DENDRITIC SPINES IN THE HIPPOCAMPUS OF GENETIC ABSENCE EPILEPSY RATS (GAERS): AN ULTRASTRUCTURAL QUANTITATIVE ANALYSIS

Serap Şirvancı, M.D.* / Şükrü Midillioğlu, M.D.* / Charles K. Meshul, Ph.D.**
Filiz Onat, M.D.,Ph.D.* / Tangül Şan, Ph.D.***

* *Department of Histology and Embryology, School of Medicine, Marmara University, Istanbul, Turkey.*

** *Electron Microscopy Laboratory and Department of Behavioral Neuroscience and Pathology, VA Medical Center, Oregon Health Science University, Portland, OR, USA.*

*** *Department of Pharmacology and Clinical Pharmacology, School of Medicine, Marmara University, Istanbul, Turkey.*

ABSTRACT

Objective: The aim of the present study was to analyze the area of glutamate immunoreactive (-ir) dendritic spines in CA3 and dentate gyrus hilar regions in Genetic Absence Epilepsy Rats from Strasbourg (GAERS) hippocampus by electron microscopy in order to investigate possible glutamate-induced morphological changes in dendritic spines.

Methods: GAERS and non-epileptic control (NEC) rats were used. The brains were removed after perfusion fixation and CA3 and dentate hilar regions of the hippocampus were studied using immunogold electron microscopical methods. The area of dendritic spines was measured with the program NIH Image Analysis.

Results: There was no significant difference in the mean number of glutamate-ir spines making asymmetric synapses per mossy terminal in CA3 and dentate hilar regions compared to the control group. No significant difference in the area of glutamate-ir dendritic spines making asymmetric synapses with mossy fiber terminals between NEC and GAERS groups was found.

Conclusion: Dendritic spines in the hippocampus seem not to be affected in absence epilepsy, as opposed to those in temporal lobe epilepsy (TLE). This may be due to differences in pathogenetic mechanisms between TLE and absence epilepsy.

Key Words: Hippocampus, GAERS, Ultrastructure, Dendritic spine

INTRODUCTION

Typical absence epilepsy is characterized by a sudden interruption of behavioral activity and responsiveness associated with bilaterally synchronous spike and wave discharges in the EEG. Genetic absence epilepsy rats from Strasbourg (GAERS), a well-established model

Marmara Medical Journal 2003;16(3):195-200

Correspondence to: Serap Şirvancı, M.D., - Department of Histology and Embryology, School of Medicine, Marmara University, Haydarpaşa, 34668 Istanbul, Turkey
e.mail address: ssirvanci@yahoo.com

of human absence epilepsy on the basis of neurophysiological, pharmacological and genetic studies (1,2), is a strain of Wistar rats with genetically determined seizures. Although spike and wave discharges have been demonstrated to originate from the thalamus and cortex, other brain regions such as the hippocampus may also be involved in the cellular and molecular mechanisms underlying absence epilepsy. In a previous study, basal levels of extracellular glutamate were shown to be three times higher in GAERS compared to non-epileptic controls. In addition, no difference in GABA levels within the hippocampus between GAERS and the non-epileptic control group was reported in the same study (3). Metabolic activity was increased in many brain regions of adult GAERS and in limbic structures of immature GAERS including the hippocampus (4).

Spines were proposed to have roles both in storing memory and protecting neurons from toxic insults associated with the raised Ca^{+2} that follows synaptic activity (5). They also mediate synaptic plasticity and more than 90% of dendritic spines in the mammalian CNS are contacted by excitatory synapses (6). It was reported that excessive activation of postsynaptic glutamate receptors caused formation of cell body swelling, chromatin clumping, development of dendritic varicosities and swelling or loss of dendritic spines (7). Alterations in postsynaptic intracellular Ca^{+2} concentration regulates the morphology of dendritic spines and seizures cause spine shrinkage and an increase in spine density (8). Spine density in the proximal dendrites of the granule cells in the hippocampus of TLE patients was shown to be increased (9). Dendritic spine degeneration was also reported in different epilepsy models such as the kainate (10,11), pilocarpine (12,13) and tetanus toxin induced models (14,15). Although dendritic shrinkage in CA1 and CA3c pyramidal neurons in tetanus toxin-induced seizures was reported in previous studies, other reports have shown dendritic hypertrophy of basal dendrites of granule cells in dentate gyrus in both animal models and humans (9,16-18).

