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Comparing glutamatergic neuron population in the mediodorsal thalamic nucleus of genetic absence epilepsy rats from strasbourg (GAERS) and normal control Wistar rats

Safiye Çavdar[a,*], Merve Özgür[a], Özlem Kirazlı[b], Serçin Karahüseyinoğlu[c], Filiz Onat[d]

[a] Department of Anatomy, School of Medicine, Koç University, Sarıyer, Istanbul, Turkey
[b] Department of Anatomy, School of Medicine, Marmara University, Istanbul, Turkey
[c] Department of Histology-Embryology, School of Medicine, Koç University, Sarıyer, Istanbul, Turkey
[d] Department of Pharmacology and Clinic Pharmacology, School of Medicine, Marmara University, Istanbul, Turkey

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ABSTRACT

An imbalance between GABAergic inhibition and glutamatergic excitation is suspected to play a role in the genesis of epileptic processes. In the present study we quantified the number of glutamate+ve neurons in the mediodorsal thalamic nucleus (MD) of genetic absence epilepsy rats from Strasbourg (GAERS) and compared these with values for normal Wistar rats.

The MD thalamic nucleus was removed from each animal and the glutamatergic neurons were labelled using light-microscopy glutamate immunohistochemistry. The disector method was used to quantify the glutamate+ve neurons in the MD thalamic nucleus of GAERS and Wistar rats. The data were statistically analyzed.

In the Wistar animals glutamate+ve neurons formed 89% and in GAERS 92.3% of the total neurons in 1000 μm^3 of MD thalamic nucleus. In GAERS glutamate+ve neurons showed statistically significant increase in the MD thalamic nucleus compared to Wistar animals. In Wistar animals the glutamate−ve neurons formed 11% and in GAERS 7.7% of the total neurons in 1000 μm^3 of MD thalamic. No significant difference was observed in glutamate−ve neurons between the two strains. The average diameter of glutamate+ve neurons showed no significance, while glutamate−ve neurons were significant between the two strains.

The results of the present study, on genetic absence epilepsy model, GAERS, confirms the role of MD thalamic nucleus in chemically induced absence epilepsy.

1. Introduction

The electroencephalogram (EEG) of absence epilepsy of both GAERS and humans shows bilateral spike-and-wave discharges (SWD's). The seizures arise synchronously in both hemispheres on the EEG with no identifiable macroscopic or microscopic structural brain abnormalities (Danober et al., 1998; Snead, 1995; Marescaux and Vergnes, 1995).

Models that display the clinical and pharmacological characteristics of absence seizures, are either experimentally induced (γ-hydroxbutyric acid, pentylenetetrazole, GABA agonists or penicillin models) or genetic model (GAERS and WAG/Rij) (Vergnes et al., 1982; Snead, 1994; Benerjee and Snead, 1995).

In the present study the genetic absence epilepsy rats from Strasbourg (GAERS) were used. This is a genetic model with clinical and pharmacological characteristics similar to those occurring in human. It represents a selected inbred strain of Wistar rats and is a well-validated genetic model of typical absence epilepsy for neurological, behavioral, and pharmacological studies (Snead, 1995; Polack et al., 2007; Vergnes et al., 1987). Epileptic seizures, concomitant with loss of consciousness and behavioral arrest, start 40 days after birth in this strain and persist throughout life (Vergnes et al., 1986).

Abbreviations: LV, lateral ventricle; 3V, third ventricle; ic, internal capsule; Rt, thalamic reticular nucleus; VL, Ventrolateral thalamic nucleus; AD, Anterodorsal thalamic nucleus; AV, Anteroventral thalamic nucleus; MD, Mediodorsal thalamic nucleus; PV, Paraventriculat thalamic nucleus; PT, Paratenial thalamic nucleus; sm, striamedullaris thalami; mt, mammilothalamic tract; Glut+ve, Glutamate+neurons; Glut-ve, Glutamate-neurons.

* Corresponding author at: Koç University, School of Medicine, Department of Anatomy, 34450 Sarıyer, Istanbul, Turkey.
E-mail address: scavdar@ku.edu.tr (S. Çavdar).

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There is evidence supporting the role of thalamus and cortex for both GAERS and the penicillin model of absence epilepsy (Vergnes et al., 1987; Avoli and Gloor, 1982). Studies have shown 16–50% increases in the local metabolic rates for glucose in the neocortex and thalamus in GAERS (Nehlig et al., 1991). Further, thalamic lesions suppressed cortical SWD's (Vergnes and Marescaux, 1992). Transection of the corpus callosum and/or midline cuts of the thalamus of GAERS animals showed the involvement of corpus callosum in the lateralization of SWD's. However, the midline thalamus played a minor role in the bilateral transfer of SWD's and was responsible for the development of synchronization (Snead, 1995). Bilateral lesions in mediodorsal thalamic nucleus (MD) abolished SWDs from both cortex and thalamus in two experimental rat models of absence seizures (pentylenetetrazole and γ-hydroxybutyric acid model) (Benerjee and Snead, 1994). These data supported the role of the MD thalamic nucleus in the control of seizures (Bertram and Scott, 2000; Kubová et al., 2001). There are two accepted hypotheses for the production of the SWD's in absence epilepsy: either excessive thalamic oscillation, due to neuronal hypersynchronization under GABAergic inhibitory control, or glutamatergic cortical hyperexcitability (Danober et al., 1998; McCormick, 1992; Crunelli and Leresche, 1991).

In the present study we quantified the number of glutamate+ve and glutamate−ve neurons in 1000 μm^3 of MD thalamic nucleus of genetic absence epilepsy rats from Strasbourg (GAERS) and compared this with comparable figures for normal Wistar rats.

