, indicating a reduction in the neuroinflammatory response within the dentate gyrus. These Fluorojade B staining results demonstrate that kainic acid and PTZ treatment induced acute neuronal degeneration within the dentate gyrus, with some signs of partial recovery after 8 weeks. However, residual neuronal degeneration and a reduced but still present neuroinflammatory

response were observed within this specific hippocampal subregion. These findings underscore the susceptibility of the dentate gyrus to the treatment and its potential for limited recovery over an extended period in rats. **(Fig. 1)**.

In the TUNEL assay, a mean of 1.24±0.18 apoptotic nuclei was observed per section in the control brain. At 48 hr after SE, a significant increase, i.e., 2.36±0.17 ($P<0.05$), nuclei per section in DG, CA3, and the hilar region was detected (Fig. 2A). In PTZ-treated rats, also a significant increase, i.e., 2.28±0.12 nuclei per section ($P<0.05$), was seen. After 8 weeks of SE or kindling, a significant ($P<0.05$) increase in apoptotic nuclei was observed in both the groups, i.e., 3.82±0.6 and 4.13±0.8 nuclei per section, respectively (Fig. 2B).

Hippocampal cell proliferation: The control brain showed 15.2±1.772 BrdU+ cells per section (Fig. 3A), primarily in the subgranular zone (SGZ). After 48 hours of KA treatment, a significant ($P<0.001$) threefold increase was observed, with 45.20±3.499 BrdU+ cells per section in both hippocampal lobes (Fig. 3A) compared to the control brain. Many BrdU+ cells were detected in the hilar region of DG and the CA3 field, along with the SGZ, while fewer cells were observed in CA1. Similarly, in the PTZ-kindled brain, there was about a five-fold increase (70.40±3.82 per section, $P < 0.001$) in BrdU+ cells compared to the control brain (Fig. 3B).

To examine whether this increased cell proliferation in both groups persists for a long time, we injected BrdU into KA- and PTZ-treated rats 8 weeks after SE or kindling and sacrificed them 24 hr later. Results showed that cell proliferation in the SGZ was significantly decreased and reduced to half by 8 weeks following SE (8.20±1.42 BrdU+ cells per section; $P < 0.05$) compared to the control brain (Fig. 3A). The number of BrdU+ cells was still significantly high 8 weeks after kindling (28.40±1.93 BrdU+ cells per section; $P < 0.001$) compared to the control brain (Fig. 3B). However, this was significantly less than the cell proliferation observed 48 hr after kindling. A small number of BrdU+ cells were also found in the hilar CA3-CA2 and CA1 fields in addition to the SGZ.

Cell differentiation

Phenotype of cells born after 48 hr of SE or kindling: In age-matched control rats, the majority of BrdU+ cells surviving up to 4 weeks were co-labelled with calbindin (13.16±0.48 cells per section), while some were co-labelled with GFAP (4.86±0.27; Fig. 4A and 5), primarily localized in the SGZ and granule cell layer (GCL) region. Among the total differentiated cells counted, 73.03% were identified as mature neurons and 26.97% were identified as mature astrocytes.

In the hippocampus of KA-treated rats, 77.76% of BrdU+ cells were co-labelled with GFAP (29.58±1.21 cells per section), and only 22.23% of cells were co-labelled with calbindin (8.46±0.85; Fig. 4A and 5).

In PTZ-induced kindled brains, the majority of BrdU+ cells were co-labelled with calbindin (44.78±2.26 cells per section), which accounted for 79.65% of total cells. Only 20.34% of cells (11.44±0.62 cells per section) differentiated into astrocytes and were co-labelled with GFAP. Neurogenesis was 340.20% ($P<0.001$), whereas gliosis was 235.39% ($P < 0.001$).

Phenotype of cells born after 8 weeks of SE or kindling: Cell proliferation decreased drastically in both SE- and kindled rats after 8 weeks. In the hippocampus of KA-treated rats, 27.58% (1.60±0.80 cells per section) of BrdU+ cells

were co-labelled with GFAP, and 72.41%

(4.20±0.70 cells per section) cells were co-labelled with calbindin (Fig. 4B and 5). Neurogenesis was thus reduced to 31% (P<0.001), whereas gliosis was reduced to 32.92% (P<0.001) in control rats. In contrast to control rats, most of these cells were distributed over the hilar region and CA3 field. A smaller number of cells were also found in the SGZ and GCL.

In PTZ-induced kindled rats, the differentiation pattern of cells, which were born after 8 weeks of kindling, was similar to that of the control rats. The majority of BrdU+ cells were co-labelled with calbindin (24.45±2.8 cells per section; Fig. 4B), accounting for 79.77% of the total cells identified as differentiated. Only 20.22% of cells (6.20±0.6 cells per section) differentiated into astrocytes and were co-labelled with GFAP (Fig. 4 B and 5). Neurogenesis was increased to 185.00% (P<0.001), whereas gliosis was increased to 127.57% (P<0.05) compared to control rats.

When the magnitude of neurogenesis was compared between two time points, i.e., 48 hr and 8 weeks post SE, we observed that after 8 weeks it reduced to 49.64% (after 48 hr, P<0.01). Similarly, in kindled rats, neurogenesis decreased to 54.60% (P<0.001) after 48 hr. When the magnitude of gliosis was compared, we observed that after 8 weeks it reduced to 5.40% of the gliosis that had occurred after 48 hr (P<0.0001). In kindled rats, gliosis decreased to 54.38% (P < 0.05) after 48 hr.

