

**THE EFFECT OF PTZ - INDUCED SEIZURES ON NEURONAL DAMAGE
IN AMYGDALA OF RATS**

Fariba Karimzadeh¹, Hassan Alavi¹, Ali Reza Mahmoudian¹, Gholam Reza Hassanzadeh², Mahmoud Hosseini³

- 1- Dept. of Anatomy, School of medicine, Mashhad University of Medical Sciences, Mashhad, IRAN
- 2- Dept. of Anatomy, School of medicine, Tehran University of Medical Sciences , Tehran, IRAN
- 3- Dept. of Physiology, School of medicine, Mashhad University of Medical Sciences, Mashhad, IRAN

Summary

Dark neurons are seen in neurosurgical biopsies, experimental ischemia, hypoglycemia, and epilepsy. This study was aimed to investigate probable damage of amygdala due to pentylenetetrazol (PTZ) - induced acute seizures in rat. Male Wistar rats were divided into control, sham, and PTZ groups. EEG recordings were performed before and after the PTZ injection in PTZ group. Dark neuron numbers in amygdala were compared. Dark neurons were seen in PTZ group; however, there was no significant difference between PTZ, control and sham groups. The result of present study showed that amygdala maybe damage in acute PTZ induced seizures.

Keywords: Dark neuron; Seizure; *amygdala*; Pentylenetetrazol

Corresponding author: Mahmoud Hosseini

Dept. of Physiology

School of medicine

Mashhad University of Medical sciences

Mashhad, Iran.

E-mail: hosseinim@MUMS.ac.ir

Tel:98 511 8828564-5

Introduction

Dark neurons were noticed to occur in neurosurgical biopsies and it was hypothesized that they were produced by mechanical stress forces (1,2,3). It has been reported that dark neurons maybe produce under other conditions such as hypoglycemia, ischemia, and epilepsy (3,4,5) without any trauma or mechanical forces. Epilepsy produces widespread dark neurons through out the brain, even under excellent fixation (5,6).

Brain damage due to various kind of epileptic models such as temporal lobe epilepsy (7, 8, 9), partial (10) and febrile seizure (11) has been widely reported. It has been shown that some regions of the amygdala are very sensitive to seizure-induced damage (8,9,12,13,14,15), however, Tuunanen (2000) reported total neuronal number in the amygdala or hilus were not reduced after few amygdala kindling-induced seizures (16). This study has been aimed to investigate probable damage of amygdala in pentylentetrazol-induced acute seizure in rat.

Materials and methods

Animals and chemicals

PTZ was purchased from Sigma and other materials purchased from Merck Company. Adult male Wistar rats, weighing 250–300g each, were used throughout the study. All of them were housed in the same room under a constant temperature (22 ± 2 C) and illuminated 7:00 A.M. to 7:00 P.M., with food pellets and water available. They were randomly divided as follows:

- 1- Control group: the animals of this group didn't receive any surgery procedure or EEG recording, the brains were only removed and histological studies were carried out.
- 2- Sham Group: the animals of this group underwent the surgery procedure, the electrodes were implanted on the duramater and EEG was recorded, and the brains were removed and histological studies were carried out.
- 3- PTZ group: the electrodes were implanted as same as group 2, EEG was recorded before and after PTZ injection and the brains were removed for histological studies.

PTZ-induced seizures and EEG recording

Recording electrodes were implanted on the dura mater under intraperitoneal (i.p.) urethane anesthesia (1.2 g/kg) (17) and stereotaxic apparatus and fixed by dental cement. EEG recordings were performed for 10 min before (baseline) and then after the PTZ (120 mg/kg, i.p) injection (18).

