Special Issue - Neuroscience

Age-dependent Electroencephalographic Differences in the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) Model of Absence Epilepsy

Mohammad Rafiqul Islam[1,2], Jafri Malin Abdullah[1,3]

Submitted: 3 Nov 2014
Accepted: 12 Nov 2014

[1] *Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*

[2] *Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*

[3] *Center for Neuroscience Services and Research, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*

Abstract

Genetic Absence Epilepsy Rats from Strasbourg (GAERS) are a prognostic genetic model of absence epilepsy. This model displays the electro-clinical, behavioural, and pharmacological features of absence seizures. Although GAERS share typical characteristics, including spike-and-wave discharges (SWDs) in the electroencephalography (EEG), age-dependent studies with these animals have not yet been reported. The aim of the present study is to perform a systematic comparison contrasting the SWDs of young and older GAERS, in terms of the number, duration, frequency, and waveform morphology of the discharges, as well as the pre-SWD EEG characteristics, using identical measurement and analysis techniques. The number, cumulative total duration and mean duration of SWDs were significantly higher in young GAERS (4 to 6 months) compared to older GAERS (12 to 14 months). Furthermore, the SWD spectra and average SWD waveforms indicated that a single cycle of the SWD contains more energy in faster components, such as increased spikes and higher power, in the SWDs of the young GAERS. Additionally, older GAERS showed weak amplitude spikes in SWDs and higher power pre-SWDs. These clear morphological differences in the EEGs of young and older GAERS rats should be further examined in future studies that explore new dimensions of genetic absence epilepsy.

Keywords: *epilepsy, electroencephalography, frequency, seizure, rat*

Introduction

Typical absence epilepsy is a type of non-convulsive epilepsy that differs in many respects from other forms of epileptic seizures. Typical absence seizures are characterised by a brief unresponsiveness to environmental stimuli and cessation of activity, which can be accompanied by automatisms or moderate tonic or clonic components affecting the limbs, eyeballs or eyelids. Clinically, typical absence epilepsy is associated on electroencephalography (EEG) with bilateral, synchronous and regular, spike and wave discharges (SWDs) that start and end abruptly (1,2). SWDs are regular, symmetrical, generalised, and transient electroencephalographic patterns observed in many types of epilepsy, particularly typical absence seizures. In human absence seizures, SWDs typically recur at a slow frequency of approximately 3 Hz, whereas in the genetic model absence epileptic rat strains, they occur faster (7–11 Hz) (3,4).

No structural lesion of any type has ever been identified as the substrate of typical absence epilepsy (1,5). Their cause is increasingly regarded as genetic (6–8). Because typical absence epilepsy mainly affects children and adolescents with moderate consequences, studies of the pathophysiological mechanisms cannot be conducted in humans for ethical reasons. Therefore, much of the recent information available about the pathophysiology of absence seizures was derived from studies in animal models. To be valid as a model of human disease, animal models should ideally exhibit similar clinical (isomorphism) and pharmacological (predictivity) characteristics to those observed in humans. In addition, they should have a similar

aetiology (homology) to the human disease (9). Models displaying clinical and pharmacological characteristics of absence seizures are either experimentally induced or genetically determined. SWDs can be pharmacologically induced in rodents, cats or primates by injection of pentylenetetrazol, penicillin, gamma-hydroxybutyrate or GABA agonists (10,11).

Two independent genetic models (WAG/Rij and the GAERS strain) are available for describing absence epilepsy phenotypes. Animal models for seizures and epilepsy have played a fundamental role in advancing our understanding of the basic mechanisms underlying ictogenesis and epileptogenesis and have been instrumental in the discovery and preclinical development of novel antiepileptic drugs. Despite the successful development of various new antiepileptic drugs in recent decades, the search for new therapies with better efficacy and tolerability remains an important goal (12). The discovery and development of new antiepileptic drugs rely heavily on the preclinical use of animal models to establish efficacy and safety prior to the first trials in humans (13).