NADPH-d positive cell count: Every third coronal section passing through the hippocampus (bregma - 1.8 to -5.8) was selected for counting of NADPH-d positive neurons in all hippocampal subregions (CA1–CA4, DG). Two types of positive neurons were recognizable - one was darkly stained cell bodies with clearly visible dendrites, and the other had diffusely stained cell bodies without visible dendrites (Fig. 5A). The cell number was higher in the CA1 and CA3 regions compared to CA4. Few NADPH-d positive cells were also observed in the hilus and granular cell layer within the DG. A statistically significant increase (P < 0.05) in the number of NADPH-d positive neurons was observed in both the KA (10.40±1.65) and PTZ (8.5±1.3) treated brains when compared with the control brain (5.24±0.98; Fig. 4B) after 48 hr of SE and kindling. Furthermore, the NADPH-d positive cell count in the KA-treated group was significantly higher than in the PTZ-treated group. This increase was persistent after 8 weeks of SE or kindling (Fig. 6B).

GABA immunostaining: In control rats, GABA immunopositive neurons were observed throughout all fields of the hippocampus, including the hilar region and all seven layers. After 48 hr of SE, a decline in GABA immunostaining was observed in the hippocampus of PTZ-treated brains, while it remained unchanged in KA-treated brains compared with the control brain. Interestingly, divergent outcomes were observed in GABA immunostaining between the two groups after a duration of 8 weeks. The brain subjected to PTZ treatment exhibited higher intensity compared to the rats treated with KA.

BDNF and NGF expression (Fig. 7): In control rats, BDNF and NGF immunoreactivity was present in pyramidal neurons and granule cells of Dg. BDNF expression was higher in KA- and PTZ-treated brains compared to control brains after 48 hr of KA or PTZ administration. After 8 weeks of administration, BDNF expression decreased. Similar results were were obtained in the immunoblotting study. Lane 3 and 5 show increased expression of BDNF after 48 hr of KA and PTZ treatment, respectively, compared to control rats (lane 1). Eight weeks after administration, there was a significant decrease in the expression of BDNF in both groups, and it was lower than that observed in the control rats (lanes 2 and

4).

An increase in NGF immunoreactivity was observed in KA (Fig. 6) and PTZ-treated brains after 48 hr of kindling. NGF expression decreased after 8 weeks of KA and PTZ administration compared to 48 hr. Immunoblotting showed increased expression of NGF in Lanes 2 and 4 after 48 hr of KA and PTZ administration, respectively, as compared to control (lane 1). In lanes 3 and 5, a noteworthy reduction in NGF expression was evident after 8 weeks. The levels of expression were below those observed in the control rats.

Discussion

The present study showed that systemic administration of KA and PTZ elicited seizures in rats, but marked differences were observed in intensity, duration, and frequency. Administration of KA led to generalized tonic seizures, referred to as SE, followed by SRS after a latent period of a few weeks. In contrast, administration of PTZ initially resulted in a freezing response, but with repetitive injections, the seizure response altered dramatically and finally increased in duration and intensity.

Neuronal loss in Dg and other fields of the hippocampus was studied after 48 hr and 8 weeks of SE, and kindling discerned major differences in magnitude and mechanism between the two groups. Nissl and Fluorojade B staining results in KA-treated brains showed cell loss after 48 hr, primarily in the CA3 and hilar region. However, very few degenerating neurons were observed by the TUNEL assay, suggesting that cell death takes place primarily by necrosis. After 8 weeks, few degenerating neurons were observed by Nissl and Fluorojade B staining, which were equivalent to those observed by the TUNEL assay, suggesting that by the 8 weeks following SE, cell death occurs primarily by apoptosis.

In contrast to KA, PTZ-treated rats showed an insignificant increase in the number of degenerating cells at 48 hr post-kindling compared to control brains. However, the number of apoptotic cell bodies was higher than in control brains and was equivalent to that in KA-treated brains. Furthermore, unlike SE, after 8 weeks of kindling, the number of apoptotic cell bodies remained the same; this suggests that 48 hr and 8 weeks after kindling, neuronal loss occurs consistently at a slow rate, primarily by apoptosis. The differences in the magnitude and mechanism of neurodegeneration and in seizure types after administration of KA and PTZ can be explained by their different modes of action on hippocampal neurons. KA induces significant excitotoxicity by selectively activating KA receptors in the hippocampal CA1 and CA3 subfields, which are preferentially expressed on CA3 pyramidal neurons[18].

Cell proliferation following KA and PTZ treatment was also examined at 48 hr and 8 weeks post-SE and kindling. A threefold and about fivefold increase in the rate of cell proliferation was seen after 48 hr of SE and kindling, respectively. The rate of cell proliferation radically decreased in both groups by the end of 8 weeks. It reached below the basal level in the KA brain but was still significantly high in the PTZ brain as compared to the control brain.

It can be concluded that kindling exerted a more profound effect on cell proliferation. KA may directly act upon neural stem

cells (NSCs) in the SGZ. On the other hand, PTZ, which is a GABA antagonist, can directly promote cell proliferation by removing the inhibitory effects of GABA[19][20]. We hypothesize negative regulation of GABA on Neuronal Progenitor Cells (NPCs) proliferation, and a GABA antagonist like PTZ may promote NPCs proliferation.

To further prove this hypothesis, changes in GABA levels were examined. Results showed that GABA immunoreactivity was decreased after 48 hr of kindling. This suggests that increased cell proliferation could be a result of the endogenous decrease of GABA and the presence of PTZ. However, PTZ lasts for only two hours in the body. Still, we observed increased cell proliferation at 48 hr after the last PTZ injection. This observation suggests the role of other factors like seizures in cell proliferation.

