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Electron Microscopic Gaba Evaluation in Hippocampal Mossy Terminals of Genetic Absence Epilepsy Rats Receiving Kindling Stimulations

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ABSTRACT

Objective: The hypotheses related to the fact of epileptic mechanisms are mainly based on excitation-inhibition imbalance in central nervous system. GAERS (Genetic Absence Epilepsy Rats from Strasbourg) is a well-known animal model of absence epilepsy, and frequently used in experimental studies. In the present study, we aimed to examine possible morphological and gamma-aminobutyric acid (GABA) density changes in GAERS hippocampus after electrical kindling stimulations.

Methods: All control and test group rats received 6 kindling stimulations. Rats were decapitated 1 h after the last stimulation. Ultrastructural GABA immunocytochemistry was used to evaluate GABA density quantitatively in mossy terminals of hippocampal CA3 region.

Results: GABA levels were less in kindling groups compared to their controls, and in GAERS groups compared to Wistar groups; mitochondrial and dendritic spine area ratios were greater in GAERS groups compared to Wistar groups, although all these evaluations were statistically nonsignificant. Depletion of synaptic vesicles was evident in the mossy terminals of kindling groups.

Conclusion: The reason of decreased levels of GABA found in the present study might be that GABA has been released from the synaptic pool rapidly at an early time period after the last stimulation, for compansation mechanisms. Depletion of synaptic vesicles observed in kindling groups shows that even 6 kindling stimulations have an impact of changing hippocampal morphology in trisynaptic cycle. The increased mitochondrial area in GAERS might be related to the increased mitochondrial activity. The increased dendritic spine area might be related to the increased performance of learning in GAERS. Our findings indicating that absence epilepsy and temporal lobe epilepsy have different mechanisms of epileptogenesis might be a basis for further experimental studies.

Keywords: Absence epilepsy, GAERS, hippocampus, kindling, GABA, immunocytochemistry

1. INTRODUCTION

Epilepsy is a common neurological disorder with a prevelance of 1% worldwide (1, 2). According to WHO data, there are about 50 million patients with epilepsy. The most common type is temporal lobe epilepsy (TLE) arising from limbic system. Another group is idiopathic generalized epilepsy. In the pathogenesis of absence epilepsy, included in this group, cortico-thalamo-cortical circuit plays a role.

Absence epilepsy and TLE is not commonly seen in the same patient and the reason is not well understood (3, 4). Genetic absence epilepsy rats from Strasbourg (GAERS), a well known animal model of absence epilepsy, is frequently used in epilepsy studies. In the present study, kindling application, which is used for generating TLE model, in GAERS is used to examine possible morphological and gamma-aminobutyric acid (GABA) density changes in hippocampal mossy fiber terminals (MFTs) at the electron microscopical level.

In a previous study, focal seizures in GAERS are investigated by using TLE model and it was demonstrated that Wistar controls had grade 5 seizures, however, GAERS had only grade 2 seizures although they were given 30 kindling stimulations (5). According to the data obtained from Marmara University, School of Medicine, Department of Medical Pharmacology, stimulating Wistar rats 6 times at their after discharge thresholds triggers grade 2 limbic seizures. This number of stimulations provides the same number of stimulations with that of GAERS and staying at the same seizure stage. Thus, in the present study, we tried to standardize possible morphological and GABA density changes in two groups.

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Hippocampal MFTs are giant terminals and contain both GABA and glutamate (6). It was shown that seizures induce a transient increase in glutamic acid decarboxlase 67 (GAD67), GABA synthesizing enzyme, and GABA levels 6 and 24 h after seizures (7).

Cortico-thalamo-cortical circuits in absence epilepsy and limbic structures in TLE were reported to play a role (8, 9). Although seizure circuits of these diseases are thought to be independent from each other, the fact that genetic absence epilepsy rats are resistant to kindling, points that related regions of the brain interact with each other. In the present study, we aimed to investigate possible morphological and GABA density levels at the electron microscopic level and enlighten the neuronal circuits playing role in the mechanisms of these diseases.