It has recently been reported that glutamate immunoreactivity in mossy terminals in the CA3 region in the hippocampus was significantly increased in GAERS compared to the non-epileptic control group (19). The present study was performed to analyze the area of glutamate immunoreactive (-ir) dendritic spines in CA3 and dentate gyrus hilar regions in GAERS hippocampus by electron microscopy in order to investigate possible glutamate-induced morphological changes in dendritic spines.

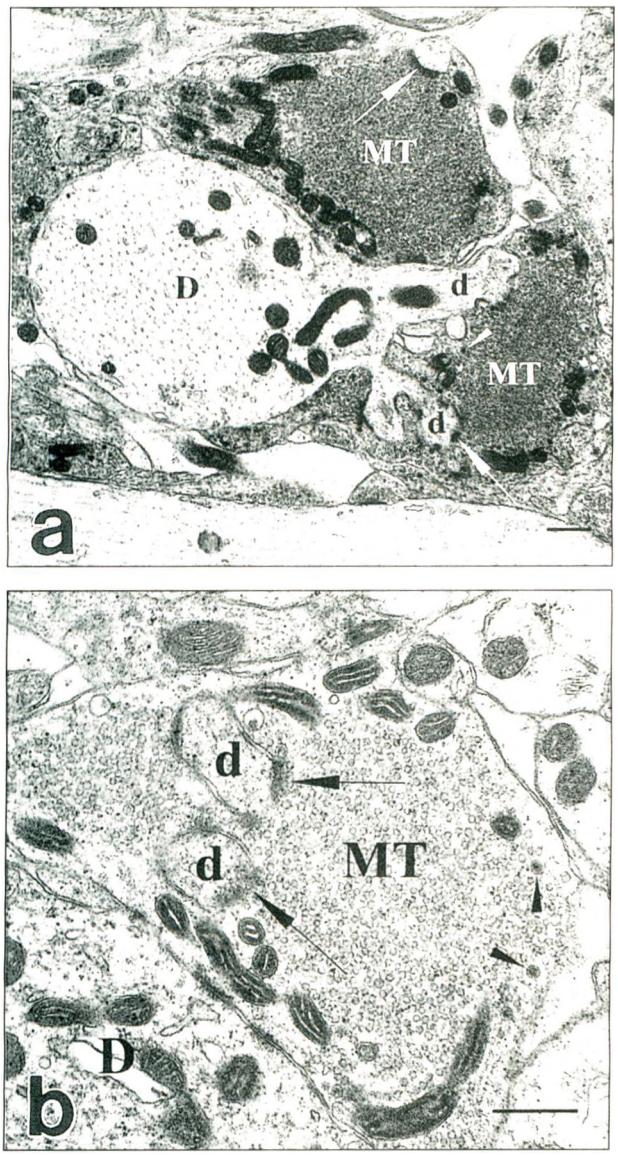
MATERIALS AND METHODS

Adult, non-epileptic control Wistar rats (NEC) and GAERS (250-300 g, 6-12 months old) having absence seizures on EEG were used. The animals were housed in a temperature-controlled room (20±3°C) with a 12 h light / dark cycle and fed a standard diet. Full approval for the experimental study was obtained from the Animal Care and Use Committee of Marmara University (40.2000.mar).