Administration of KA and PTZ both elicited seizures, though the intensity, duration, and frequency of seizures were different. A proliferative surge occurs in NSCs of the SGZ shortly after seizures, leading to an increased production of new neurons.

Multiple studies demonstrate that several factors, including NGF, fibroblast growth factor-2 (FGF-2)[21], BDNF [22], and vascular endothelial growth factor (VEGF), are up-regulated in the hippocampus after acute seizures and are known to promote NSC proliferation and neuron survival. We studied the expression of BDNF and NGF after 48 hr and 8 weeks of SE and kindling. Results showed an increase in the expression of both BDNF and NGF, as revealed by immunohistochemistry and immunoblotting. Expression of these factors decreased after 8 weeks of SE and kindling. These results establish a positive correlation between growth factor expression and cell proliferation.

Thus, it appears that multiple mechanisms underlie the increased hippocampal cell proliferation observed at 48 hr post-SE and kindling. The difference in the magnitude of cell proliferation can be attributed to the different types of seizures elicited by KA and PTZ. Induced cell proliferation may be a consequence of neurodegeneration. However, results strongly suggest that these two processes of neurodegeneration and cell proliferation may be differentially regulated and that neuronal death is not a major cause of the enhanced cell proliferation in the hippocampus; despite very small neuronal injury, the PTZ-treated brain showed a higher rate of cell proliferation than the KA-treated brain.

Cell proliferation was decreased by 8 weeks following KA and PTZ treatment relative to 48 hr post-treatment. The rate of cell proliferation in the KA-treated brain was even lower than in the control brain, while in PTZ-treated brains, it was significantly higher after 8 weeks, but this was half of the rate observed after 48 hr of PTZ treatment. This suggests that the rate of cell proliferation is continuously declining. This may be due to the reversal of all the events that occurred shortly after SE or kindling. This observation is supported by the decrease in BDNF and NGF expression observed after 8 weeks.

We also observed BrdU+ cells in the hilar and CA3 regions at 48 hr post-SE or kindling. This is noteworthy because the rats were sacrificed only 24 hr after BrdU injection, which is a very short time period for a cell to migrate from the SGZ to the hilar region or from the SVZ to the CA3 field. The only possible explanation for the presence of BrdU+ cells in these ectopic areas is activation of quiescent progenitor cells, a phenomenon reported by Reitze et al., 2000.

To determine the phenotypes of cells that were born 48 hr and 8 weeks post-SE or kindling, rats were pulsed with BrdU

for five consecutive days starting from day 2 or 9th week following SE or kindling [23]. Rats were then allowed to survive for an additional 28 days after the last BrdU injection. The phenotypes of BrdU-labeled cells were assessed by double immunostaining using the mature neuron marker Calbindin or the astrocyte marker GFAP.

Results showed that in the control brain, 73% of cells born at 48 hr were differentiated into neurons and 27% into astrocytes. KA treatment drastically decreased neurogenesis and increased gliosis, as only 23% of cells differentiated into neurons and 77% into astrocytes. PTZ treatment did not significantly alter the ratio between neurogenesis and gliosis. Eight weeks after SE or kindling, the ratio between neurogenesis and gliosis was similar for control, KA, and PTZ brains. The major difference observed was the decreased neurogenesis and increased gliosis in KA-treated brains shortly after SE. It is plausible that the microenvironment of the hippocampus after SE is adequate for maintaining significant proliferation of NSCs in the SGZ, but it is not conducive to the neuronal differentiation of newly born cells, while the neurogenic potential in the hippocampus of PTZ remains intact. We suggest that the initial severe seizure induced by KA and the neuronal damage start a switch in cell fate, resulting in gliosis but not neurogenesis [23].

We also examined the effects of KA and PTZ on nNOS, an enzyme which synthesizes NO. We identified nNOS-positive cells using a histochemical method for NADPH-d [24]. Results showed a significant increase in NADPH-d-positive cells in both KA- and PTZ-treated brains at 48 hr post-SE or kindling. This increase persisted for 8 weeks following SE or kindling. However, previous studies have demonstrated that NO is a physiological inhibitor of neurogenesis in the adult mouse SVZ and DG [25][26]. In light of these findings, we suggest that the inhibitory effect of NO may not overcome the proliferative effect of other mitogenic factors which were released shortly after SE or kindling. However, as time passes after SE or kindling, the level of mitogenic factors decreases and the inhibitory property of NO becomes effective.

Spontaneous recurrent seizures (SRS) were observed in the KA-treated brain but not in the PTZ-treated brain. This can be attributed to gliosis, which occurred only in the KA-treated brain shortly after its commencement. These newly born glial cells were found in the GCL, hilar region, CA3, and CA1 area. The presence of these ectopic glial cells in the hippocampus may contribute to SRS.

In conclusion, we assert that initial seizures are not the result of neurodegeneration, as seizures were also observed in PTZ-treated rats. The onset of seizures in both KA and PTZ models may be the result of a neurotransmitter imbalance. Increased cell proliferation is a consequence of seizure activity and not due to neurodegenerative changes, as evidenced by more pronounced cell proliferation in PTZ-treated rats, which showed less neurodegeneration and more frequent seizures.

The observed nNOS expression and increased NO synthesis observed shortly after seizures, lasting up to 8 weeks, may inhibit cell proliferation. Seizure activity also increases the expression of NGF and BDNF levels shortly after SE or kindling, potentially promoting cell proliferation. Neuronal loss and ectopic gliosis could be a primary cause of SRS, as SRS was only observed in KA-treated rats that showed neurodegeneration and increased gliosis.