Histological studies

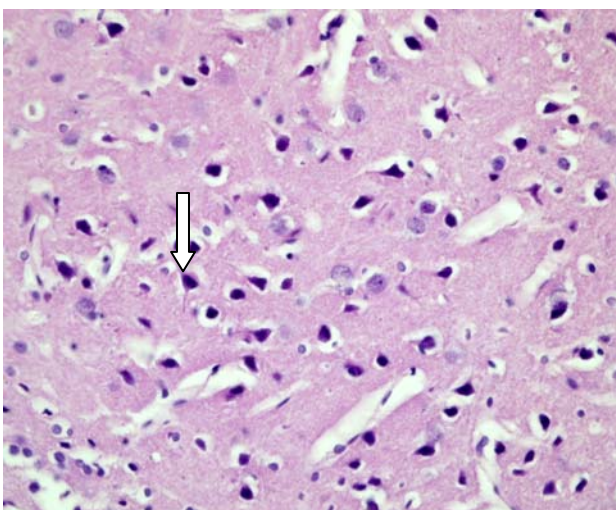
Immediately after the tests, all rats were given a high dose of urethane and transcardially perfused with 100 ml of saline followed by 100 ml fixative solution (glutaraldehyde 1.25% and paraformaldehyde %1 in 0.2 mol buffer phosphate at pH=7.4, T=4⁰ C) for one hour(19,20). The brains were removed just after perfusion and immersed in fixative solution, in room temperature, for 10 days. Three series of 10- μ m thick coronal sections were cut every 100 - μ m from 2.3 to 3.3 mm posterior to the bregma (2). Each series of sections was used for Hematoxilin & Eosin stain, Crysel violet and also Toluidine blue staining (2). Slides were examined with light microscope and digital photographs were taken from amygdala basolateral nuclei of both hemispheres. Dark neurons were counted using image tools 2 software (19).

Statistical analysis

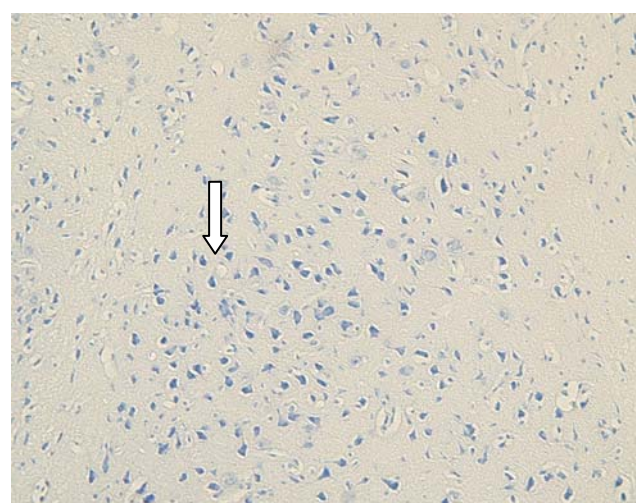
Data were expressed as means \pm SEM. Dark neuron numbers were compared between all 3 groups. Analysis of variance (ANOVA), followed by Tukey's test, were used for statistical evaluation. The *p*-values less than 0.05 were considered to be statistically significant.

Results

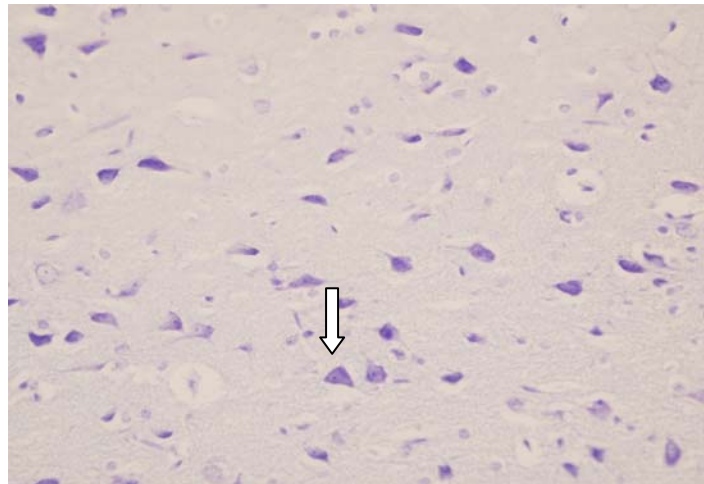
Injection of 120 mg/kg of PTZ results in seizures in rat. PTZ induces seizures also results in dark neurons production in amygdala. The number of dark neurons in basolateral nuclei of amygdala in PTZ group was not significantly more than sham and control groups (Fig 2).



