Neural Circuits (2017) Volume 1 Issue 1, pp.x–x.
doi: 10.24294/nc.v1i1.61

ORIGINAL RESEARCH ARTICLE

A brief review of practical epileptology in the field of basic science: Epileptic mutant animals

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ABSTRACT

The need for epileptic mutant animals is on a revived trend. The concept of epilepsy is a process in which genetic, developmental and acquired insults lead to recurrent/spontaneous seizures (epilepsy) through latency. Drug-induced epilepsy models or kindling models are not preferable in detecting changes in the development. On the contrary, following the development of epileptic mutant itself means searching for the epileptogenesis. Using epileptic mutant animal models may be better for searching promising biomarkers of epilepsy.

Keywords: *epileptic mutant animals; biomarker of epilepsy*

ARTICLE INFO
Received: July 2, 2017
Accepted: September 24, 2017
Available online: November 23, 2017

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CITATION
Murashima YL. A brief review of practical epileptology in the field of basic science: Epileptic mutant animals. Neural Circuits 2017; 1(1): x–x.
doi: 10.63018/nc.v1i1.61.

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Introduction

Epileptic mutant animals are normally kept in frozen embryo to spare the costs and manpower. However, the seizure thresholds of epileptic mutants are very delicate and the reproducibility of seizure threshold through frozen embryo procedures is unstable. The best way is to keep animals in a systemic inbred strain. However, the technical difficulties made the demand lesser. The International League Against Epilepsy (ILAE) and Workshop on Neurobiology of Epilepsy (WONOEP) raised the priority of "biological marker of epilepsy (BME)" in 2015. The need for epileptic mutant animals is on a revived trend. The concept of epilepsy is a process in which genetic, developmental and acquired insults lead to recurrent/spontaneous seizures (epilepsy) through latency (Figure 1 and Figure 2). Drug-induced epilepsy models or kindling models are not preferable in detecting changes in the development. On the contrary, following the development of epileptic mutant itself means searching for the epileptogenesis. This paper presents a typical example: one of the most commonly used epileptic animal models may be the "pilocarpine model". Once pilocarpine is applied, the animal shows status epilepticus instantly, followed by a silent period (latency) and then establishes spontaneous seizures. During the latency and just after the status epilepticus, one can study the mechanism of epileptogenesis and one can analyze the mechanism of ictogenesis and the transition from ictogenesis to epileptogenesis. However, it will be impossible to detect the relationship between the development and the expression of BME (otherwise, it is very attractive that instantly starting experimental epileptology is possible).

Evolution of Concept

1. Epilepsy does not start with the first seizure.
2. Symptomatic seizures are not epilepsy.
3. During human/rodent development, biomarker can change.

Figure 1. Basic definition for epilepsy and biomarker of epilepsy (WONOEPXIII, Istanbul, 4th September 2015)

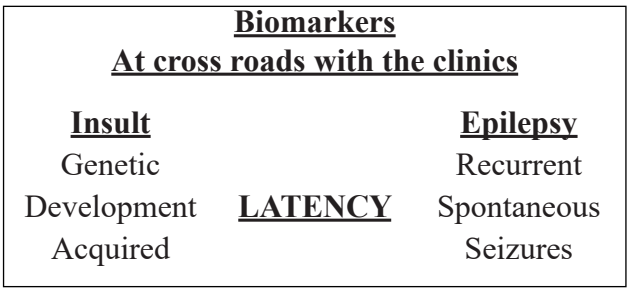

Figure 2. Requirements for biomarker of epilepsy from the aspects of human epilepsy (WONOEPXIII, Istanbul, 4th September 2015)

Epileptic mutants are divided into two groups: one is single-gene group whose responsible gene is uniquely identified, and the other is polygene group whose responsible genes are heterogeneous and not identified uniquely. The typical former one is spontaneously epileptic rat (SER), which has been established in Japan; Genetic absence epilepsy rat from Strasbourg (GAERS), a model of absence seizure, has been established in France. An example of the latter one is EL which was also established in Japan.

How to breed, proliferate and maintain these strains and, furthermore, how to use these animals in the experiments are described here in detail to maximize the merits of these epileptic mutants of epilepsy.

SER/*Kyo*

Origin: SER/*Kyo* was developed at the animal center of the medical school in Kyoto University, Japan. Two different type of mutant strains were established from genetic crossing of tremor rat (inbred TRM) and zitter rat (inbred ZI)[1]. The pathogenic rat SER/*Kyo* is autosomal recessive. It is a double mutant with homozygous mutant genes tremor (*tm*) and zitter (*zi*) together. *Tm* was lacking 200-kb including *Aspa* (*Aspartoacylase*) gene. *Zi* is known to be an 8-bp deletion of *Atm* (*attractin*) gene.

Characteristics: SER/*Kyo* shows severe absence seizures and tonic seizures spontaneously[1,2]. From developmental aspects, **SER/*Kyo*** shows tremor from the age of 2–11 weeks. Absence seizure is characterized by 5–7 Hz spike and wave complex after five weeks of age[3]. Tonic seizures are observed after six weeks of age. Additionally, wild jumping and running are observed. Pharmacological studies have clarified that tonic seizures of SER are compatible with human grand mal seizures and absence seizures of SER are compatible with human petit mal absence[2]. Also, SER shows severe spongiform brain degeneration, hypomyelinogenesis[4], auditory disturbance and behavioral abnormalities due to the TRM.