Increased neurogenesis was observed in kindling, but the development of chronic epilepsy was not observed; this suggests that aberrant neurogenesis does not contribute to the development of recurrent seizures. The present study

enables us to identify and understand the factors involved in the onset of seizures and the development of chronic epilepsy. This understanding will aid in formulating the strategies for prevention and treatment of epilepsy. Further investigations will be needed to elucidate the possible role of newly born cells in the development of epilepsy.

Figures

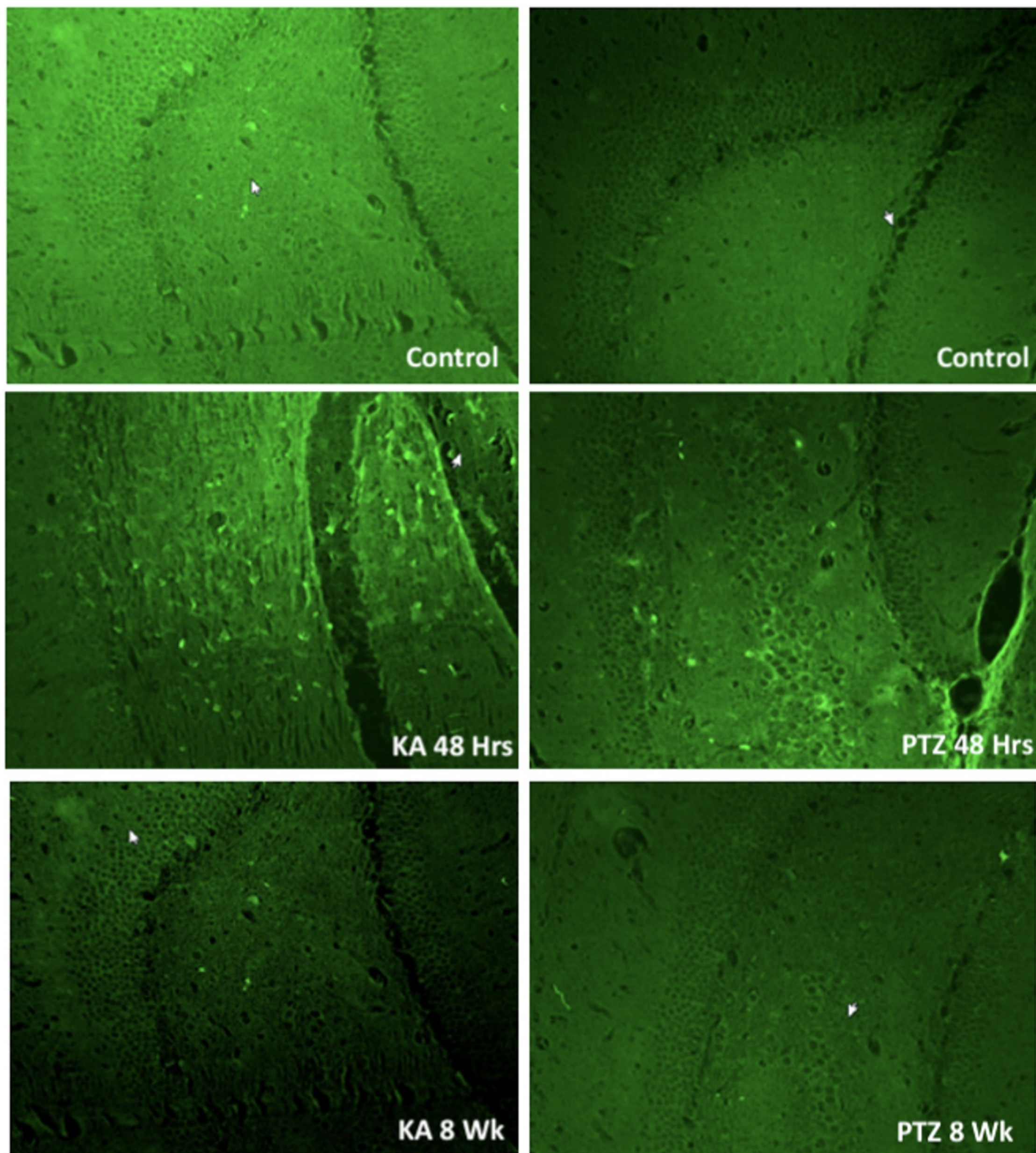


Fig. 1. Fluorojade-B staining in the Dentate gyrus (Dg) of control, KA-treated rats after 48 hrs and 8 weeks, and PTZ-treated rats after 48 hrs and 8 weeks