Greater insight into the pathophysiology underlying epilepsy can be achieved by the use and study of experimental animal models (14). Genetic Absence Epilepsy Rats from Strasbourg (GAERS) are a strain of Wistar rats that was originally bred in Strasbourg (15), and these animals are generally accepted as an experimental animal model of human absence seizure because GAERS share many clinical characteristics with typical human absence epilepsy (16). All GAERS show idiopathic (genetic) generalised seizures characterised by paroxysmal unresponsiveness to environmental stimuli and a cessation of ongoing activity. During the epileptic episodes, the EEG of GAERS displays the bilateral and synchronous spike and wave discharges (SWDs), predominantly over the fronto-parietal cortex (17). The number, duration, and frequency of SWDs increase to reach a maximum at the age of four to six months, at which point, 100% of GAERS have developed SWDs (18). GAERS represent a useful animal model for the evaluation and development of new antiepileptic drugs, as it is known that drugs that are effective against human absence seizures also exhibit dose-dependent suppression of SWDs in GAERS (19). The aim of this study was to perform a systematic comparison contrasting the SWDs of young and older GAERS, in terms of the number, duration, frequency, waveform discharge morphology and pre-SWD EEG characteristics to identify the age-dependent SWD differences that should be further examined in future studies of absence epilepsy.

Materials and Methods

Animal subjects

For the present experiments, 4- to 6-month-old male GAERS (young; n = 8) and 12- to 14-month-old male GAERS (older; n = 8), weighing 220–325 g, were used. The parent GAERS were gifted from Kyoto University, Japan. The animals were bred in our laboratory and maintained under environmentally controlled conditions (12 hr light/dark cycles, 20–22 °C) in the animal facility house of the Universiti Sains Malaysia (USM) Health Campus, with food and water *ad libitum*. All experiments were conducted according to the guidelines approved by the Animal Ethics Committee of USM.

Surgery: Implantation of Data Sciences International (DSI) telemetry

Rats were anesthetised with ketamine (80 mg/kg, i.p.) and xylazine (7.5 mg/kg, i.p.) with additional ketamine (5 mg/kg, i.p.) given during surgery when sensorial pain stimulated by squeezing the footpad elicited a motor reflex (15). After adequate anaesthesia, the fur on the head and back was clipped rostral to the medial canthus of the eyes to immediately cranial to the last cervical vertebra in a strip approximately 3 cm wide. The animals were placed on a heating pad and secured into a stereotaxic apparatus (Stoelting Model 51600; Illinois, U.S.A.). The surgical site and surrounding area were swabbed with 70% ethyl alcohol and scrubbed with a 4% chlorhexidine solution. A 3-4 cm mid-sagittal incision was made into the scalp, and the skin was reflected with haemostats to expose the entire dorsal portion of the skull. The periosteum was removed and homeostasis achieved with sterile cotton-tip applicators. Bregma was marked, and two holes were bored through the skull with a drill (# 105 drill bit). Stainless steel epidural recording electrodes (DSI Model F40-EET; St. Paul, MN, U.S.A.) that were insulated except at the tip were implanted bilaterally into the parietal cortex. The other two electrodes were placed into the neck muscle for electromyography EMG recording to compare with the EEG spikes. The EEG electrodes were fixed to the skull with dental acrylic. The radio telemetry unit was placed subcutaneously into a pocket over and caudal to the scapula. Using blunt-ended scissors, a subcutaneous pocket was made caudally from the incision by pushing aside connective tissue and then skin was sutured

(Figure 1). The method of telemetry implantation followed from previous study (13,20). The surgical procedures of our experiment were considered to elicit minimum to mild pain according to the pain assessment, and this pain was managed by local anaesthesia (21). Following surgery, the rats were allowed a 1 week recovery period before starting EEG recordings.

Recording of EEGs

The rats were habituated to EEG recording system and connected to leads a day before the recording session. The next day, after 1 h adaptation period, the EEG recordings were taken for 6 h (between 9 am and 3 pm).

The EEG activity was acquired by DSI Dataquest telemetry software and analysed off-line using DSI Neuro-Score software (St. Paul, MN, USA), which was configured for automatic detection and saving SWDs. EEG analysis was performed by a 'blinded' unbiased investigator. All SWDs of the EEG were revised manually.

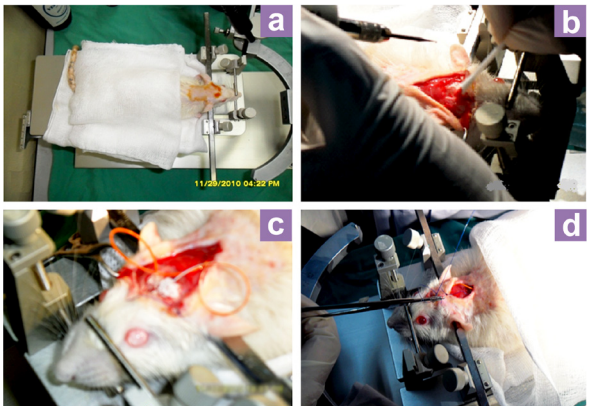

Figure 1: Implantation of Data Sciences International (DSI) telemetry system into GAERS rat.

