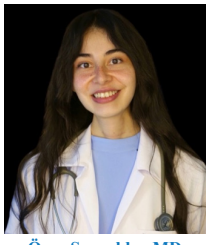
Amygdala Kindling Resistance in Rats with Genetic Absence Epilepsy: Role of Sex Differences

Özge Sarıyıldız[1], Elif Tuğçe Erdeve[2], Nursima Mutlu[1,3], Talat Taygun Turan[1], Nihan Çarçak Yılmaz[1,4], Filiz Yılmaz Onat[1,5]

[1]Acıbadem University Institute of Health Sciences, Department of Neuroscience, İstanbul, Türkiye
[2]İstanbul University Institute of Health Sciences, Department of Pharmacology, İstanbul, Türkiye
[3]İstanbul University Institute of Science, Department of Molecular Biotechnology and Genetics, İstanbul, Türkiye
[4]İstanbul University Faculty of Pharmacy, Department of Pharmacology, İstanbul, Türkiye
[5]Acıbadem University Faculty of Medicine, Department of Medical Pharmacology, İstanbul, Türkiye



Özge Sarıyıldız, MD

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Corresponding Author: Filiz Yılmaz Onat, MD, Prof., Acıbadem University Institute of Health Sciences, Department of Neuroscience; Acıbadem University Faculty of Medicine, Department of Medical Pharmacology, İstanbul, Türkiye, E-mail: filiz.onat@acibadem.edu.tr

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Abstract

Objective: Genetic Absence Epilepsy Rats from Strasbourg (GAERS) exhibit notable resistance to amygdala kindling-induced seizures compared to Wistar rats. However, the influence of sex difference on kindling resistance in GAERS remains unexplored. This study aims to evaluate whether male and female GAERS differ in their susceptibility to kindling.

Methods: Three-to-4-month-old female (n=6) and male (n=6) GAERS and male Wistar rats as control (n=6) were implanted with a stimulation electrode stereotaxically into the basolateral amygdala and four recording electrodes on the cortex. After one-week recovery, animals were stimulated at the afterdischarge (AD) threshold twice a day for kindling until the maximum number of 15 stimulations or three consecutive stage 5 seizures according to Racine's scale. Stage and cumulative amygdala AD duration were analysed using GraphPad Prism with a one-way ANOVA test.

Results: At the end of the kindling procedure, all Wistar rats exhibited stage 5 seizures, whereas neither male nor female GAERS rats progressed to stage 5 ($p<0.001$). Cumulative amygdala AD duration was significantly higher in male Wistar rats compared to male GAERS ($p=0.001$) and female GAERS ($p=0.01$). However, no significant difference in cumulative amygdala AD duration was observed between male and female GAERS ($p=0.94$).

Conclusion: We have confirmed that this resistance also applies to female GAERS. This finding implies the importance of studying sex differences in epilepsy, as most existing research has focused predominantly on males.

Keywords: Absence epilepsy, kindling resistance, epilepsy rat model

INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by spontaneous and recurrent seizures. It remains a significant global health issue, affecting approximately 50 million people worldwide.[1] Understanding the mechanisms underlying epilepsy development and progression, known as epileptogenesis, is critical for developing more effective treatments.[2] One widely used experimental model to study epileptogenesis and seizure dynamics is the kindling model.[3] In this model, repeated sub-treshold electrical stimulation of a specific brain region, often the amygdala, leads to the gradual development of seizures. Over time, this results in the generation of permanent changes in brain excitability, ultimately causing spontaneous seizures.[4,5] The Racine scale, which ranges from stage 1 (mild facial clonus) to stage 5 (generalized tonic-clonic seizures), is commonly employed to classify seizure severity in kindling studies.[6] The kindling model has been extensively studied in various rodent strains, each offering distinct insights into seizure susceptibility, progression, and resistance.[7] Genetic Absence Epilepsy Rats from Strasbourg (GAERS) is a well-established animal model for absence epilepsy, characterized by spontaneous spike-and-wave discharges (SWDs) seen on the electroencephalogram (EEG), which are also observed in human absence epilepsy.[8] GAERS have traditionally been considered resistant to convulsive seizures,[5,9,10] making them an interesting model to study epileptogenesis.[11] In contrast,