2. Materials and methods

Adult (6–12 months old) Wistar albino control rats (n = 5) and GAERS (n = 5) weighing 250–300 g were used. The animals were kept under controlled environmental conditions (12/12 h light/dark cycle, 20 ± 3 °C) with standard laboratory chow and tap water ad libitum. All animals were treated according to the policies of the Animal Care and Use Committee of Marmara University.

2.1. Tissue preparation

The GAERS and Wistar rats were deeply anesthetized with intraperitoneal ketamine (100 mg/kg) and chlorpromazine (1 mg/kg) and sacrificed by transcardiac perfusion with 4–5 ml heparin solution (2 ml heparin in 8 ml 0.1 mol/L HEPES, pH 7.6) and a fixative containing 2.5% glutaraldehyde, 0.5% paraformaldehyde, and 0.1% picric acid in 0.1 mol/L HEPES at pH 7.6. After fixation, the animals were decapitated and the brains were removed. The entire brain was immersed overnight in the same fixative solution at 4 °C, and then washed several times in 0.1 mol/L HEPES, pH 7.3. Coronal sections were cut at 300 μm on a vibratome (Leica VT 100S). Using the Paxinos and Watson rat brain atlas we have removed cylinders of MD tissue from the 300 μm sections (Fig. 1) using a sharpened trocar, with visual control under a dissection microscope (X4 magnification). These cylinders were postfixed in 1% osmium tetroxide and 1.5% potassium ferricyanide (1:1) for 30 min at room

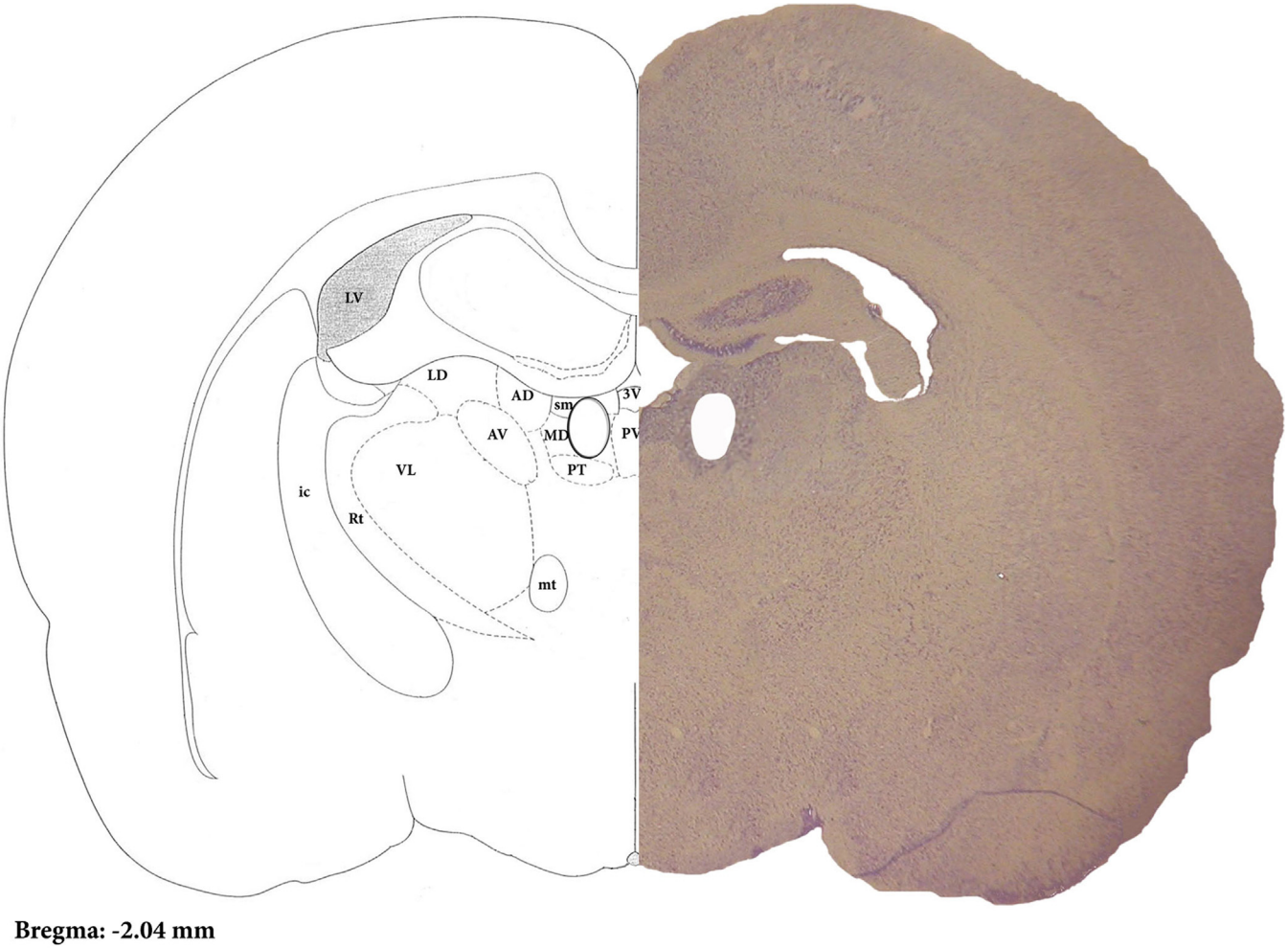

Fig. 1. Representative coronal vibratome sections and schematic illustration showing the MD thalamic area sampled for the study. The cylinders of tissue were removed from the sections with a sharpened trocar, with visual control under a dissection microscope (X4 magnification).