

A New View of The Racine Scoring System in The Pentylene-tetrazol Epilepsy Model

Pentilentetrazol Epilepsi Modelinde Racine Skorlama Sistemine Yeni Bir Bakış

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Abstract

Background: Pentylene-tetrazol (PTZ) is the predominantly used chemical in studies to develop antiepileptic drugs and to investigate the mechanism of epilepsy. As PTZ is given systematically, it leads to generalized seizures, first creating myoclonic contractions and following generalized tonic-clonic seizures. According to the kindling model of epilepsy, repeated chemical stimulation to animals causes electrophysiological changes in local tissue. It was aimed to better observe and score the seizures of experimental animals by placing intermediate-phase levels between the phases.

Materials and Methods: The study was performed by intraperitoneal administration of PTZ to female (n=7) and male (n=7) Wistar-Albino rats. PTZ agent administered at a dose of 35 mg/kg every two days allowed the model to kindle at a final dose of 50 mg/kg at the end of one month. The seizures were scored according to the Racine scoring method and recorded on video.

Results: According to the results, there was a significant difference between phase values of original Racine scoring and those of new suggested Racine scoring for both female and male epilepsy groups

Conclusions: The importance of the developed method is to determine a more valid and more useful model by adding intermediate levels to all grades of the animal model proposing to change the degree of scoring in the literature.

Key Words: Kindled, Epilepsy model, Rats, Racine

Öz.

Amaç: Pentilentetrazol (PTZ), antiepileptik ilaçlar geliştirmek ve epilepsi mekanizmasını araştırmak için çalışmalarda ağırlıklı olarak kullanılan kimyasaldır. PTZ sistematik olarak verildiğinden, ilk önce miyoklonik kasılmalar yaratarak genelleşmiş tonik-klonik nöbetleri takiben genel nöbetlere yol açar. Epilepsi tutuşma modeline göre, hayvanlara tekrarlanan kimyasal ile stimülasyon, lokal dokuda elektrofizyolojik değişikliklere neden olur.

Materyal ve Metod: Çalışma dişi (n=7) ve erkek (n=7) Wistar-Albino sıçanlarına intraperitoneal PTZ uygulaması gerçekleştirildi. İki günde bir 35 mg/kg'lık bir dozda uygulanan PTZ ajanı, modelin 1 ayın sonunda 50 mg/kg'lık son dozda tutuşmasını sağladı. Nöbetler Racine skorlama sistemine göre puanlandı ve videoya kaydedildi. Fazlar arasına ara-faz seviyeleri eklenerek deney hayvanlarının nöbetlerinin daha iyi gözlemlenmesi ve puanlandırılması amaçlanmıştır.

Bulgular: Sonuçlara göre hem dişi hem de erkek epilepsi gruplarında önceki Racine skorlama sistemindeki analize göre anlamlı bir fark vardı.

Sonuç: Geliştirilen yöntemin önemi, hayvan modelinin tüm derecelerine ara seviyeler ekleyerek daha geçerli ve kullanışlı bir model belirlemektir.

Anahtar kelimeler: Tutuşma, Epilepsi modeli, Sıçanlar, Racine

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Introduction

Epilepsy is a neurological disease of the central nervous system that affects more than 100 million people worldwide. It is considered that epilepsy is resolved as a person has been seizure-free for at least 5 years with anti-seizure medication during the last 10 years (1). Epilepsy is also defined by at least two non-provoked (or reflex) seizures occurring for more than 24 hours or diagnosis of an epilepsy syndrome (2).

In order to develop antiepileptic drugs and to investigate the mechanism of epilepsy, animal experiments are performed due to inability of the conduction experiments in intact human brain. The predominant used chemical substance in animal experiments is pentylenetetrazol (PTZ) (3) and given systematically of it may lead to generalized seizures, and result in creating myoclonic contractions and finally generalized tonic-clonic seizures (4, 5).

According to the kindling model of epilepsy, repeated chemical stimulation to animals causes electro-physiological changes in local tissue. In animals, the amygdala is almost the same to the psychomotor epilepsy observed in humans in terms of kindled, EEG structure, behavior and response to drugs. In addition, this method has been used to investigate neuronal damage after epileptic seizures, because the histological changes observed in the brains tissues of epilepsy patients also occur in the brain regions of chemically kindled animals. Therefore, this protocol is useful to produce animal models of epilepsy appropriately (6).