Wistar rats, commonly used as a control group, are susceptible to kindling, which produces convulsive seizures.[7,12,13] The differences in seizure susceptibility between these strains provide a valuable framework to investigate the underlying mechanisms of resistance and progression in kindling.[14,15] Previous studies have reported that GAERS rats exhibit slower kindling progression and lower seizure severity compared to non-epileptic controls.[1,5,15] The specific mechanisms behind this resistance are not fully understood, but it is hypothesized that both genetic and neurobiological factors contribute to their kindling resistance.[16,17] Additionally, sex differences in seizure susceptibility have been a topic of increasing interest, as hormonal influences and neuroanatomical differences between male and female rats may play a significant role in modulating seizure activity.[18,19] Understanding whether sex affects kindling resistance in GAERS rats is crucial, as it can provide insight into potential sex-specific therapeutic strategies for epilepsy.[7,12,20]

In this study, we investigated kindling resistance by comparing seizure progression and the duration of amygdala afterdischarge (AD) among male Wistar rats (control group), male GAERS rats, and female GAERS rats. We hypothesized that Wistar rats would exhibit faster kindling progression and longer AD durations compared to GAERS rats. We also aimed to explore potential sex differences in kindling dynamics within the GAERS population. By analyzing seizure stages, AD durations, and progression patterns, we sought to delineate the factors contributing to kindling resistance and their potential implications for understanding the broader mechanisms of epileptogenesis. Understanding these dynamics has significant implications for epilepsy research. For instance, investigating the differences between these strains could reveal potential targets for therapeutic interventions aimed at increasing seizure resistance. Additionally, understanding the sex-related factors influencing seizure dynamics could lead to personalized treatment strategies, considering sex-specific neurological and hormonal factors. This study provides new insights into the kindling resistance observed in GAERS rats and explores how both strain and sex contribute to differences in seizure dynamics. These findings are important for advancing our understanding of epilepsy and for developing novel and targeted approaches to managing this complex neurological disorder.

METHODS

Animals

Experiments were carried out with non-epileptic control male Wistar rats (n=6) and male (n=6) and female (n=6) GAERS rats aged 3 to 4 months. The animals were kept in a temperature-controlled room (20±3 °C) with a 12-h light-dark cycle. All animals were allowed free access to commercial rat pellets and tap water. All rats were housed in separate cages. The experimental protocol was approved by the Acıbadem Mehmet Ali Aydınlar University Ethical Committee for Experimental Animals (decision no: ACU-HADYEK 2023/39, date: 21.06.2023).

MAIN POINTS

- This study highlights the importance of studying sex differences in epilepsy research.
- Both male and female Genetic Absence Epilepsy Rats from Strasbourg (GAERS) show kindling resistance, with no significant difference between the sexes.
- Cumulative amygdala afterdischarge durations were significantly shorter in both male and female GAERS when compared to Wistar rats, suggesting greater seizure resistance in GAERS.

Stereotaxic Surgery

Animals were anesthetized using inhalation isoflurane (2.5-3%; the flow rate of oxygen was ~0.8 L/min) anesthesia and placed into a stereotaxic instrument (Stoelting Model 51,600, Stoelting Co. Illinois, USA). The scalp was longitudinally incised and the skull was leveled between lambda and bregma. A bipolar twisted stainless-steel electrode (MS303/1 twisted; Plastics One Inc., Roanoke, VA, USA), insulated except at the tip for stimulation, was implanted into the right basolateral amygdala (BLA) at coordinates anteroposterior (AP): -2.6 mm and lateral (L): 4.8 mm from bregma, and dorsoventral: -8.5 mm from the surface of the skull. For unilateral cortical EEG recording stainless steel screws were placed on the dura over the left frontal (AP: +2.0 mm and L: ±1.7 mm from bregma) and left occipital cortex (AP: -6.3 mm and L: ±4.0 mm from bregma). A ground electrode was placed over the cerebellum. Coordinates were obtained from the stereotaxic atlas of Paxinos and Watson,[21] and the bregma was used as the reference point. Electrodes were connected by insulated wires to a microconnector for EEG recordings, fixing them to the skull with dental acrylic following the surgery, the animals were returned to their cages for routine care, each housed individually, and allowed to recover for one week before the EEG recordings. The experimental study design is presented in Figure 1 and was created via BioRender.com.