In early 1980s, Vergnes and Marescaux discovered that 30% of control Wistar rats showed bilateral synchronized spike and wave discharges on cortical EEG and inbred this strain and named them as GAERS (10). Although the mechanism of seizures in absence epilepsy is excessive GABAergic interactions in thalamo-cortico-thalamic circuit, studies reported the role of neuronal network in the hippocampus. Cellular and molecular mechanisms underlying epilepsy may also present themselves in other brain regions such as the hippocampus. Nehlig et al. found out that energy metabolism and metabolic activity increased in the limbic structures including hippocampus in adult and immature GAERS (11,12). Cerebral glucose utilization was shown to be increased in the primary limbic structures including the hippocampus in immature GAERS, before the appearance of spike and wave discharges (11). Another study demonstrated that basal levels of extracellular glutamate significantly increased in GAERS compared to nonepileptic control rats (13).

Eşkazan et al. reported that although Wistar control rats had stage 5 seizures, GAERS only had stage 2 seizures (5). It was suggested that converse mechanisms may occur in turning of limbic focal seizures in motor seizures, and underlying pathophysiological mechanisms of secondary generalized convulsions might differ from that of absence seizures. They also concluded that mechanisms of generalized absence seizures might be responsible for the resistance of secondary generalization of limbic seizures.

Disturbance underlying spike and wave discharges, especially a possible alteration in GABA function and/or cortical excitability, may change seizure susceptibility to different convulsive agents (14). Previous studies showed that GAERS were more susceptible to focal seizures induced by cortical GABA deprivation and generalized seizures induced by pentylenetetrazole, compared to the controls (15). Therefore, Vergnes et al. investigated susceptibility of GAERS and control rats to seizures by different convulsive agents (16). However, thalamocortical absence seizures are increased by increased GABAergic transmission (17) and they are thought as the seizures of inhibitory GABAergic system (16, 18, 19). It was reported that increase in seizure susceptibility in cortex and/or aberrant susceptibility to the drugs interacting with GABAergic transmission might point to the possible abnormalities which have a role in the generation of spontaneous spike and wave discharges in GAERS. It was also reported that a developmental imbalance between excitation and inhibition might be parallel in the generation of spike and wave discharges.

2. METHODS

Six-month-old male nonepileptic Wistar and GAERS rats were used in the present study. Animals were obtained from Marmara University, The Experimental Animal Implementation and Research Center. All experiments were done according to the national guidelines on animal experimentation and were approved by the Marmara University Local Ethical Committee for Experimental Animals (57.2012.mar). The animals were housed with free access to water and food in a 12-h light/dark cycle controlled room at 20±3 °C. Groups were as follows:

1. Sham-operated Wistar group (n= 4)
2. Sham-operated GAERS group (n= 4)
3. Kindling Wistar group (n= 4)
4. Kindling GAERS group (n= 4)

2.1. Stereotaxic Operation

Animals in the kindling group were anesthetized with ketamine (100 mg/kg) and xylazine hydrochloride (10 mg/kg), and stimulation/recording electrodes were placed into the basolateral amygdala and recording electrodes into the cortex with the aid of a stereotaxic instrument according to Paxinos and Watson Atlas (1998). Then, the animals were allowed for a recovery period of 10 days.

Electrode and cannula coordinates were as follows for EEG recordings: AP 2.0 mm, L 3.5 mm (frontal region), AP – 6.0, L 2.0 mm (parieto-occipital region). Tripolar recording electrodes were placed as referance electrodes on the cerebellum. For kindling procedure, bipolar stimulation/recording electrodes were placed into the basolateral amygdala (AP – 2.6 mm, L 4.8 mm, V – 8.5 mm).

2.2. Kindling

After the recovery period from stereotaxic surgery, 3 h of EEG recordings were obtained for determining the number and duration of basal spike and wave discharges. After that, at the beginning, a stimulation of 50 µA (80 Hz, 1 msn square waves, total duration 2 sn) was given for determining the afterdischarge threshold and continued until the first after discharge by increments of 25 µA. Stimulations were applied two times a day at the intensity of after discharge threshold determined for each animal. Seizures were evaluated according to Racine's seizure scale (20, 21). A total of 6 stimulations were given at the intensity of after discharge threshold for ensuring that all animals had stage 2 seizure.

One hour after the last stimulation, perfusion fixation procedure was applied under deep anesthesia.