Analysis of SWD intensities

An SWD was identified as a characteristic train of sharp asymmetric large-amplitude spikes and slow waves lasting at least 1 s (22). The first and last spike of a SWD complex with amplitudes at least 2-fold higher than the basal EEG signal were accepted as the SWD onset and offset. The intensity of the SWDs was analysed over a 6 h period divided into 1 h intervals. The cumulative total duration and number of SWDs were measured over 1 h intervals. The mean duration of the SWDs was calculated as the ratio of the cumulative total duration to the number of SWDs.

Statistical analysis

The results were expressed as the mean (SD). Student's *t* test was used to evaluate the significance of the differences between the means of the cumulative total duration, the duration and the number of SWDs of both groups.

Results

SWDs intensities

The hourly number, cumulative total duration and mean duration of SWDs from young GAERS and older GAERS are shown in Figure 2. In 4- to 6-month-old GAERS, SWDs start and end abruptly on a normal EEG background, and the peaks were approximately three times higher than the baseline signal, whereas in the older GAERS, pre-SWDs were strong and the peaks were approximately two times higher than the baseline signal (Figure 3). The voltage varied

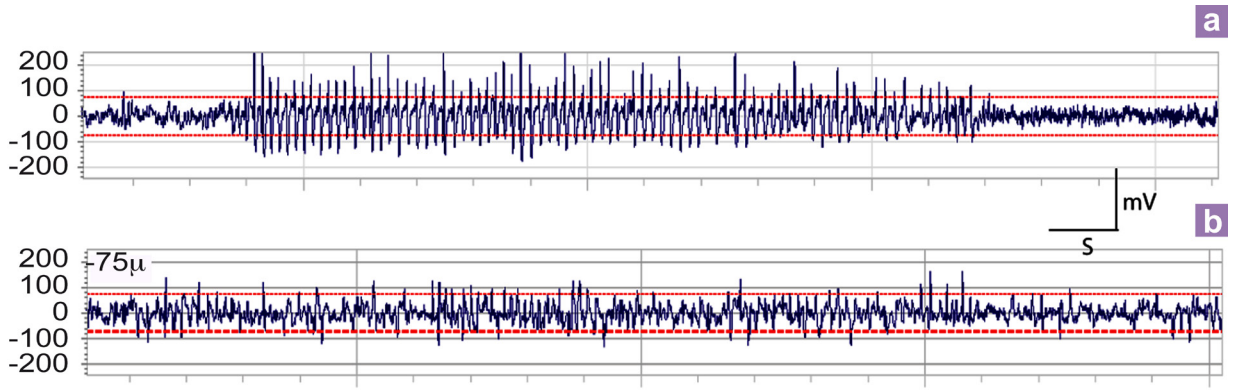

Figure 2: (a) Electroencephalographic activity recorded from the young GAERS and old GAERS (b) that show spikes and wave discharges (SWDs) surrounded by normal background electroencephalography (EEG).

from 300 to 1000 mV. These parameters were significantly higher in 4- to 6-month-old young GAERS compared to (12- to 14-month-old) older GAERS ($P < 0.05$).

Frequency characteristics of SWDs

The spectral characteristics of SWDs in the young and older GAERS were analysed by computing the power spectra using the short-time Fourier transform (17). Because the discharge frequency decreases along each SWD complex, the average power-spectra of the first, middle and last 2 s segments of the SWDs were computed for each group. The average power-spectra of 20 SWD complexes were computed for each animal.

In addition to the SWDs, the power spectrum of the transitional periods of 1 s duration immediately before each SWD (pre-SWD) was computed. The baseline spectra were also computed from an awake EEG signal that was at least 10 s away from any SWD pattern. These spectra were compared within each group to monitor the pattern in the frequency content of the EEG during the pre-SWD period. Additionally, both the baseline and pre-SWD spectra were compared between the two groups.