Kindling

On the day of the experiment, the animals were placed in plexiglass cages and electrical stimulations were delivered from an isolated constant current stimulator (Accupulser A310, Stimulus isolator A365; World Precision Instrument, Sarasota, FL, USA). Following a 30-minute habituation period, basal EEG was recorded for 30 minutes. Then, the rats were stimulated with an initial stimulus of 50 μA (biphasic square wave pulses of 80 Hz, each 1 msec in duration, for a total duration of 2 seconds) and continued with 50 μA increments until an initial AD was obtained. The AD activity was defined as spike discharges lasting 2 seconds or more following the stimulation. The animals were stimulated twice daily with the determined current that produces AD in the EEG. Stimulations were given during a period that lacked SWDs in GAERS. If a SWD was observed in the EEG when the animal was connected to the stimulator, a waiting period was allowed before the stimulation was delivered. Seizure stages observed after each stimulus were classified using Racine's[6] standard 5-stage scale: stage 1, facial clonus; stage 2, rhythmic head movements, head nodding; stage 3, unilateral forelimb clonus; stage 4, bilateral forelimb clonus and rearing; stage 5, falling, rearing and tonic-clonic convulsion. If an animal had not reached stage 5 seizures in kindling development groups, electrical stimulation was terminated following the 15th stimulus. Thus, the maximum number of stimulations was 15 in the groups. Electrical activity in the amygdala and cortex was recorded with a PowerLab System before and after each stimulus. Amygdala AD durations were assessed from the EEG recordings offline.

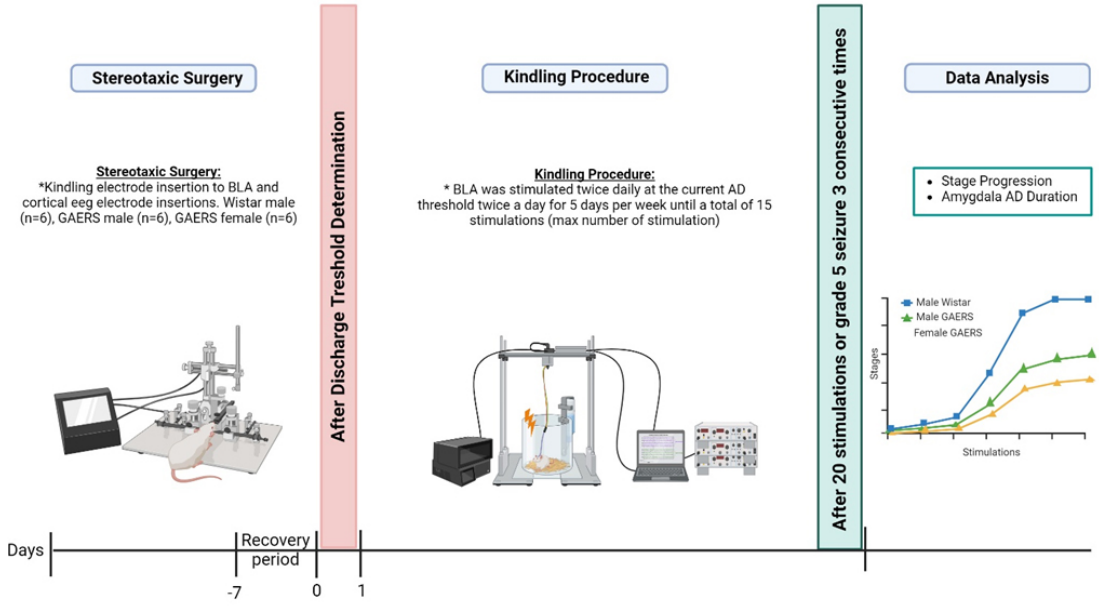

Figure 1. Experimental study design
GAERS: Genetic Absence Epilepsy Rats from Strasbourg, BLA: Basolateral amygdala, AD: Afterdischarge

EEG Analysis

Basal EEG was recorded from all Wistar rats to exclude any animal that could have absence-like activity. Electrical activity of the cortex and of the stimulated region of the amygdala was amplified through a BioAmp ML 136 amplifier, with bandpass filter settings at 1-40 Hz and recorded using Chart v.8.1 (PowerLab 8S ADInstruments, Oxfordshire, UK). A SWD complex in GAERS was identified as such if its duration was at least 1 s with a train of sharp spikes and slow waves (7-11 Hz) and amplitude of at least twice the background amplitude of the EEG.[10,22] The criteria for AD activity were defined as spike discharges lasting 2 seconds or more following stimulation, consistent with the guidelines.[6,16] The AD duration was measured as the total duration of spikes recorded on the EEG from the BLA electrode after the stimulation ended.

Histological Verification

At the end of the experiment, the animals were decapitated to verify the electrode placements. The brains were removed and placed in a formalin/sucrose mixture. Frozen sections were cut at 40 µm on a cryostat and stained with thionine. Only the animals with correct BLA electrode placement were included in this study (Figure 2).