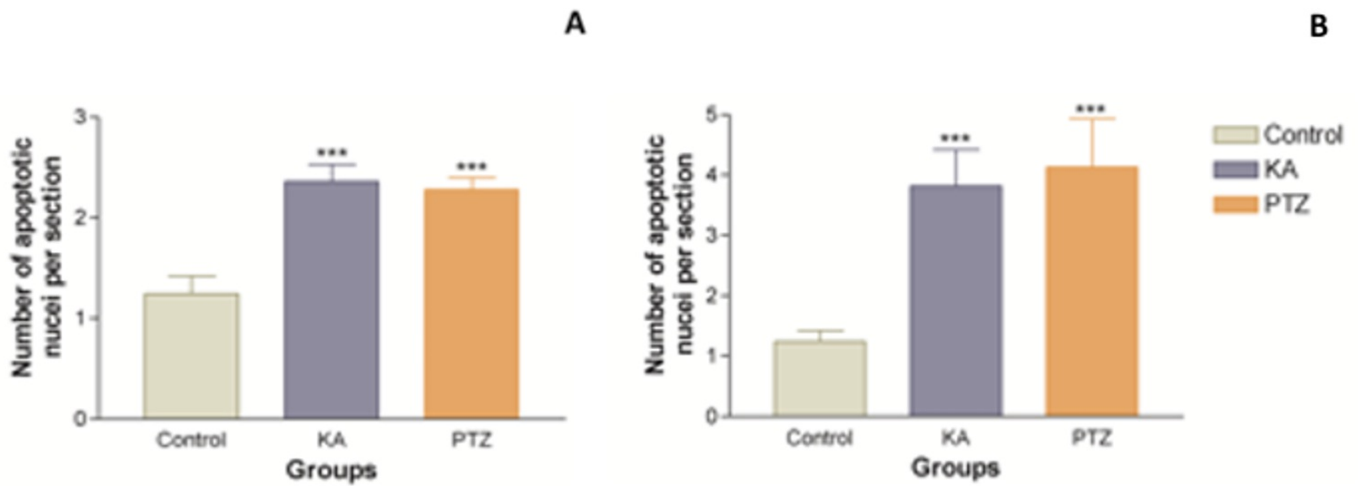


Fig. 2. Graphs representing the number of cells positive for the TUNEL assay

A. Representing the number of apoptotic cells in the Dg region of the hippocampus after 48 hrs of KA and PTZ treatment.

B. Representing the number of apoptotic cells in the Dg region of the hippocampus after 8 weeks of KA and PTZ treatment.

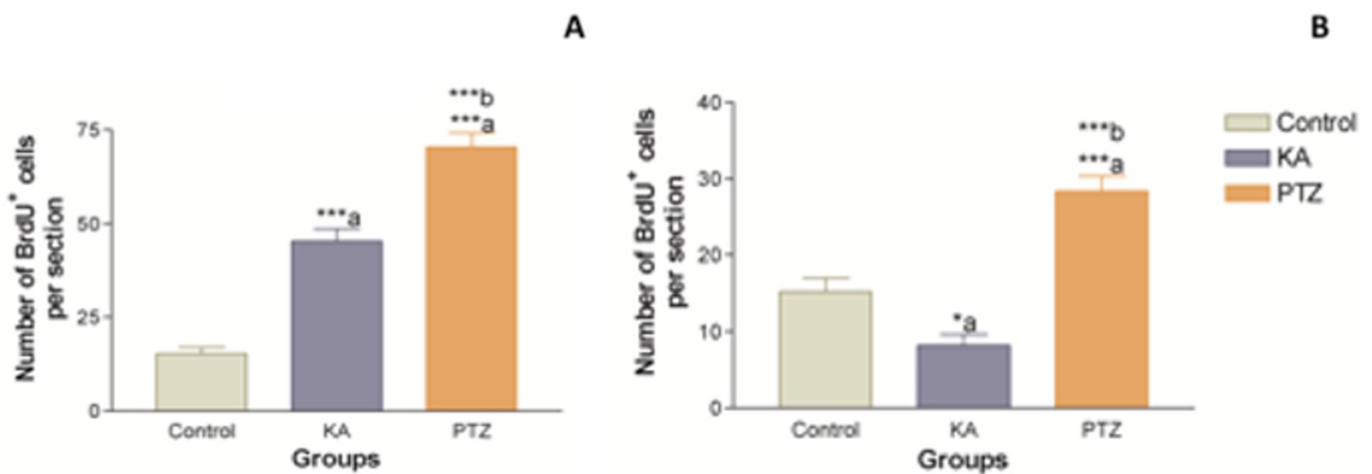


Fig. 3. Graphs representing the number of Brdu+ cells

A. Representing the number of Brdu+ cells in the Dg region of the hippocampus after 48 hrs of KA and PTZ treatment.

B. Representing the number of Brdu+ cells in the Dg region of the hippocampus after 8 weeks of KA and PTZ treatment.

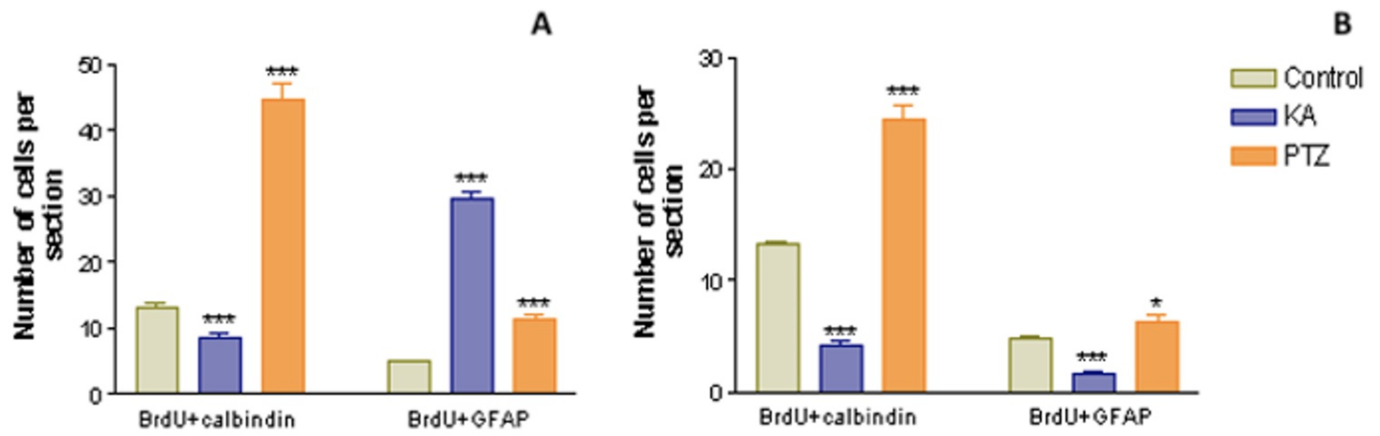


Fig. 4. Graphs representing the number of cells co-labelled Brdu+-calbindin and Brdu+ - GFAP

A. Graphs representing the number of cells co-labelled Brdu+-calbindin and Brdu+ - GFAP 48 hrs after KA and PTZ treatment.