To avoid the effects of any large-scale amplitude variability among the EEGs of individual animals, the spectra were normalised by dividing the power at each frequency by the

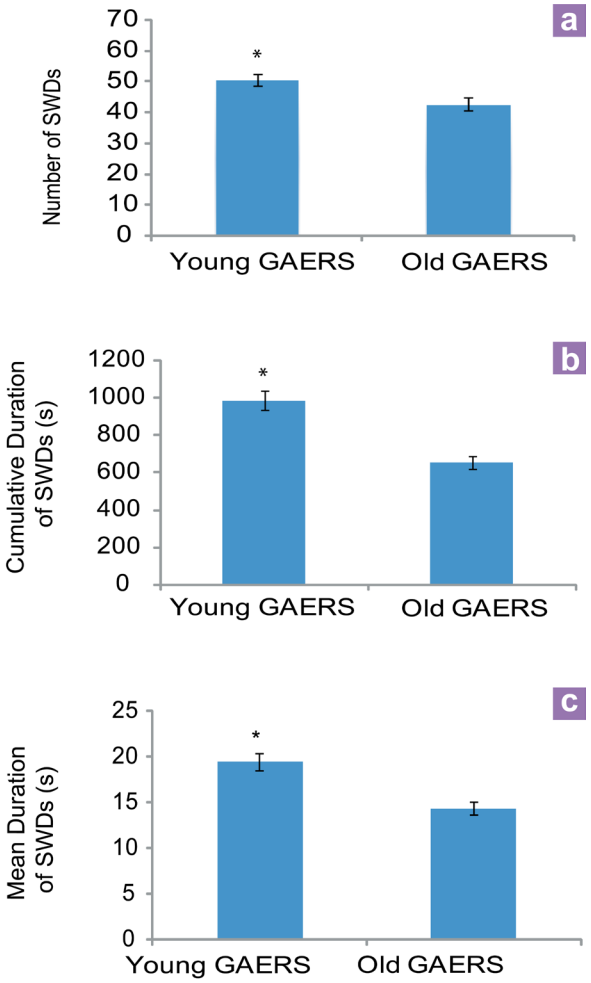

Figure 3: (a) The mean number, (b) cumulative total duration and mean duration (c) of SWDs for 1-h period in young and old GAERS rats. *$P < 0.05$ significance differences are present when young GAERS are compared to old aged GAERS rat.

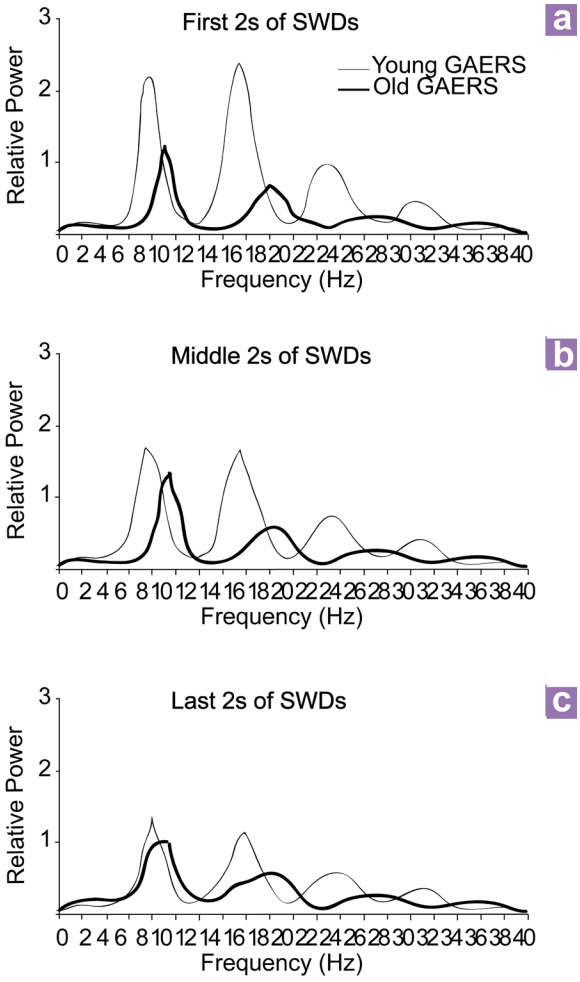

Figure 4: (a) The power spectra of the first, (b) middle and last (c) 2 s of the SWDs in young and old aged GAERS rats. Relative powers were calculated by dividing the power at each frequency by the sum of the power between 0 and 40 Hz in the baseline EEG.

sum of the power of the baseline EEG between 0 and 40 Hz.