2.3. Transmission Electron Microscopy

Deep anesthesia was performed by applying ketamine (100 mg/kg) and xylazine hydrochloride (10 mg/kg) for perfusion fixation and decapitation. Then, perfusion was performed by a perfusion pump at a speed of 50 ml/min by giving a fixative solution containing 0.5% paraformaldehyde, 2.5% glutaraldehyde and 0.1% picric acid in 0.1 M HEPES (pH 7.4). After that, animals were decapitated and brains were obtained and left in the same fixative at 4°C overnight. On the following day, 300-µm-thick sections were obtained by a vibratome (Leica VT1000S) and CA3 region of the hippocampus was dissected under a stereomicroscope. Tissues were post-fixed in 1% osmium tetroxide/1.5% potassium ferricyanide (1:1) for 30 min. Sections were incubated in 0.5% uranyl acetate for 30 min at room temperature in dark and then dehydrated in increasing series of ethanol and cleared in propylene oxide. Sections were incubated in propylene oxide/epon (1:1) mixture overnight. On the following day, sections were incubated in pure epon for 3 h and embedded in Epon 812. Polymerization was achieved by an incubator at 60°C for 24 h. One-µm-thick semi-thin sections were obtained by an ultramicrotome (Leica Ultracut R) and stained with toluidine blue for proper orientation. Thin sections were obtained on grids coated with Coat-Quick 'G' pen.

2.4. Immunogold Method

After drying the sections, they were washed in TBST tampon (0.1% Triton X-100, 0.9% sodium chloride, 0.05 M Tris tampon, pH 7.6). Grids were then incubated in anti-GABA antibody (Sigma A2052, 1:20000) at room temperature. Then the sections were washed in TBST pH 7.6 and pH 8.2 and incubated in 10 nm gold conjugated goat anti-rabbit IgG secondary antibody (Sigma G7402, 1:50) for 90 min. Sections were washed in TBST pH 7.6 and air-dried. After contrasting with uranyl aceate and lead citrate, sections were examined with a SIS Morada CCD camera attached JEOL 1200 EXII transmission electron microscope. MFTs of hippocampal CA3 region were examined for GABA density. For negative controls, primary antibody was omitted.

2.5. Quantitative and Statistical Analysis

MFTs in the stratum lucidum of CA3 region were determined and 10 MFTs were photographed from each animal. Micrographs were evaluated for GABA density with NIH Image Analysis (Image J) program. Terminal areas were calculated and 10 nm gold particles were counted. GABA density was calculated by dividing the number of gold particles by the terminal area. GABA density inside the vessel lumens were calculated for background staining. Final GABA density was determined by subtracting the bacground density from terminal GABA density for each terminal. Mitochondrial, vesicular and cytoplasmic GABA densities were calculated separately. Total mitochondrial area in each terminal was also determined. Similarly, dendritic spine areas synapsing with each terminals were calculated. Data were interpreted as mean±S.E.M. and evaluated with One-Way ANOVA and Tukey multiple comparison tests. Significance level was determined as $p<0.05$.

3. RESULTS

3.1. Light Microscopy

Normal hippocampal structure was observed in sham-operated Wistar (Figure 1a) and GAERS (Figure 1c) groups. Neuronal structure was appeared to be normal in both groups. Dark neurons were observed in kindling Wistar (Figure 1b) and kindling GAERS (Figure 1d) groups and these were interpreted as necrotic neurons.

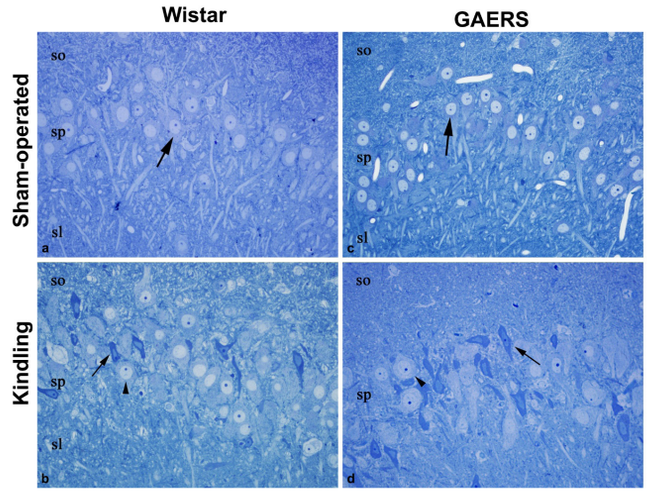

Figure 1. *Semi-thin sections of hippocampal CA3 regions. (a) Sham-operated Wistar group. Normal appearing neuronal cells (arrow). (b) Kindling Wistar group. Arrow: Necrotic dark neuron, arrowhead: normal appearing pyramidal cell. (c) Sham-operated GAERS group. Arrow: Normal appearing pyramidal cell. (d) Kindling GAERS group. Arrow: Necrotic dark neuron, arrowhead: normal appearing pyramidal cell. so: stratum oriens, sp: stratum pyramidale, sl: stratum lucidum. Toluidine blue staining. Original magnification: x400.*