For ultrastructural observation and immunocytochemistry, NEC (n=4) and GAERS (n=4) rats were sacrificed by transaortic perfusion with a fixative solution (2.5% glutaraldehyde, 0.5% paraformaldehyde, and 0.1% picric acid in 0.1 M HEPES buffer, pH 7.3). The animals were decapitated and the entire brain left overnight in the same fixative at 4°C. The brains were washed several times in 0.1 M HEPES, pH 7.3, and cut into 300 μm slices using a vibratome in the following day. The CA3 and dentate gyrus regions of the hippocampus were dissected and incubated in 1% osmium tetroxide / 1.5% potassium ferricyanide for 30 min at room temperature. The samples were then washed several times in deionized water and stained en block with aqueous 0.5% uranyl acetate for 30 min at room temperature in the dark. The tissue was then dehydrated in a graded series of ethanol, cleared in propylene oxide and embedded in Epon for 24 h at 60°C. Semi-thin sections (1 μm) were cut on a Leica Ultracut R ultramicrotome and stained with toluidine blue and viewed with the light microscope for proper orientation. The tissue was then thin sectioned (60 nm), collected on 200 mesh nickel grids coated with a Coat-Quick 'G' pen (Kiyota International, Elk Grove, IL, USA) and air-dried for 3-4 h.

The grids containing thin sections were washed in Tris-buffered saline, pH 7.6, containing 0.1%

Triton X-100 (TBST 7.6) and incubated in the primary antibody overnight in a moist chamber at room temperature. The anti-glutamate antibody (Biogenesis) was diluted 1:1.600.000 in TBST 7.6. Aspartate (1 mM) was added to the antibody solution to prevent cross-reactivity with aspartate within the tissue. The samples were washed several times in TBST 7.6 and TBST 8.2 and incubated for 90 min in goat anti-rabbit IgG