The power spectra of the first, middle and last 2 s of the SWD complexes consisted mainly of peaks at the discharge frequency and its harmonics. The peaks at the fundamental frequency and first 2 harmonics could be easily identified in the spectra of both young and old GAERS groups (Figure 4). In both groups, the discharge frequency decreased along the SWD complex. Changes in the mean discharge frequency among the first, middle and last 2 s of the SWD complexes for the young and old GAERS groups are shown in Figure 5. The old GAERS group showed a sharper decrease in discharge frequency along the SWD complex compared with the young GAERS group. To better understand this effect along the three periods of the SWDs, we pair-wise compared the SWD frequency in each period between both groups with post hoc t-tests. The mean SWD frequency was 7.58 Hz (SD 0.29) for young GAERS and 9.35 Hz (SD 0.35) for old GAERS during the first 2 s period of the SWDs; 7.00 (SD 0.24) for young and 7.55 (SD 0.31) for old GAERS during the middle 2 s of the SWDs; and 5.55 Hz (SD 0.21) for young GAERS and 6.75 Hz (SD 0.45) for old GAERS during the last 2 s period of the SWDs, confirming that the significantly higher frequency in the first part of the SWDs in old aged GAERS decreases more sharply than that in GAERS along the SWD, until both age groups fail to show a significant difference in SWD frequency during the last 2 s period.

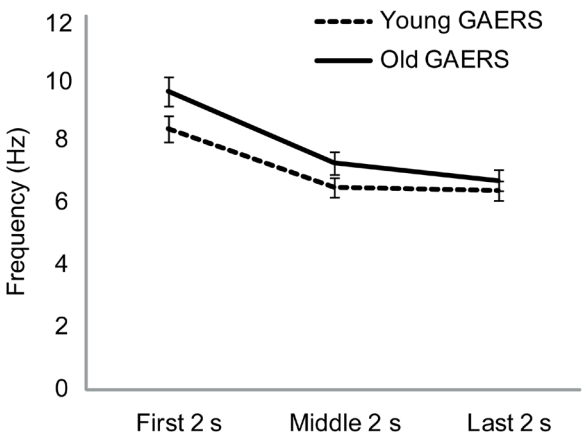

Figure 5: Change of the mean discharge frequency among the first, middle and last 2 s of the SWDs between two groups. The old GAERS showed a stronger decrease in the discharge frequency along the SWDs compared with the young GAERS rats.

Discussion

The intensity according to the number, cumulative duration and mean duration of SWDs was significantly higher in young GAERS compared to older GAERS. In the spectra, we found that the peak frequency was higher in the older GAERS during the beginning of SWDs. To the best of our knowledge, this is the first report to compare the spectral characteristics of SWDs between young GAERS and older GAERS and shows a statistically significant difference in the SWD frequency.

A sharper decrease in the SWD frequency coinciding with a shorter discharge duration was observed by (23), which resembles our findings of a sharper frequency decrease coinciding with a shorter SWD duration in old GAERS compared with young GAERS. However, the summed power at the fundamental frequency and at the 1st and 2nd harmonics was significantly higher in the young GAERS during the first 2 s of the SWDs, which could be attributed to the higher power at the 1st harmonic (which was even stronger than the power at the fundamental frequency in GAERS). The spike and late positive transient were larger in the young GAERS group, while the slower wave component was of similar shape and amplitude in older GAERS.

Relative Amplitudes of Spikes and Waves

Like our findings in young GAERS, (15,24) showed that in GAERS, SWDs start and end abruptly on a normal EEG background. In 4-month-old GAERS from the 30th generation, the mean frequency of spike and wave complexes within a discharge was 920.5 c/s, and their voltage varied from 300 to 1,000 mV. Van Hense et al. (2003) (17) observed from the cortical epidural EEG of GAERS that the fundamental frequency was from 7 to 11 Hz ; the amplitude varied from 300 to 1000 mV; and the mean duration of SWDs was 10.0 s (SD 8.5) (1-65 s). In young GAERS, we also observed similar results.