3.2. Transmission Electron Microscopy

MFTs were differentiated as giant terminals including dense core vesicles (Figure 2a-f). They were observed to be GABA (+) in all groups. GABA immunoreactivity was positive on vesicle mebranes, in the vesicles, in the terminal cytoplasm, and on the mitochondria.

In kindling Wistar group, severe vesicle loss in MFTs was observed and the vesicles were seen as clusters (Figure 2b). Some vesicle clusters in or out of the MFTs were surrounded by membranes. Some MFTs were dark and appearead as degenerated (Figure 2c).

Vesicles were seen as clusters in MFTs in kindling GAERS group (Figure 2f) and some terminals appeared as degenerated

(Figure 2e). There were membrane clusters around MFTs, which appeared to be detached from the terminal. Vesicle clusters in or out of the MFTs were surrounded by lamellar membranes in some terminals.

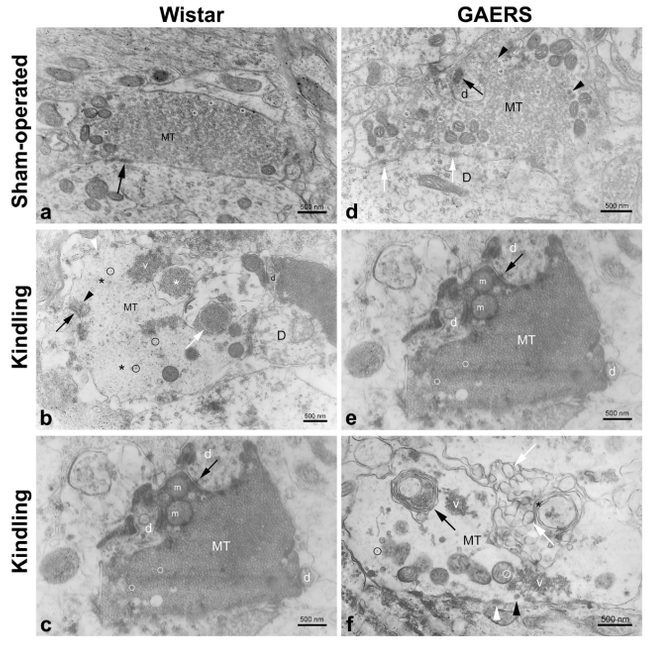

Figure 2. *Transmission electron micrographs. (a) Sham-operated Wistar group. Arrow: Desmosome-like junction between a MFT (MT) and a dendrite, circle: GABA immunoreactivity. (b) Kindling Wistar group. Degenerated MFT (MT). Black arrow: Asymmetric synapse between MFT and a dendritic spine, white arrow: vesicle cluster surrounded by a membrane, arrowhead: synaptic vesicles clustered around a synapse, asterisk: vesicle loss in MFT, v: synaptic vesicles, circle: GABA immunoreactivity, D: dendritic shaft, d: dendritic spine. (c) Kindling Wistar group. Degenerated MFT (MT). Arrow: Asymmetric synapse between MFT and a dendritic spine, m: mitochondria, circle: GABA immunoreactivity, d: dendritic spine. (d) Sham-operated GAERS group. MT: Normal appearing MFT, white arrows: desmosome-like junctions between MFT (MT) and a dendrite, black arrow: asymmetric synapse between MFT and a dendritic spine, circle: GABA immunoreactivity, arrowhead: dense core vesicle, D: dendritic shaft, d: dendritic spine. (e) Kindling GAERS group. MT: Degenerating MFT, black arrow: asymmetric synapse between MFT and a dendritic spine, circle: GABA immunoreactivity, d: dendritic spine, m: mitochondria. (f) Kindling GAERS group. Degenerating MFT (MT), v: vesicle clusters, black arrow: vesicle cluster inside MFT surrounded by lamellar membrane, white arrow: membrane fragments thought to be detached from MFT, asterisk: lamellar membranes out of MFT, black circle: cytoplasmic GABA immunoreactivity, white circle: mitochondrial GABA immunoreactivity, black arrowhead: dense core vesicle, white arrowhead: desmosome-like junction between MFT and a dendrite. Bars: 500 nm.*