Keeping strain methods: Inbreed with homo *zi* and hetero *tm*.

Genetic absence epilepsy rat from Strasbourg (GAERS)

Origin: Vergnes *et al*. have established it from the inbred stains, which show frequent bursts of high voltage spike and wave (SW) with motor arrest[5,6].

Clinical electroencephalogram (EEG) findings: GAERS show 7–11 Hz SW bursts at least over once a minute. Every burst continues over 15 seconds. However, background EEG is normal. GAERS show these findings after 30 days of age and show maximum at four months of age, because these clinical symptoms are completely dependent on the development. These clinical findings never change after four months till death.

Pharmacopathological findings: GABAergic systems, especially $GABA_B$ receptors abnormalities, are suspected as may be the cause of SW discharge[5]. Especially, the involvement of reticular thalamic nuclei (Rt) is suspected. Rt neuron recording by the patch-clamp method elucidates that Rt low-threshold Ca^{2+} current causes over-burst discharges, which in turn show SW discharges through abnormal rhythmic firings[5].

EL mouse

Background: Imaizumi *et al*.[7] found seizure strain mice in the hydrocephalus II DDY mouse strain. At first, he suspected that EL showed seizure because of the infection. He inbred the strain and maintained it for a year. After that, the infection of EL was denied, and he proposed the "Ep" (epileptic mouse) strain internationally. However, Ep was misunderstood as the initials for epidermis. In 1960, Ep was changed as El. Nevertheless, with the propagation of citation system, and "El" being also a Spanish word, a big confusion occurred. In 1980, an international symposium on El mice organized by Mori A, president of the Japan Neurosciences Research Association, finally name of this epileptic mutant as EL. In the huge works by Seyfried *et al*. and The Jackson Laboratory Neuroscience Mutagenesis Facility, the mutation gene was not identified. They have introduced the idea of multiple

quantitative loci. The mode of inheritance of EL is autosomal dominant and the penetration rate is 100%. During the development, every EL show tonic clonic seizures.

Induction of seizure and characteristics: After three weeks of age, EL mice pick up tail and rotate the body quickly once a week. EL mice then show initial tonic clonic seizure at 8–12 weeks of age. Once EL mice show seizure, they would show seizure every week during seizure induction procedure. After 12 weeks of age, EL mice show automatic seizures only changing ages till the end of their lives every once a week. The response to antiepileptic drugs is very good. EL is not intractable. Also, further neural stem cells transplantation for dorsal hippocampus can control seizures completely in a week[8]. Behaviorally, EL show abnormal midnight hyperactivity at 1–2 a.m. Protein level or gene level abnormalities are listed in Figure 3[9–13]. Almost all candidates of functional proteins related to epileptogenesis and ictogenesis are listed. These are characteristics for the polygene type of epileptic mutant animals.

How to keep, proliferate and maintain epileptic mutant animals for the experiments

Do not use mutant animals immediately after the introduction!

Epileptic mutants are very feasible for the environments. Even if the produced mutant shows stable seizure susceptibility, the seizure susceptibility of the mutant animals is unstable just after the introduction. Especially, animals obtained from frozen embryo should not be used directly. If transported by air, the seizure threshold of the mutant animals will increase. After at least four weeks, the seizure threshold in the animal room should be observed and the threshold should be determined as the same as that of the producer's records. We suggest that the mutant animals should be used for the experiments at least after 3F inbred and the confirmation of stability of the seizure threshold.

Epileptic mutants do not proliferate constantly!

"Slope breeding" (keeping the animals in about 30° inclination under the cage putting cage cover) is very useful to increase the mating rate. For instance, EL deliver a maximum of 12 babies at first delivery (after second delivery, the number of babies dramatically decreased to 3 to 5). It is the best use to utilize the first 12 animals to decrease the individual variation for the one-series experiments. As the animal societies of all over the world recommend the reduction of the number of animals, this method agrees the ethical problems of animal experiments.

Never use all the mutant animals immediately after the production!

In our animal laboratory, the best proliferation last year is in April with 58 babies and the least is in February with only eight babies. The constant strain inbred and how many animals to be used should always be considered. Especially in case of the experiments using newborn animals, the used animals should never be utilized for proliferation. Additionally, the timing of epileptogenesis starts at about the newborn age. Young animals are always attractive. Avoid "Big success experiment, however, the animals disappeared." We have successfully done the experiment of newborn animals in only April from the 58 babies, *i.e.* 12 × 4. The essence is always keeping the mouse pyramid shape with a long slope. Absolute number has no meaning because young adults cannot deliver over 12 L, and sooner or later the total animals will disappear. It is always the best to meet the born/wean ratio.

Is it necessary to make such a hard work to maintain the mutant animals?