Sitnikova and van Luijtelaar (2007) (25) categorised the waveform morphology of SWD in the EEG of the WAG/Rij rats according to the definitions of transient wave components of SWD first described by Weir (1965) (26), who explained that spike 1, an early positive transient, spike 2, a

late positive transient and the wave are sequential components of the SWD pattern. Sitnikova and van Luijtelaar (2007) observed that the largest component of type I SWD of WAG/Rij rats, spike 2, is most pronounced in the frontal cortex, suggesting that the amplitude of this feature is mainly controlled by local processes within the anterior cortex, while the late positive transient is generated by the projection of spike 2 via dense descending pathways of the frontal cortex to the thalamus (25). In the present study, we clarified that spike 2 and the late positive transient were clearly higher in amplitude in the GAERS rats. The finding that relative amplitudes of the spike vs. wave components differed across age groups might be an important feature of the SWDs that reflects differences in frontal cortex excitability.

Conclusion

Under the same experimental conditions and using identical measurement and analysis techniques, we found significant differences in the number, mean duration, cumulative duration and spike-wave frequency of the SWDs, as well as in the spectral characteristics of the pre-SWD EEGs, in the absence epilepsy rat model of young and old GAERS. Furthermore, we found that the relative amplitudes of the spike vs. wave components within a single SWD cycle were also significantly different according to age. The assessment of these findings suggests that each of these variables represents a phenotypic feature of SWDs that is age-dependent in GAERS, which should be further examined in future studies of absence epilepsy.

Acknowledgement

We acknowledge the distributor of above GAERS rats are used in this experiments from the Institute of Laboratory Animal, Graduate School of Medicine, Kyoto University, Japan and this work was supported by an institutional grant from USM Research University Individual grant (No. 1001/PSKBP/812019).

Conflict of Interest

None.

Funds

USM Research University Individual grant (No. 1001/PSKBP/812019).

Authors' Contributions

Conception and design, statistical expertise, analysis and interpretation of the data, final approval of the article: MRI, JMA
Drafting of the article: MRI
Critical revision of the article: JMA

Correspondence

Professor Dato' Jafri Malin Abdullah
MD, PhD, FRCS (Ed), FACS, DSCN (Belgium)
Center for Neuroscience Services and Research
Universiti Sains Malaysia
16150 Kubang Kerian
Kelantan, Malaysia
Tel: +609-767 2083
Fax: +609-767 2084
Email: brainsciences@gmail.com

References

1. Niedermeyer E. Primary (idiopathic) generalized epilepsy and underlying mechanisms. *Clinic Electroencephalograph*. 1996;**27(1)**:1–21.
2. Panayiotopoulos CP. Typical absence seizures and their treatment. *Arch Dis Child*. 1999;**81(4)**:351–355. doi: 10.1136/adc.81.4.351.
3. Drinkenburg WH, van Luijtelaar EL, van Schaijk WJ, Coenen AM. Aberrant transients in the EEG of epileptic rats: a spectral analytical approach. P*hysiol Behav*. 1993;**54**:779–783.
4. Panayiotopoulos CP. Typical absence seizures and related epileptic syndromes: assessment of current state and directions for future research. *Epilepsia*. 2008;**49(12)**:2131–2139. doi: 10.1111/j.1528-1167. 2008.01777.
5. Berkovic SF, Andermann F, Andermann E, Gloor P. Concepts of absence epilepsies: discrete syndrome or biological continuum? *Neurol*. 1987;**37**:993–1000.
6. Lennox WG, Lennox MA. *In: Epilepsy and related disorders. Volume 1&2*. Boston (MA): Little Brown & Co;1960.
7. Doose H, Gerkin T, Horstmann T, Volzke E. Genetic factors in spike-wave absences. *Epilepsia*. 1973;**14**:57–75.
8. McNamara JO. Cellular and molecular basis of epilepsy. *J Neurosci*. 1994;**14(6)**:3413-3425.
9. Kornetsky C. *Animal models: promises and problems*. In: Hanin I, Udsin E, editors.. Animal models in Psychiatry and Neurology. Oxford (OX): Pergamon Press; 1977. p. 1-7.
10. Snead OC. Gamma-hydroxybutyrate model of generalized absence seizures: Further characterization and comparison with other absence models. *Epilepsia*. 1988;**29(4)**:361–368.

11. Snead OC. P*athophysiological mechanisms of experimental generalized absence seizures in rats*. In: Malafosse A, Genton P, Hirsch E, Marescaux C, Broglin D, Bernasconi R, editors. Idiopathic Generalized Epilepsies: Clinic Exp Genetic Aspects. London (LN): John Libbey; 1994. p. 133–150.