3.3. Statistical Analysis

No significance was determined between the groups in parameters. However, the highest terminal area was determined in kindling Wistar group. A tendency to decrease in cytoplasmic, vesicular, and total GABA densities was found in kindling groups compared to their corresponding control groups, and in GAERS groups compared to Wistar groups; and a tendency to increase in mean ratio of mitochondrial area/MFT area and in ratio of spine area/MFT area in GAERS groups compared to Wistar groups.

Mean MFT area was 8.14±1.16 μm^2, mean vesicular GABA density 4.42±1.66, mean cytoplasmic GABA density 3.41±1.75, mean mitochondrial GABA density 1.25±0.43 particle/μm^2, mean ratio of mitochondrial area/MFT area 0.59±0.005, and mean spine area/MFT area 0.07±0.01 in sham-operated Wistar group (Figure 3).

Mean MFT area was 8.08±0.78 μm^2, mean vesicular GABA density 2.50±0.68, mean cytoplasmic GABA density 2.42±0.76, mean mitochondrial GABA density 0.81±0.28 particle/μm^2, mean ratio of mitochondrial area/MFT area 0.07±0.01, and mean spine area/MFT area 0.17±0.17 in sham-operated GAERS group (Figure 3).

Mean MFT area was 10.30±1.09 μm^2, mean vesicular GABA density 2.99±1.64, mean cytoplasmic GABA density 2.75±1.46, mean mitochondrial GABA density 0.58±0.18 particle/μm^2, mean ratio of mitochondrial area/MFT area 0.06±0.001, and mean spine area/MFT area 0.08±0.02 in kindling Wistar group (Figure 3).

Mean MFT area was 7.89±0.76 μm^2, mean vesicular GABA density 1.60±0.31, mean cytoplasmic GABA density 2.11±0.70, mean mitochondrial GABA density 0.52±0.14 particle/μm^2, mean ratio of mitochondrial area/MFT area 0.07±0.006, and mean spine area/MFT area 0.11±0.02 in kindling GAERS group (Figure 3).

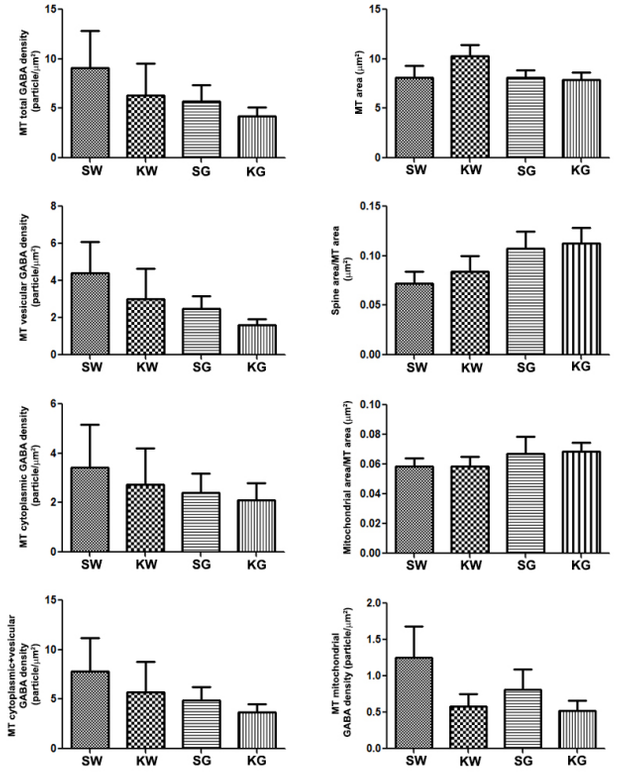

Figure 3. *Statistical analysis. SW: Sham-operated Wistar group, KW: kindling Wistar group, SG: sham-operated GAERS group, KG: kindling GAERS group.*