Data Analysis

The results were expressed as "mean±standard error of the mean" and statistically evaluated using repeated measures ANOVA (GraphPad Prism, version 10.3.0, San Diego, CA, USA). One-way ANOVA was followed by Dunnett's post-hoc multiple comparison test. This was used to compare the mean numbers of stimulations to reach the first stage 2, 3, 4, and 5 seizures, among more than two groups. Statistical significance was determined at $p<0.05$.

RESULTS

The mean number of stimulations to display the first stage 5 seizure was 8.2±2.6 in Wistar control rats, as shown in Figure 3.

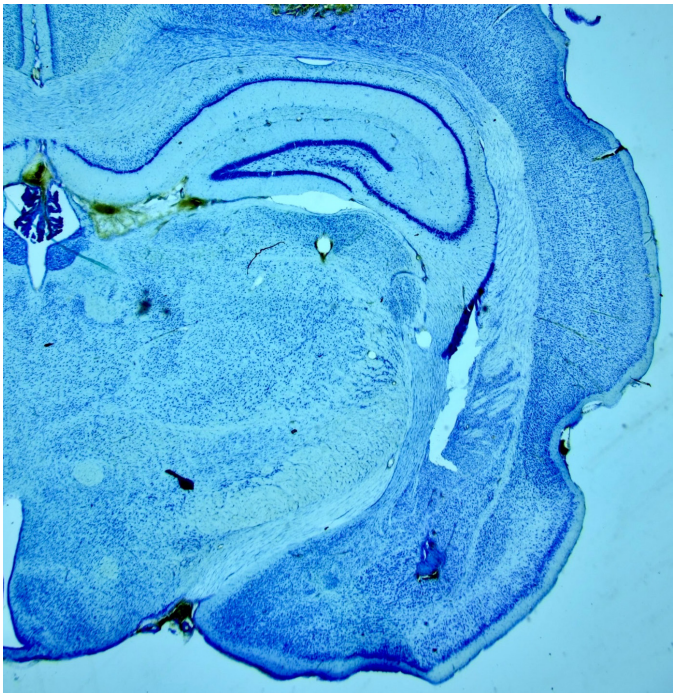

Figure 2. Histological verification of BLA electrode placement with thionine staining. The arrowhead in this coronal section indicates the location of BLA
GAERS: Genetic Absence Epilepsy Rats from Strasbourg, BLA: Basolateral amygdala

In contrast to Wistar rats, both male and female GAERS never showed stage 5 seizures following 15 stimulations (Figure 3). Therefore, all GAERS were stimulated until the maximum number of stimulations (15) was reached.

Kindling stimulation with currents at AD threshold produced AD that was recorded simultaneously from the amygdala and

frontoparietal cortices of both groups. Cumulative AD durations in the amygdala of Wistar rats (317.0±17.3s) were significantly longer than those in both male GAERS (91.22±23.47s) and female GAERS (82.28±14.44s). No significant difference in the cumulative AD duration in the amygdala was observed between male GAERS (91.22±23.47s) and female GAERS (82.28±14.44s), as shown in Figure 4.

DISCUSSION

The results of our study are consistent with previous studies and provide further evidence that while Wistar rats rapidly progress to stage 5 seizures and have longer cumulative AD durations, GAERS never progress to stage 3-5.[5,9] This indicates that GAERS rats have an enhanced resistance to kindling-induced seizure development. The absence of significant differences in stage progression and cumulative AD durations between male and female GAERS supports the hypothesis that genetic factors in GAERS rats provide resistance against kindling, regardless of sex.

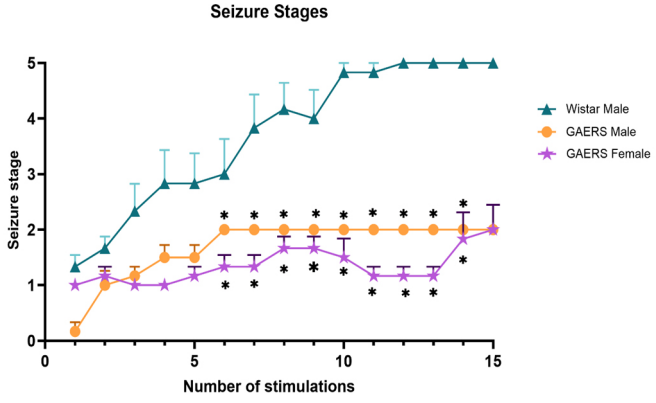

Figure 3. Amygdala kindling progression of male Wistar rats and male and female GAERS rats. Values are represented as mean±standard error of the mean (*p≤0.05)
GAERS: Genetic Absence Epilepsy Rats from Strasbourg, AD: Afterdischarge