12. Bialer M, White HS. Key factors in the discovery and development of new antiepileptic drugs. *Nat Rev Drug Discov*. 2010;**9(1)**:68–82. doi: 10.1038/nrd2997.

13. White AM, Williams PA, Ferraro DJ, Clark S, Kadam SD, Dudek FE, et al. Efficient unsupervised algorithms for the detection of seizures in continuous EEG recordings from rats after brain injury. *Neurosci Methods*. 2006;**152(1–2)**:255–266. doi: 10.1016/j.jneumeth.2005.09.014

14. Dedeurwaerdere S, Boon P, De Smedt T, Claeys P, Raedt R, Bosman T, et al. Chronic levetiracetam treatment early in the life decreases epileptiform events in young GAERS, but does not prevent the expression of spike and wave discharges during adulthood. *Seizure*. 2005;**14(6)**:403–411. doi: 10.1016/j.seizure.2005.07.001.

15. Marescaux C, Vergnes M, Depaulis A. Genetic absence epilepsy in rats from Strasbourg-A review. *J Neural Transmiss*. 1992;**35**:37–69.

16. Danober L, Deransart C, Depaulis A, Vergnes M, Marescaux C. Pathophysiological mechanisms of genetic absence epilepsy in the rat. *Prog Neurobiol*. 1998;**55(1)**:27–57. doi: 10.1016/S0301-0082(97)00091-9.

17. Van Hese P, Marten JP, Dedeurwaerdere S, Boon P, Lamahieu et al. Detection of SWD in the cortical EEG of GAERS. *Phys Med Biol*. 2003;**48(12)**:1685–1700. doi:10.1088/0031-9155/48/12/302.

18. Pinault D, Vergnes M, Marescaux C. Medium-voltage 5–9 Hz oscillations give rise to spike-and-wave discharges in a genetic model of absence epilepsy: in vivo dual extracellular recording of thalamic relay and reticular neurons. *Neurosci*. 2001;**105**:181–201. doi: 10.1016/s0306-4522(01)00182-8.

19. Large CH, Sokal DM, Nehlig A, Gunthorpe MJ, Sankar R, Crean CS, et al. The spectrum of anticovulsant efficacy of retibine (ezogabine) in animal models: Implications for clinical use. *Epilepsia*. 2012;**53(3)**:425–436. doi: 10.1111/j.1528-1167.2011.03364.x.

20. White A, Williams PA, Hellier JL, Clark S, Dudek FE, Staley KJ. EEG spike activity precedes epilepsy after kainite-induced status epilepticus. *Epilepsia*. 2010;**51(3)**:371–383. doi: 10.1111/j.1528-1167.2009.02339.

21. Kohn DF, Martin TE, Foley PL, Morris TH, Swindle MM, Vogler GA, et al. Public statement: guidelines for the assessment and management of pain in rodents and rabbits. *J Am Assoc Lab Anim Sci*. 2007;**46(2)**:97–108.

22. Midzianovskaia IS, Kuznetsova GD, Coenen AM, Spiridonov AM, van Luijtelaar EL. Electrophysiological and pharmacological characteristics of two types of spike-wave discharges in WAG/Rij rats. *Brain Res*. 2001;**911(1)**:62–70. doi: 10.1016/S0006-8993(01)02705-6.

23. Bosnyakova D, Gabova A, Zharikova A, Gnezditski V, Kuznetsova G, van Luijtelaar G. Some peculiarities of time-frequency dynamics of spike-wave discharges in humans and rats. *Clin Neurophysiol*. 2007;**118(8)**:1736–1743. doi: 10.1016/j.clinph.2007.04.013.

24. Vergnes M, Boehrer A, Simler S, Bernasconi R, Marescaux C. Opposite effects of GABAB receptor antagonists on absences and convulsive seizures. *Eur J Pharmacol*. 1997;**332(3)**:245–255. doi: 10.1016/S0014-2999(97)01085-6.

25. Sitnikova E, van Luijtelaar G. Electroencephalographic characterization of spike-wave discharges in cortex and thalamus in WAG/Rij rats. *Epilepsia*. 2007;**48(12)**:2296–2311. doi: 10.1111/j.1528-1167.2007.01250.

26. Weir B. The morphology of the spike-wave complex. Electroencephalogr. *Clin Neurophysiol*. 1965;**19(3)**:284–290.