Behavioral scoring is widely used to achieve seizure levels in different seizure models. Originally developed for the amygdala-kindling model, the Racine scale is widely used as a level measure in other models of experimental epileptic seizures (7). However, the Racine scale has always contradictions due to insufficient explanation of epileptic behaviors (8). It was showed that rats experiencing retention in other limbic regions may have different responses than those described in Racine system (9). Moreover, different density classification systems have been established for audiogenic seizures, indicating that the Racine scale is not suitable for other experimental models that do not rely on kindled (10). Experimental animals observed in the studies are scored according to these behaviors, but weakness of the model emerges as the deviation in the epileptic degrees of the animals increase.

In this study, it was aimed to have more competent observing and scoring in the seizures of experimental animals by placing intermediate-phase levels between the stages determined in the Racine scoring system. According to the results, it was determined that PTZ kindled model in the following periods needed intermediate degrees in the system.

Materials and Methods

Animals

Animals were cared in terms of the NIH Animal Care and Use Guide. All procedures applied on animals were approved by the Animal Ethics Committee of the university (ethics committee decision number: 2019/027). Additionally, whole observations and video recordings were performed under healthy conditions.

The experiment was performed using 280-350 g male/female Wistar albino rats obtained from the research center of university. The rats were placed in cages at controlled temperature ($24\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$), free access to water and food, and kept in a 12-hour light-dark cycle. The animals were randomly divided into two groups; male/female PTZ kindling epilepsy groups contained 7 rats each ($n=14$). The animals were administered 35 mg/kg of PTZ dissolved in 0.9% NaCl.

Pentylenetetrazol (PTZ) kindling epilepsy model

A GABA_A receptor antagonist pentylenetetrazol (PTZ) (P6500, Sigma, St. Louis, MO, USA) was dissolved in 0.9% saline and injected as intraperitoneal at a dose of 35 mg/kg. Injections were administered every two days for one month to kindle the model on the rat and behaviors of the rats were observed for 30 minutes individually to score according to the following system in terms of epileptic seizure scoring (11).

- Phase 0: No response to PTZ
- Phase 1: Continuous ear and facial twitching
- Phase 2: Myoclonic body jerks
- Phase 3: Clonic forelimb convulsions
- Phase 4: Tonic-clonic seizures
- Phase 5: Generalized tonic-clonic seizures
- Phase 6: Death.

50 mg/kg high dose PTZ was given to animals 1 week after the last PTZ injection to demonstrate improved seizure sensitivity in both female and male epilepsy groups. Animals with phase 4 or phase 5 were considered to be completely kindled.

Suggested Racine scoring system

After the problem of Racine scoring system was recognized, it was decided to develop a new system (Figure 1). Accordingly, significant differences in the behavior of animals during seizure were evaluated by the intermediate phases.

All new phases are listed as follows:

Phase 0: No response to PTZ,

Phase 0.5: Short-term twitching of the ear and face,

Phase 1: Long-term twitching of the ear and face,

Phase 1.5: Twitching from the ear and face to the body,

Phase 2: Short-term myoclonic reflexes in the body,
 Phase 2.5: Recurrent quick and severe myoclonic reflexes in the body,
 Phase 3: Short-term rearing-up on hind-legs,
 Phase 3.5: Severe rearing-up on hind-legs and transition to clonic seizure,
 Phase 4: Short-term tonic-clonic seizures and normalization,
 Phase 4.5: Long-term tonic-clonic seizures,
 Phase 5: Severe recurrent generalized tonic-clonic seizures,
 Phase 5.5: Prolonged generalized tonic-clonic seizures and unconsciousness
 Phase 6: Death