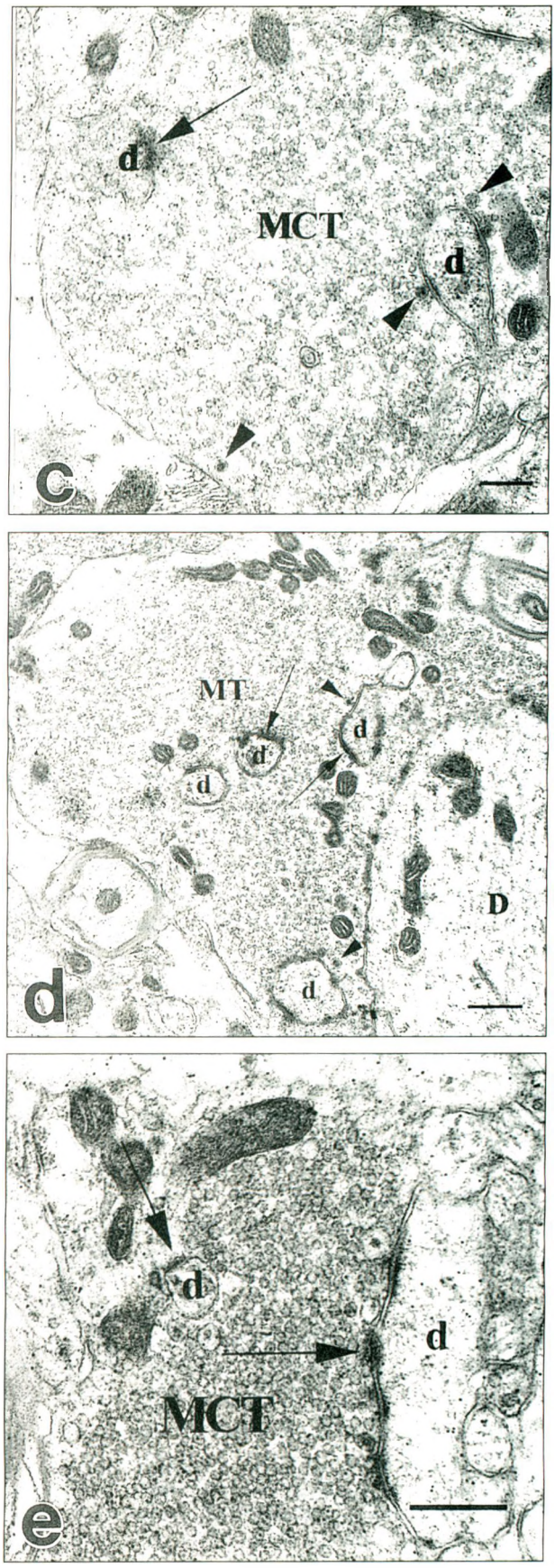


Fig. 1: Dendritic spines synapsing with mossy terminals in control **(a,b,c)** and GAERS **(d,e)** groups in CA3 **(a,b,d)** and dentate hilar **(c,e)** regions. (**MT:** mossy terminal; **D:** dendritic shaft; **d:** dendritic spine; **MCT:** mossy fiber collateral terminal; **Black/white arrow:** asymmetric synapses between dendritic spines and mossy terminals; **Black/white arrowhead:** dense core vesicle). Bar: 500 nm.

(Sigma) conjugated to 10 nm gold, which was diluted 1:50 in TBST 8.2. The samples were then washed in TBST 7.6 and deionized water. The sections were counterstained with uranyl acetate and lead citrate before being viewed and photographed on the transmission electron microscope. All of the samples were processed simultaneously with the same antibody solutions to avoid day-to-day differences. Sections were viewed and photographed using a JEOL 1200 EX transmission electron microscope.

The photographs were taken randomly within the same region of the stratum lucidum layer of the CA3 subfield where the synapses between mossy fiber terminals and dendritic spines of CA3 pyramidal neurons occur and within the hilar region of the dentate gyrus of the hippocampus. Asymmetric synapses were identified by an accumulation of synaptic vesicles within the presynaptic nerve terminal and a prominent postsynaptic density which is wider than the width of the synaptic cleft.

For the quantitative analysis, the area of dendritic spines was determined using the program NIH Image Analysis. The number of spines per mossy fiber terminal was also calculated and a comparison was made between GAERS and the control group. The data were analyzed using a Mann-Whitney U-test and are presented as mean ± standard deviation.

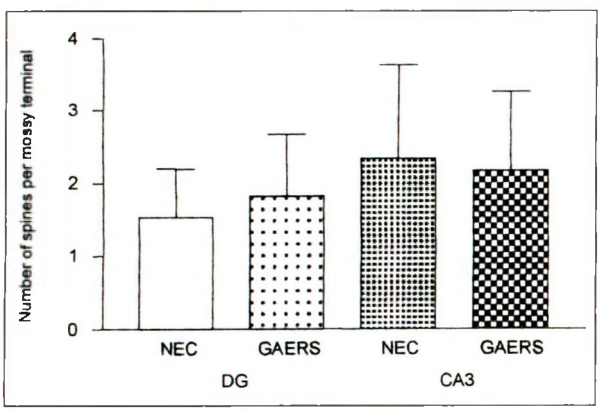


Fig.2: Number of glutamate-ir dendritic spines making asymmetric synapse per mossy terminal in CA3 and dentate hilar regions in GAERS and NEC. (**NEC:** Non-epileptic control rat).

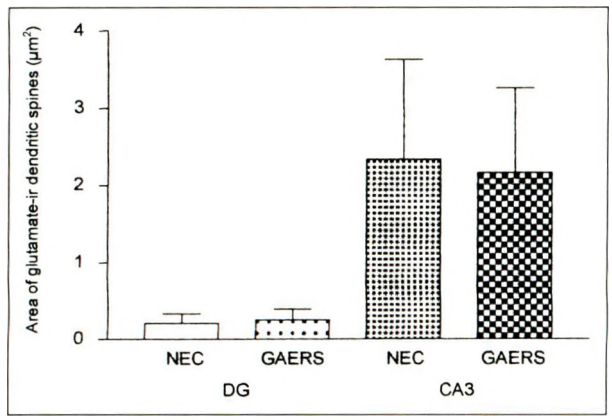


Fig.3: The area of glutamate-ir dendritic spines in CA3 and dentate hilar regions in GAERS and NEC. (**NEC:** Non-epileptic control rat).