H&E (A)



Toluidine blue (B)



Crysyl violet (C)

Fig 1: Light-microscopic appearance of Hematoxilin & Eosin (A), Toluidine blue (B) Crysyl violet (B) and stained dark neurons in 10- μ m sections of the amygdala basolateral nuclei in rats. Scale bars: \sim 100 μ m. White arrows show dark neurons.

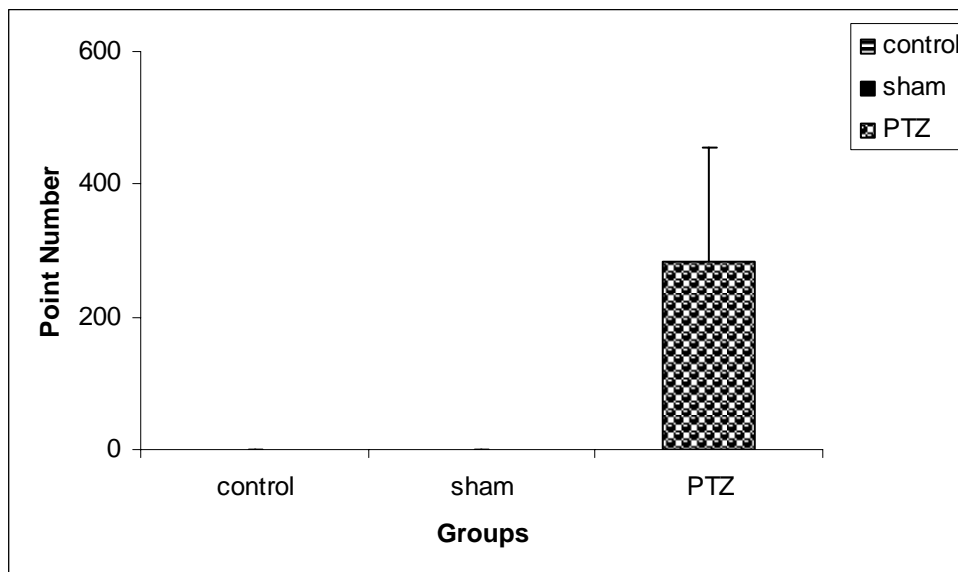


Fig 2: Comparison of dark neuron numbers in the amygdala basolateral nuclei among control, sham and PTZ. The brain of control group was removed without EEG electrode implantation and PTZ injection. The electrodes were implanted on the dura of the animals in sham and PTZ groups and EEG was recorded after PTZ injection. Saline (1ml/kg) was injected instead of PTZ in sham group. Finally, the brains of all animals were removed and histological studies were carried out.

Discussion

The relationship between seizures and subsequent pathological conditions in central nervous system has been widely investigated. Animal and human studies have provided clear evidences that seizures can cause brain damage(11,21,22). Progressive and permanent, functional and structural abnormalities in the amygdala, and memory deficits after repeated brief seizures have been widely reported(23,24,25). It has been shown that seizures could produce morphological changes in brain tissue such as dark neurons. Dark neurons, previously, were introduced as histological artifacts that were seen in neurosurgical biopsies (2, 26). But it was hypothesized that dark neurons were produced after brain trauma (1, 2). Currently, it has been well documented that dark neurons also appear under conditions where no trauma or mechanical forces were applied to the brain (3,18). Dark neurons have basophilic appearance with typical morphological changes following many kinds of insults such as hypoglycemia, ischemia , stress and epilepsy (3,4,5,27,28). Several studies have shown one of the important identified causes of dark neuron is epilepsy (5, 6, 29). These changes have been repeatedly observed using various staining methods, such as hematoxylin and eosin, Nissl, and silver stain (2, 27).