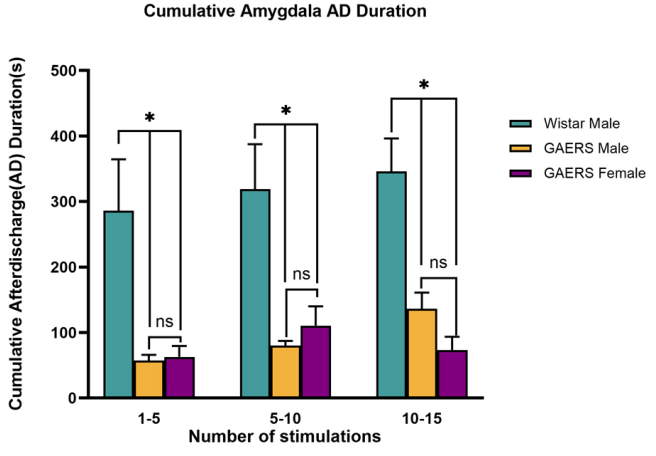

Figure 4. Cumulative amygdala AD duration of male Wistar rats and male and female GAERS rats. Values are represented as mean±standard error of the mean (*p<0.05)
GAERS: Genetic Absence Epilepsy Rats from Strasbourg, AD: Afterdischarge

In recent years, the significance of a male-female balance in preclinical research has gained prominence. Traditionally, preclinical scientific studies have involved only male subjects, which has led to obscured results and potentially dangerous consequences for women. This sex bias in research has overlooked how sex-specific factors may influence disease mechanisms and treatment responses, thus highlighting the critical need for balanced research on both sexes.

Recognizing this issue, the National Institutes of Health (NIH) has implemented policies supporting the inclusion of female subjects in preclinical experiments to ensure more accurate and comprehensive findings.[23,24] By promoting sex-balanced research, NIH aims to improve our understanding of sex-specific biological processes, which is crucial for developing targeted therapies.[23,24]

Study Limitations

A limitation of this study is that only male Wistar rats were used as controls, precluding an assessment of potential sex differences in seizure susceptibility within the Wistar group. Previous studies have demonstrated significant sex differences in drug-resistant epilepsy and in antiseizure drug pharmacokinetics, including variations in drug half-life influenced by hormonal and metabolic factors.[25,26] Therefore, to avoid redundancy, maintain focus on our primary research objectives, and minimize unnecessary animal use, in accordance with ethical principles, female Wistar rats were not included in this study. In addition, the study focused solely on behavioural and electrophysiological data and did not explore the molecular and genetic mechanisms that may underlie the observed kindling resistance in GAERS rats. Inclusion of such data could provide a deeper understanding of why GAERS resists seizure progression. Furthermore, monitoring the estrogen cycles of female GAERS rats would be essential to determine whether hormonal fluctuations affect seizure susceptibility, as estrogen has been implicated in modulating seizure activity in other epilepsy models. Together, these elements would enrich the results of the study and contribute to a more comprehensive view of seizure susceptibility and sex differences in epilepsy models.

CONCLUSION

The observed findings highlight our understanding of how kindling mechanisms may vary by sex. Further studies are needed to explore the underlying mechanisms of kindling resistance in female and male GAERS. Addressing this is crucial for developing targeted therapies and improving our understanding of sex-specific epileptic processes. Ultimately, a comprehensive examination of both sexes will enhance the efficacy of epilepsy treatments and contribute to more personalized medical approaches.

Ethics

Ethics Committee Approval: The experimental protocol was approved by the Acıbadem Mehmet Ali Aydınlar University Ethical Committee for Experimental Animals (decision no: ACU-HADYEK 2023/39, date: 21.06.2023).

Informed Consent: Animal experiment.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.S., E.T.E., N.M., T.T.T., N.Ç.Y., F.Y.O., Concept: N.Ç.Y., F.Y.O., Design: N.Ç.Y., F.Y.O., Data Collection or Processing: Ö.S., E.T.E., N.M., T.T.T., Analysis or Interpretation: Ö.S., E.T.E., N.M., T.T.T., Literature Search: Ö.S., E.T.E., N.M., T.T.T., Writing: Ö.S., N.Ç.Y., F.Y.O.

Conflict of Interest: No conflict of interest was declared by the authors.

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