4. DISCUSSION

MFTs are giant terminals with a idameter of 4-10 µm. In rats, mean MFT area was found to be about 8.2 µm^2 (22). In another study, CA3 mean MFT area was found as 4.75±0.26 µm^2 in naive Wistar control rats and 4.45±0.30 µm^2 in naive GAERS and there was no statistical difference between two groups (23). Akakın et al. found that mean MFT area in CA3 region of sham-operated Wistar rats was 4.48±0.44 µm^2, 4.07±0.50 µm^2 in sham-operated GAERS, 4.29±0.18 µm^2 in kindling Wistar (stage 5 seizure), and 4.07±0.32 µm^2 in kindling GAERS (stage 2 seizure) groups (24). In the present study, we found that mean MFT area in CA3 region was 8.14±1.16 µm^2 in sham-operated Wistar, 8.08±0.78µm^2 in sham-operated GAERS, 10.30±1.09 µm^2 in kindling Wistar groups (6 stimulations); and 7.89±0.76 µm^2 in kindling GAERS group. Although there was no statistical significance, the highest terminal area was found in kindling Wistar group. The highest terminal area in this group was 30.6 µm^2 . This is a very high number for the area of MFTs. The reason for nonsignifacence in areas between the groups may be that this high number of area was not seen in all terminals. Pierce et al. examined MFTs in dorsal and ventral hippocampal dentate area in rats given pentylenetetrazole and had grade 5 seizures, and found no significance between the groups in MFT areas in dorsal hippocampus (25). However, they showed significant increase in MFT areas in ventral hippocampus in seizure group. Other studies demonstrated increased terminal areas after cerebellar parallel fiber lesion and septohippocampal deafferentation (26, 27). This increase was reported as a mechanism of compensation. We also concluded that there may be a similar compensation mechanism in the terminals with an increased areas in kindling Wistar group. Small, membrane-coated cytoplasmic fragments around MFTs in kindling groups may have detached from neighboring degenerating MFTs. Increased areas in some MFTs may be a result of a compensation mechanism for the function of these terminals. Another possibility is that degeneration might have caused swelling in the terminal. The vesicles were observed to occupy a small space in these terminals.

Excitatory amino acids play a role in cellular abnormalities in pathological conditions, as well as in normal neuronal functions because of their high concentration and wide distribution in the central nervous system. It was shown in a previous study on the toxic effect of glutamate that there was a delayed neuronal death in neurons exposed to glutamate for a short period (9). The most vulnerable region to glutamate toxicity in epilepsy is CA3 region of the hippocampus (28, 29). Degeneration in CA3 pyramidal neurons in kindling groups in the present study might be a result of excessive glutamate release from MFTs after the stimulations.

It was shown in previous studies that MFTs contain GABA, an inhibitory neurotransmitter, as well as glutamate, an excitatory neurotransmitter. Sandler and Smith reported for the first time the presence of GABA in MFTs in 1991 (6). The presence of GABA in CA3 MFTs in GAERS rats was demonstrated in the studies of Şirvancı et al. (23, 30) and Akakın et al. (24). Similarly, we also demonstrated the presence of GABA in MFTs of sham-operated and kindling Wistar and GAERS rats.

Akakın et al. found that GABA density was higher in kindling Wistar group compared to sham-operated Wistar and kindling GAERS groups (24). In that study, kindling Wistar group animals were decapitated 24 h after the last grade 5 seizure and kindling GAERS group animals after the last grade 2 seizure. In the present study, GABA density in MFTs were determined 1 h after the last grade 2 seizure. This might be the reason for no increase in GABA density in Wistar rats having grade 2 seizures. There was reported a rapid but transient GAD67 mRNA increase in granule cells of rats 6 and 24 h after kainic acid injection (7). In the present study, we observed a tendency to decrease in GABA density in kindling groups compared to the control groups, although no statistical significance was determined. This finding suggests that in the first hours after kindling stimulations, GABA might be decreased in MFTs. The reason for this might be that only 6 stimulations were given and GABA density was examined at very early hours after the last stimulation. GABA might be rapidly released from the terminal for compensation and this might decrease the GABA pool. Another reason for no difference in GABA density between the groups in the present study might be that kindling Wistar group had only grade 2 seizures like kindling GAERS group and they did not reach stage 5 seizure state. In the study of Akakın et al., it was found that Wistar rats had grade 5 seizures and GAERS rats had grade 2 seizures, althoug they were given 30 kindling stimulations. There was no significant difference in GABA density between kindling GAERS group having grade 2 seizures and sham-operated GAERS group. Similarly, there was no significant difference between kindling groups having grade 2 seizures and their controls. We thought that GABA might be released rapidly from the terminal in the first hours after kindling stimulations and then increased. Akakın et al. found that GABA density in MFTs of Wistar rats having grade 5 seizures increased 24 hours after the last grade 5 seizure compared to kindling GAERS group having grade 2 seizures. In the present study, we observed that GABA levels were increased in kindling Wistar group compared to kindling GAERS group but no statistical significance was found. The reason for this insignificance might be that animals had only grade 2 seizures and they were sacrificed 1 h after the last seizure.