However there was no significant difference in dark neuron number in amygdala of PTZ, sham, and control groups, the results of present study showed that PTZ induced seizures results in dark neuron production amygdala. Several studies have indicated hippocampal and amygdala damages were created by seizures (8,9,11) however, Majak (2004) explained it depended on genetic background, age at the time of the epileptogenic insult, extent of brain lesion, location of seizure focus, seizure duration, seizure number, brain reserve, and environmental and social living conditions (30). Furthermore, Tuunanen (2000)showed total neuronal number in the amygdala or hilus were not reduced after few amygdale kindling-induced seizures (31). On the other hand pervious studies had investigated amygdala damages in recurrent seizures and other types of epileptic models such as partial (32) and temporal lobe(33)models but this study showed that acute PTZ induced- seizure could result in dark neuron production. In addition, amygdala damages and memory deficit in epileptic models maybe depends on location and methods of inducing seizures (30).

Acknowledgements:

Authors would like to thank the Vice Presidency of Research of Mashhad University of Medical Sciences, for financial supports.

References

- 1- Cammermeyer J. Is the solitary dark neuron a manifestation of postmortem trauma to the brain inadequately fixed by perfusion? *Histochemistry* (1978); 56(2):97-115.
- 2- Ooigawa H, Nawashiro H, Fukui S, Otani N, Osumi A, Toyooka T, Shima K. The fate of Nissl-stained dark neurons following traumatic brain injury in rats: difference between neocortex and hippocampus regarding survival rate. *Acta Neuropathol* (2006); 112:471–481.
- 3- Kherani ZS, Auer RN. Pharmacologic analysis of the mechanism of dark neuron production in cerebral cortex. *Acta Neuropathol* (2008); 116(4):447-52.
- 4- Auer RN, Kalimo H, Olsson Y, Siesjö BK. The temporal evolution of hypoglycemic brain damage. Light- and electron-microscopic findings in the rat cerebral cortex. *Acta Neuropathol.* (1985); 67(1-2):13-24.
- 5- BaracsKay P, Szepesi Z, Orbán G, Juhász G, Czurkó A. Generalization of seizures parallels the formation of "dark" neurons in the hippocampus and pontine reticular formation after focal-cortical application of 4-aminopyridine (4-AP) in the rat. *Brain Res* (2008); 1228:217-28.
- 6- Gallyas F, Kiglics V, BaracsKay P, Juhász G, Czurkó A. The mode of death of epilepsy-induced "dark" neurons is neither necrosis nor apoptosis: An electron-microscopic study. *Brain Res* (2008); [Epub ahead of print].
- 7- Kälviäinen R, Salmenperä T, Partanen K, Vainio P, Riekkinen P Sr, Pitkänen A. MRI volumetry and T2 relaxometry of the amygdala in newly diagnosed and chronic temporal lobe epilepsy. *Epilepsy Res*(1997) 28(1):39-50.
- 8- Pitkanen A, Tuunanen J, Kalviainen R, Partanen K, Salmenpera T. Amygdala damage in experimental and human temporal lobe epilepsy. *Epilepsy Res.* 1998; 32:233–253.
- 9- Bernasconi N, Bernasconi A, Caramanos Z, Antel SB, Andermann F, Arnold DL. Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala and parahippocampal region. *Brain*(2003); 126(2):462-9.
- 10- Salmenperä T, Kälviäinen R, Partanen K, Pitkänen A. Hippocampal and amygdaloid damage in partial epilepsy: a cross-sectional MRI study of 241 patients. *Epilepsy Res*(2001); 46(1):69-82.
- 11- Toth Z, Yan XX, Haftoglou S, Ribak CE, Baram TZ. Seizure-induced neuronal injury: vulnerability to febrile seizures in an immature rat model. *Neuroscience* (1998); 18(11):4285-94.
- 12- Meyer A, Beck E, Shepherd M. Unusually severe lesions in the brain following status epilepticus. *Neurol. Neurosurg Psychiatry* (1955); 18:24–33.
- 13- Meldrum BS, Brierley JB. Prolonged epileptic seizures in primates. Ischemic cell change and its relation to ictal physiological events. *Arch Neurol*(1973); 28:10–17.
- 14- Wasterlain C, Baldwin R, Itabashi H. Status epilepticus induces widespread neuronal injury in newborn marmosets. *Epilepsia*(1996); 37:141.
- 15- Park JH, Cho H, Kim H, Kim K. Repeated brief epileptic seizures by pentylene-tetrazole cause neurodegeneration and promote neurogenesis in discrete brain regions of freely moving adult rats. *Neuroscience*(2006); 140(2):673-84. Epub (2006).
- 16-Tuunanen J, Pitkänen A. Do seizures cause neuronal damage in rat amygdala kindling? *Epilepsy Res*(2000); 39(2):171-6.