RESULTS

In the present study, the area of glutamate -ir dendritic spines and the number of spines making asymmetric synapses per mossy terminal in CA3 and hilar regions in GAERS hippocampus were measured. There was no significant difference in the mean number of glutamate-ir spines making asymmetric synapses per mossy terminal in CA3 and dentate hilar regions compared to the control group (mean number of spines per mossy terminal ± SD: **CA3;** *control* [n=30], 2,33±1,30; *GAERS* [n=37], 2,16±1,09; **DG;** *control* [n=45], 1,53±0,66; *GAERS* [n=23], 1,83±0,83). No significant difference in the area of glutamate-ir dendritic spines making asymmetric synapses with mossy fiber terminals between NEC and GAERS groups was found (mean area [μm^2] ± SD: **CA3;** *control* [n=70], 0,24±0,16; *GAERS* [n=80], 0,27±0,24; **DG;** *control* [n=69], 0,21±0,12; *GAERS* [n=42], 0,25±0,14).

DISCUSSION

In the present study, a possible dendrotoxic effect of the increase in glutamate level in the CA3 region on the number and area of dendritic spines of CA3 pyramidal neurons was investigated. We also examined the same parameters in the dentate hilar region. No difference was found either in the number or the area of dendritic spines in both regions between GAERS and NEC groups. This is the first study

which reported a histomorphologic analysis of dendritic spines in GAERS hippocampus.

An increase in the glutamate density in mossy fiber terminals in the CA3 region of GAERS hippocampus compared to NEC animals has recently been reported (19). A previous study examined the early consequences of glutamate receptor activation on dendritic spine synapses and showed that cultured hippocampal neurons exposed to NMDA exhibited loss of dendritic spines. A functional relation between glutamate receptor activation and the actin-mediated shape of dendritic spines was suggested (20). Another study using tetanus toxin model of TLE, dendritic shrinkage in the apical and basal dendrites of CA1 pyramidal neurons was observed 8 weeks after the injection of the toxin (14). Previous studies showed that CA1 and CA3c pyramidal neurons and hilar neurons were lost in TLE associated with Ammon's horn sclerosis and dendritic coiling, nodulation and spine loss were also observed (14,15,21,22). In contrast, the present study did not show any evidence of dendritic swelling (7) or shrinkage (14) as previously reported and the number of spines making asymmetric synapse per mossy fiber terminal was not significantly different from the control group in both dentate and CA3 regions in GAERS hippocampus.

The data obtained in the present study suggests that although an increase in the glutamate content of mossy fiber terminals has been previously demonstrated in GAERS strain (19), dendritic spines in the hippocampus seem not to be affected in absence epilepsy. This may be due to differences in pathogenetic mechanisms between TLE and absence epilepsy.

Acknowledgements

The authors thank Cynthia Moore for assistance during routine tissue preparation and immunolabeling for electron microscopy. The authors also thank the Brain Research Society (Beyin Arastirmalari Dernegi - BAD, Istanbul, Turkey) for providing a bursary. CKM was supported by the Department of Veterans Affairs Merit Review Program.