B. Graph representing the number of co-labelled Brdu+ - Calbindin and Brdu+ - GFAP 8 weeks after KA and PTZ treatment.

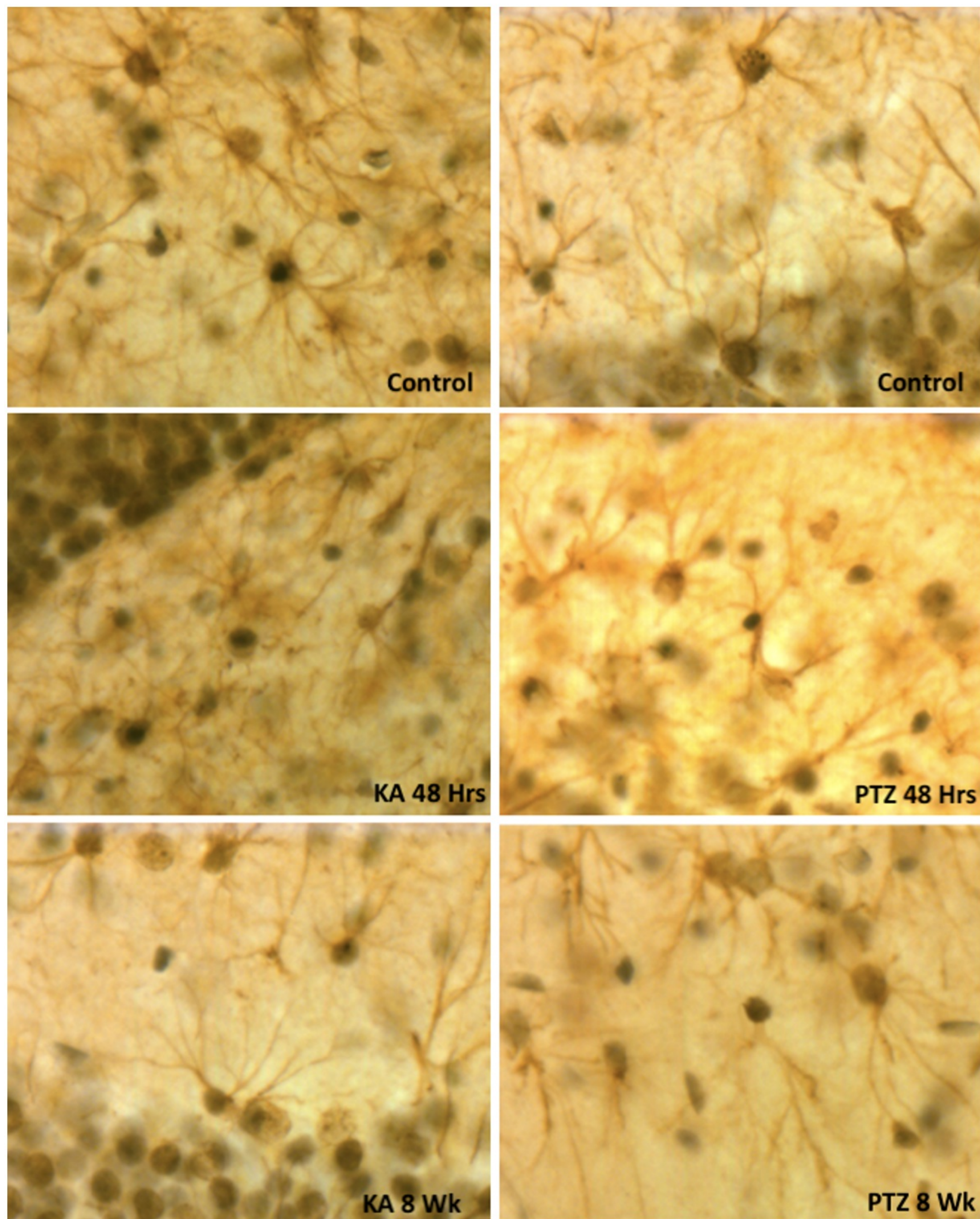
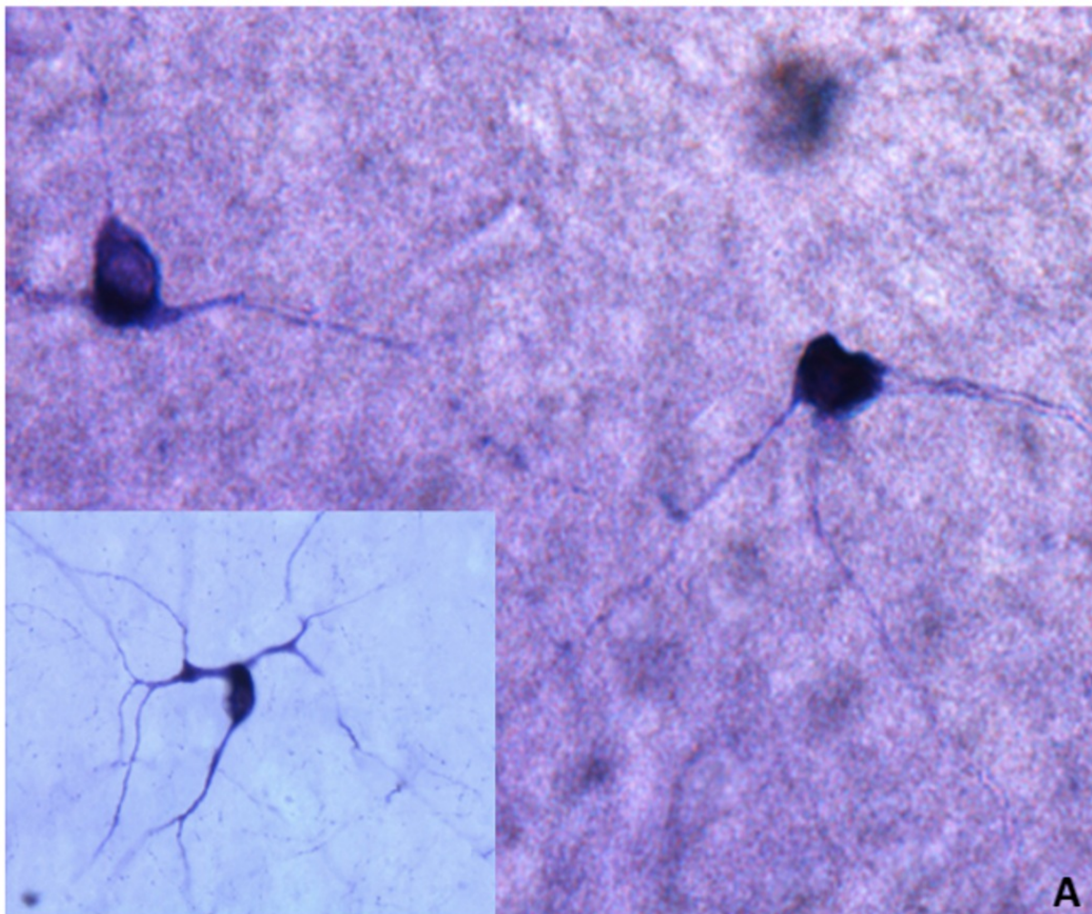