The homology with human epilepsy ILAE insist on the importance of BME. The process to epilepsy (recurrent spontaneous seizure) is satisfied with three factors (genetic, development, acquired) as insult and then with latency. Using epileptic mutant, in planning the experiments according to the characteristics of the mutant, the conditions for insult, latency, and process to epilepsy are automatically satisfied.

Epileptic mutant is a mountain of treasure for the investigation of epileptogenesis and ictogenesis. If one has strong interests in the role of specific gene function, one should better use the knock-out or transgenic mice. However, if one has some interests on the epilepsy itself, including the complications as a whole body, one should try to use epileptic mutant animals.

Additional notes

Requirements of BME accepted by the Workshop on neurobiology of epilepsy (WONOEP XIII 2015), Istanbul, Turkey (Figure 1 **to** Figure 6**)**. Lots of BME

were suggested, however, the idea that specific one gene is the BME was denied.

Clinical Useful Biomarkers

1. Those that can predict who will get epilepsy (clinical treat)
2. Who will respond to treatment
3. What would be cognitive outcome (comobidity)
4. Those that can diagnose who has epilepsy
5. Traditionally the diagnosis is made purely on clinical seizures (except new borns)
6. Those specific to epilepsy and independent of the insults
7. Stage specific

Figure 3. The clinical usefulness of biomarker of epilepsy (WONOEPXIII, Istanbul, 4th September 2015)

What is ideal biomarker

1. Non invasive – blood/CSF, EEG, PET
2. Easy applicable
3. Inexpensive
4. Those also seen in animal models so that they can be "translated"

Figure 4. Requirements for ideal biomarker of epilepsy (WONOEPXIII, Istanbul, 4th September 2015)

Factors and candidates of BME

1. Inhibitory neurotransmitter and its rate limiting enzyme (GABA, Glutamate decarboxylase (GAD))
2. Mitochondria-related energy metabolism enzymes (Glucose-6-phosphate dehydrogenase)
3. Free radical-related enzymes (Superoxide dismutase (SOD)/Mn-SOD, Cu, Zn-SOD, Nitric oxide synthetase (NOS)/nNOS, eNOS, iNOS)
4. Immediate early genes (IEG/*c-fos, zif, jun*)
5. Apoptosis-related genes (Bax, Bcl-2, BclxL)
6. Neurotrophic factors (BDNF, NT-3, FGF-2)
7. Cell cycle-related enzymes (Cyclin A,B,D,E, CDK-1,2,3)
8. Cytokines (Interleukin(IL)-1alpha,IL-1 β, IL-6, IL-receptor, IL-receptor antagonist, TNF- α)
9. Neurosteroid-related enzymes (Allopregnolone, 5α-reductase, 3β-hydroxysteroid dehydrogenase, Aromatase, P450scc)
10. $GABA_A$ receptor subunits (α1,2,4,β2,3, γ3, δ)

Figure 6. Factors and candidates of biomarker of epilepsy (BME) suggested from the aspects of polygene epileptic mutants[9–13]

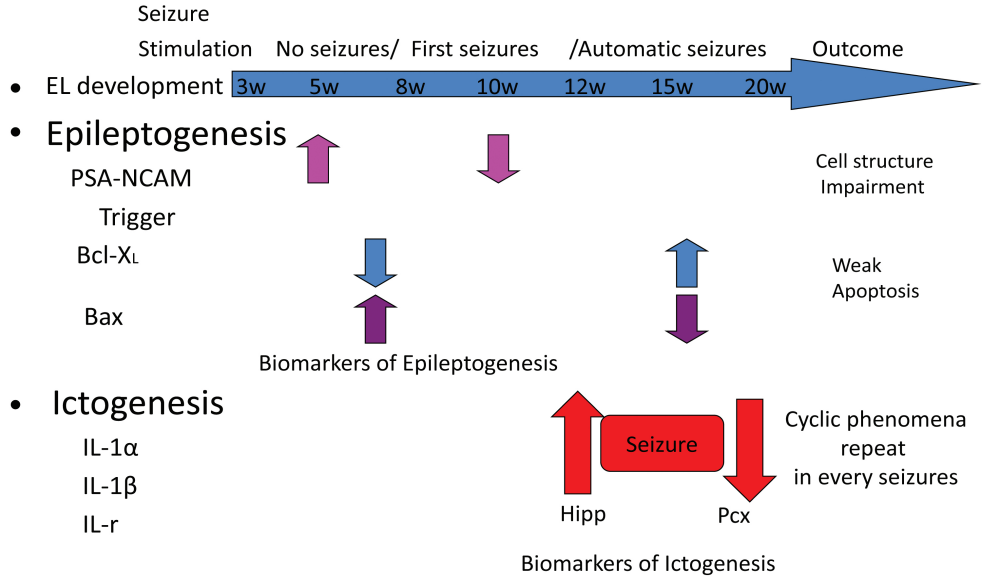

Figure 5. Biomarker of epilepsy (BME) suggested from the aspects of epileptic mutant animal, EL. (BME are satisfied with the requirements of genetic, developmental and acquired insults)

Conflict of interest

The author declares no potential conflicts of interest with respect to the research, authorship, and/or publication of their article.

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