REFERENCES

1. Danober L, Deransart C, Depaulis A, Vergnes M, Marescaux C. Pathophysiological mechanisms of genetic absence epilepsy in the rat. Prog Neurobiol 1998;55:27-57.
2. Marescaux C, Vergnes M. Genetic absence epilepsy in rats from Strasbourg (GAERS). Ital J Neurol Sci 1995;16:113-118.
3. Richards DA, Morrone LA, Bowery NG. Hippocampal extracellular amino acids and EEG spectral analysis in a genetic rat model of absence epilepsy. Neuropharmacology 2000;39:2433-2441.
4. Nehlig A, Vergnes M, Boyet S, Marescaux C. Local cerebral glucose utilization in adult and immature GAERS. Epilepsy Res 1998;32:206-212.
5. Segal M. Dendritic spines for neuroprotection: a hypothesis. Trends Neurosci 1995;18:468-471.
6. Harris KM, Kater SB. Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. Annu Rev Neurosci 1994;17:341-371.
7. Choi DW. Glutamate receptors and the induction of excitotoxic neuronal death. Prog Brain Res 1994;100:47-51.
8. Segal M, Korkotian E, Murphy DD. Dendritic spine formation and prunning: common cellular mechanisms? Trends Neurosci 2000;23:53-57.
9. Isokawa M. Preservation of dendrites with the presence of reorganized mossy fiber collaterals in hippocampal dentate granule cells in patients with temporal lobe epilepsy. Brain Res 1997;744:339-343.
10. Phelps S, Mitchell J, Wheal HV. Changes to synaptic ultrastructure in field CA1 of the rat hippocampus following intracerebroventricular injection of kainic acid. Neuroscience 1991; 40:687-699.
11. Pyapali GK, Turner DA. Denervation-induced dendritic alterations in CA1 pyramidal cells following kainic acid hippocampal lesions in rats. Brain Res 1994;652:279-290.
12. Isokawa M, Mello LEAM. NMDA receptor-mediated excitability in dendritically deformed dentate granule cells in pilocarpine-treated rats. Neurosci Lett 1991;129:609-613.
13. Isokawa M. Decreased time constant in hippocampal dentate granule cells in pilocarpine-treated rats with progressive seizure frequencies. Brain Res 1996;718:1901-1908.

14. Colling SB, Man WD-C, Draguhn A, Jefferys JGR. Dendritic shrinkage and dye-coupling between rat hippocampal CA1 pyramidal cells in the tetanus toxin model of epilepsy. Brain Res 1996;741:38-43.

15. Jiang M, Lee CL, Smith KL, Swann JW. Spine loss and other persistent alterations of hippocampal pyramidal cell dendrites in a model of early-onset epilepsy. J Neurosci 1998;18:8356-8368.

16. Spigelman I, Yan X-X, Obenaus A, Lee EY-S, Wasterlain CG, Ribak CE. Dentate granule cells form novel basal dendrites in a rat model of temporal lobe epilepsy. Neuroscience 1998;86:109-120.

17. Suzuki F, Junier M-P, Guilhem D, Sørensen J-C, Onteniente B. Morphogenetic effect of kainate on adult hippocampal neurons associated with a prolonged expression of brain-derived neurotrophic factor. Neuroscience 1995;64:665-674.

18. Von Campe G, Spencer DD, De Lanorelle NC. Morphology of dentate granule cells in the human epileptogenic hippocampus. Hippocampus 1997;7:472-488.

19. Şirvancı S, Meshul CK, Onat F, Şan T. Immunocytochemical analysis of glutamate and GABA in hippocampus of genetic absence epilepsy rats (GAERS). Brain Res 2003;988: 180-188.

20. Halpain S, Hipolito A, Saffer L. Regulation of F-actin stability in dendritic spines by glutamate receptors and calcineurin. J Neurosci 1998; 18:9835-9844.

21. Blümcke I, Zuschratter W, Schewe J-C, et al. Cellular pathology of hilar neurons in Ammon's horn sclerosis. J Comp Neurol 1999;414:437-453.

22. Vaquero J, Oya S, Cabezudo JM, Bravo G. Morphological study of human epileptic dendrites. Neurosurgery 1982;10:720-724.