In the present study, we found decreased GABA density in GAERS groups compared to Wistar groups but no statistical significance was found. MFTs synapse mostly with inhibitory neurons (31, 32-34). Şirvancı et al. examined the presence of GAD65/67, GABA synthesizing enzyme, in MFTs, and found no significant difference between the groups (35). However, there was reported to be a tendency to decrease in GAD65/67 in GAERS group. This finding is in line with the findings of the present study. Decreased GABA in MFTs of GAERS might decrease the disinhibition effect on inhibitory neurons, resulting in more effective function of these neurons. This

might be a reason for the resistance of GAERS to kindling stimulations.

GABA density in MFTs in the present study was evaluated as vesicular, cytoplasmic, and mitochondrial separately. Gold particles in the vesicles or vesicular membrane were evaluated as vesicular GABA pool, and the particles out of the vesicles as cytoplasmic pool. Gold particles on mitochondria represent mitochondrial GABA pool. Meshul et al. examined GABA pool in 3 regions, and reported that cytoplasmic pool might be evaluated together with the vesicular pool (36). Similarly, we evaluated these pools both separately and together.

In most regions of the brain, neurons are surrounded by dendritic spines. These spines are the main places of excitatory synaptic transmission and play a key role in the transmission in the brain. Distribution and structure of the spines are destroyed in many diseases and they play a role in plasticity (37-39). Şirvancı et al. found no significant difference in the area and number of dendritic spines in hippocampal CA3 and dentate regions between GAERS and nonepileptic controls (40). The reason for lack of difference in absence epilepsy was suggested to be due to different pathogenetic mechanisms in absence epilepsy and TLE. Drakew et al. generated an epileptic state with bicuculine and picrotoxin in hippocampal culture, and showed decrease in the number of dendritic spines of CA3 pyramidal neurons in the experimental group, but no difference was found in the areas between the groups (41). In line with the previous studies, in the present study, no significant difference was observed in the ratio of spine area/MFT area. However, there was a tendency to increase in ratio of spine area/MFT area in GAERS groups compared to Wistar groups. Hippocampus is involved in learning and memory (42). GAERS were reported to show better performance in learning compared to controls (43, 44). In the present study, we suggest that the tendency to increase in the ratio of spine area/MFT area in GAERS group might be related to the increased performance of this strain in learning abilities.

In the present study, we found increased ratio of mitochondrial ara/MFT area in GAERS groups compared to Wistar groups, however, no statistical difference was observed. In line with this finding, increased mitochondrial activity was reported in neurons and astrocytes in GAERS cortex (45), and increased glucose metabolism in GAERS hippocampus (46) was reported. Although the absence seizures were reported to be limited to thalamocortical circuit, this mutation in GAERS strain includes all brain cells (45). Therefore, increased ratio of mitochondrial area/MFT area in GAERS in the present study is in line with the increased mitochondrial activity and cerebral glucose utilization in this strain (11, 47).

Synaptic vesicle clustering in MFTs was previously shown in genetically epilepsy prone gerbil (48). Akakın et al. also reported vesicle loss and clustering in kindling groups (24). Similarly, in the present study, we observed severe vesicle loss in MFTs of kindling groups, and that other vesicles were clustered in some regions of the terminal. These findings show that even 6 kindling stimulations might result in structural changes in hippocampal trisynaptic circuit.

5. CONCLUSION

In conclusion, differences in the GABA density, mitochondrial area/MFT area, and spine area/MFT area between GAERS and control Wistar groups suggest that there are different epileptogenetic mechanisms between absence epilepsy and TLE. Our study might be a basis for future experimental studies.

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