- 17- Wu G, Luo F, Li Z, Zhao X, Li SJ. Transient relationships among BOLD, CBV, and CBF changes in rat brain as detected by functional MRI. *Magn Reson Med*(2002); 48(6):987-93.
- 18- File, SE. Tolerance to the anti-pentylentetrazole effects of diazepam in the mouse. *Psychopharmacol* (1983); 79(2-3):284-6
- 19- Bayat M, Hasanzadeh GR, BarzroodIpour M, Javadi M. The effect of low protein diet on thalamic projections of hippocampus in rat. *Neuroanatomy* (2005); 4: 43–48
- 20- Gost JI, Insausti R, Gonzalo LM. Production and characterization of a monoclonal antibody that selectively marks the astrocyte population in the central nervous system. *Rev Med Univ Navarra* (1993); 38(2):9-20.
- 21- Holmes GL. Seizure-induced neuronal injury: animal data. *Neurology* (2002); 59:S3-6.
- 22- Sankar R, Shin DH, Liu H, Mazarati A, Pereira de Vasconcelos A, Wasterlain CG. Patterns of status epilepticus-induced neuronal injury during development and long-term consequences. *Neuroscience* (1998); 18(20):8382-93.
- 23- Weniger G, Boucsein K, Irle E. Impaired associative memory in temporal lobe epilepsy subjects after lesions of hippocampus, parahippocampal gyrus, and amygdala. *Hippocampus*(2004); 14(6):785-96.
- 24- Zeman A. When a patient with epilepsy complains about poor memory? *Pract Neurol* (2009); 9(2):85-9.
- 25- Carreño M, Donaire A, Sánchez-Carpintero R. Cognitive disorders associated with epilepsy: diagnosis and treatment. *Neurologist*(2008); 14:S26-34
- 26- Jortner BS. The return of the dark neuron. A histological artifact complicating contemporary neurotoxicologic evaluation. *Neurotoxicology* (2006); 27(4):628-34.
- 27- Ishida K, Shimizu H, Hida H, Urakawa S, Ida K, Nishino H. Argyrophilic dark neurons represent various states of neuronal damage in brain insults: some come to die and others survive. *Neuroscience* (2004); 125(3):633-44.
- 28- Czurkó A, Nishino H. 'Collapsed' (argyrophilic, dark) neurons in rat model of transient focal cerebral ischemia. *Neuroscience Letters* 1993; 162(1-2):71-4.
- 29- Söderfeldt B, Kalimo H, Olsson Y, Siesjö BK. Bicucullineinduced epileptic brain injury. Transient and persistent cell changes in rat cerebral cortex in the early recovery period. *Acta Neuropathol*(1983); 62:87–95
- 30- Majak K, Pitkänen A. Do seizures cause irreversible cognitive damage? Evidence from animal studies. *Epilepsy Behav* (2004); 5:S35-44.
- 31-Tuunanen J, Pitkänen A. Do seizures cause neuronal damage in rat amygdala kindling? *Epilepsy Res*(2000); 39(2):171-6.
- 32- Salmenperä T, Kälviäinen R, Partanen K, Pitkänen A. Hippocampal and amygdaloid damage in partial epilepsy: a cross-sectional MRI study of 241 patients. *Epilepsy Res*(2001) ;46(1):69-82.
- 33- Pitkanen A, Tuunanen J, Kalviainen R, Partanen K, Salmenpera T. Amygdala damage in experimental and human temporal lobe epilepsy. *Epilepsy Res*(1998); 32:233–253.