Fig. 5. Double Immunostaining of GFAP (Brown) and BrDU (Gray) in the Dentate Gyrus (DG) of control, KA-treated rats after 48 hrs and 8 weeks, and PTZ-treated rats after 48 hrs and 8 weeks.



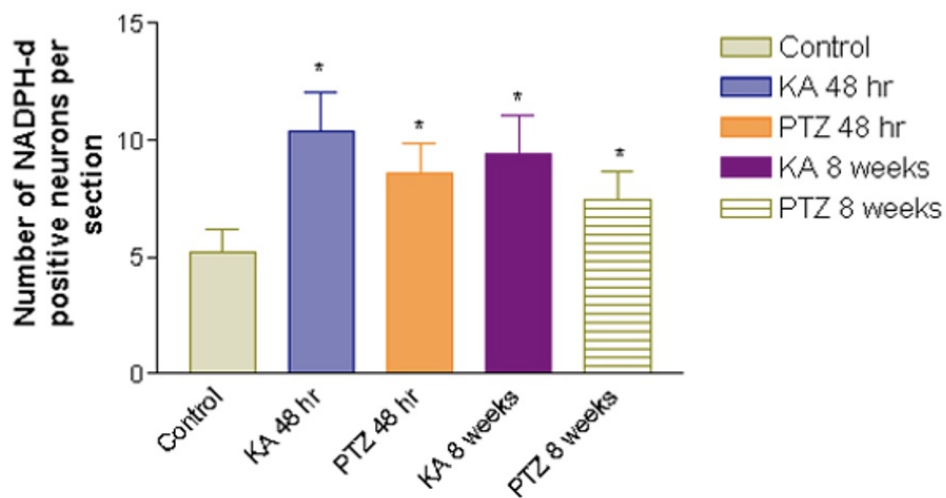
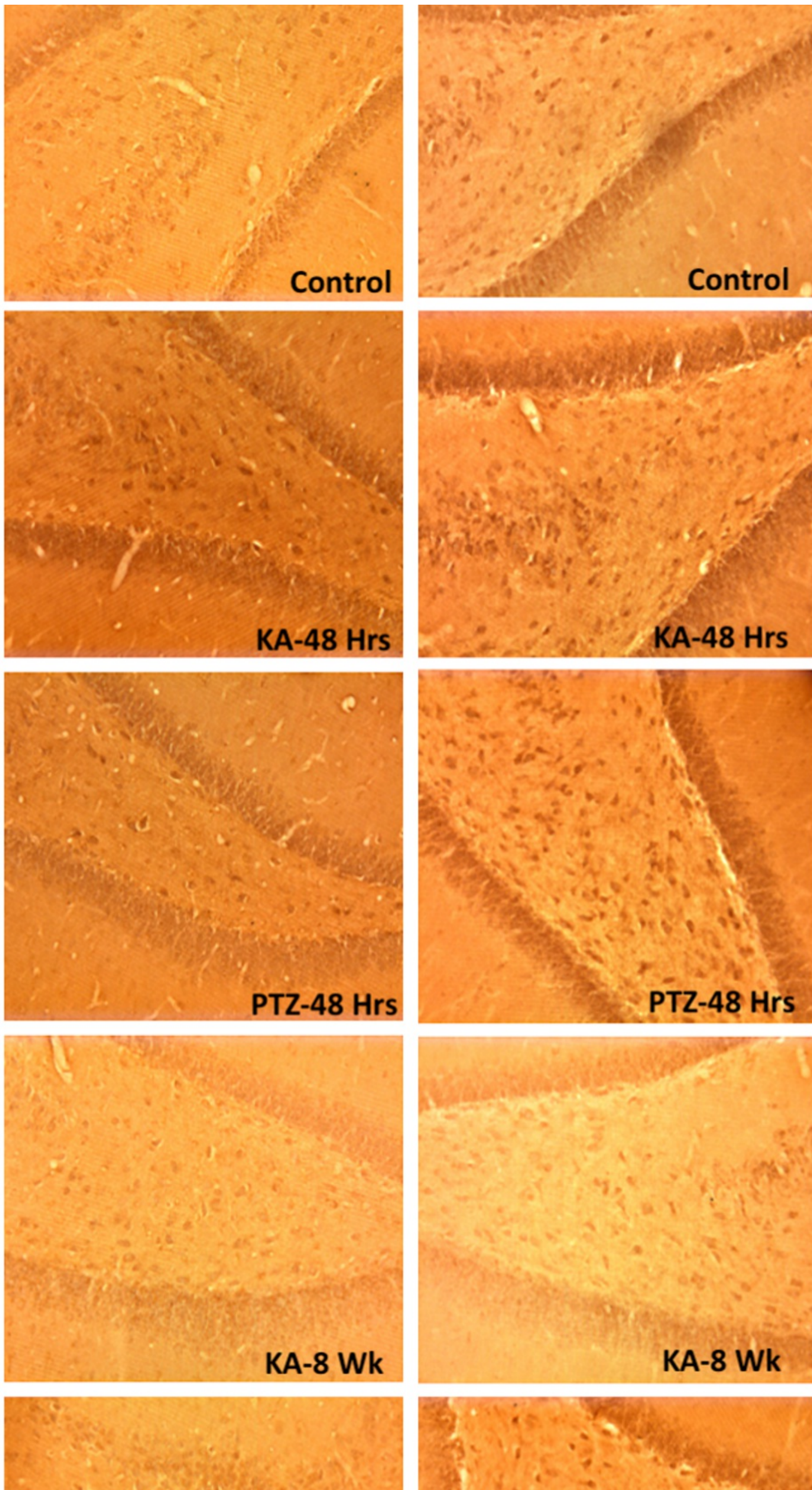


Fig. 6. NADPH-d positive neurons in control and treated rats. At two different time points of KA and PTZ treatment.

A. Representative photographs showing morphological characteristics of NADPH-d positive neurons in the DG region of the hippocampus.

B. Number of NADPH-D positive neurons per section passing through the hippocampus in the brains of control, 48 hrs, and 8 weeks after KA- and PTZ-treated rats.


Control
Control
KA-48 Hrs
KA-48 Hrs
PTZ-48 Hrs
PTZ-48 Hrs
KA-8 Wk
KA-8 Wk

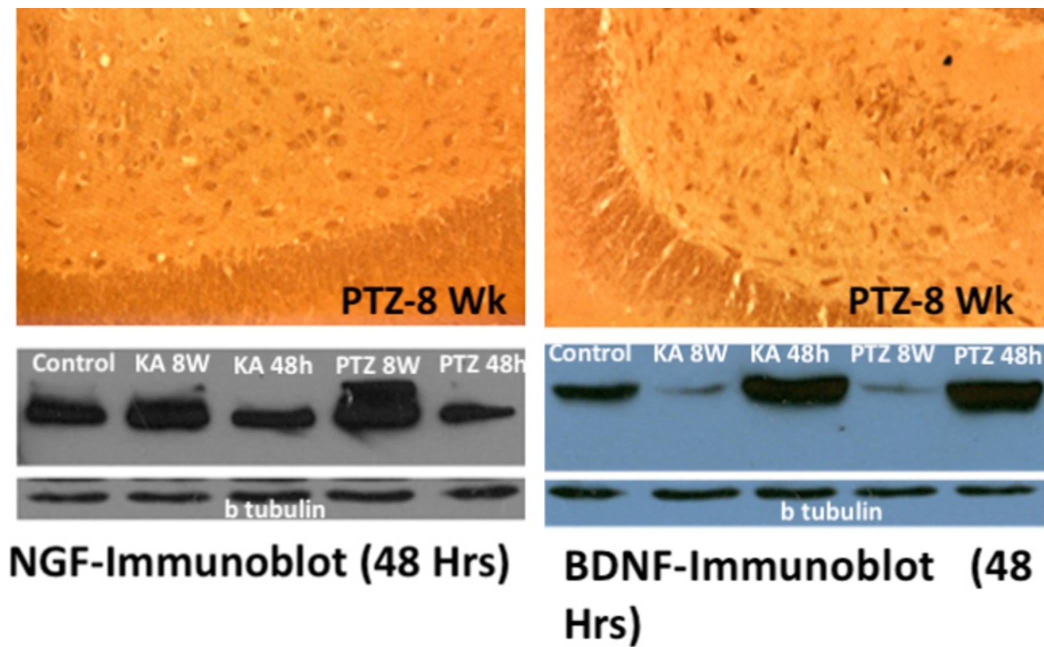


Fig. 7. Immunocytochemical localization and immunoblotting to demonstrate the NGF and BDNF in the DG region of control and KA- or PTZ-treated rats' brains.

Statements and Declarations

Conflict of Interest

There is no conflict of interest.

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