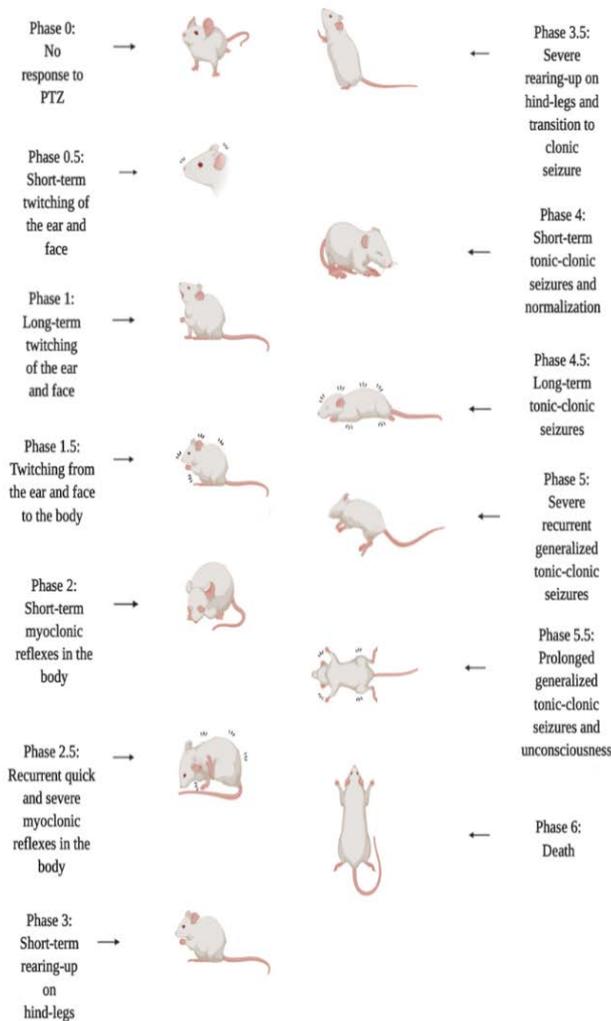


Figure 1. Suggested Racine scoring system. The behavior of animals is expressed both visually and textually. The process starting from Phase 0: No response to PTZ and completed with Phase 6: Death.

During the entire scoring, animals were recorded with a 30-minute video camera. Inter-phase scoring was also handled by a separate observer.

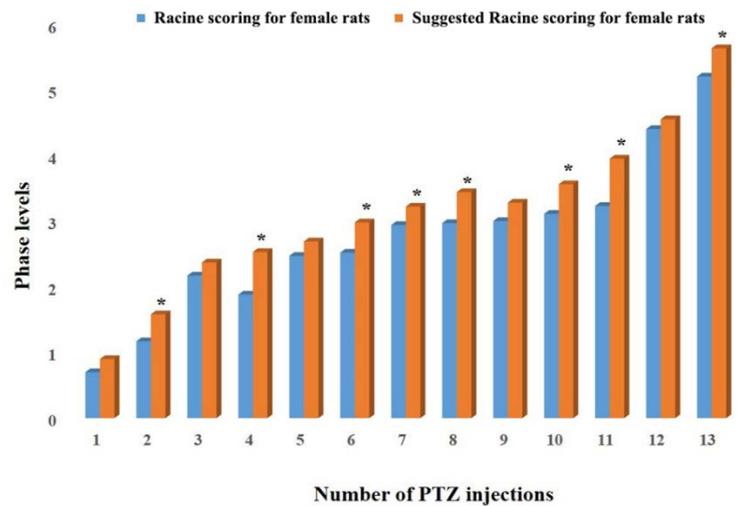


Figure 2. Racine scoring results of female rats with PTZ kindled model. The 13 numbers on the x-axis indicate the injection numbers, respectively, and the 0-6 range on the y-axis indicates the phase range. The mean phase value, which was 0.7 at the first injection, gradually reached up to 5.2 degrees. Suggested Racine scoring results of female rats with PTZ kindled model, the mean phase value, which was 0.9 at the first injection, gradually reached up to 5.63 degrees. *p values at 0.05 were accepted as statistically significant.

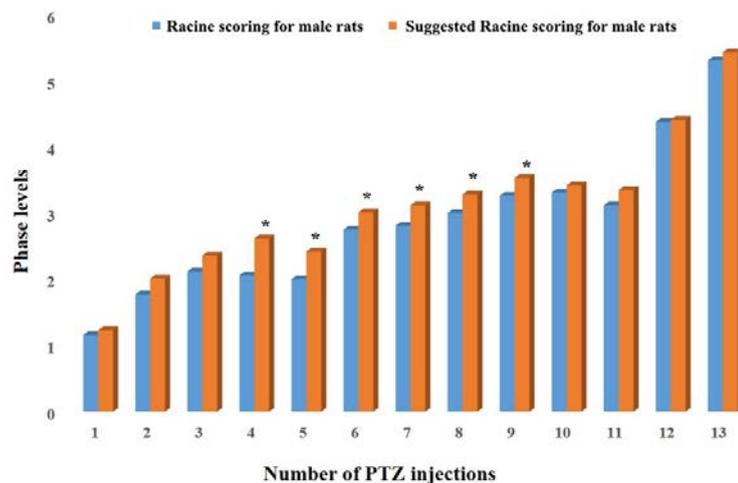


Figure 3. Racine scoring results of male rats with PTZ kindled model. The 13 numbers on the x-axis indicate the injection numbers, respectively, and the 0-6 range on the y-axis indicates the phase range. The mean phase value, which was 1.16 at the first injection, gradually reached up to 5.31 degrees. Suggested Racine scoring results of male rats with PTZ kindled model, mean phase value, which was 1.23 at the first injection, gradually reached up to 5.43 degrees. *p values at 0.05 were accepted as statistically significant.

Statistical Analysis

Standard deviation and standard mean values were calculated for the data, followed by a one-way analysis of variance (ANOVA), Mann-Whitney-U test statistical analyses, using the SPSS software. p values at 0.05 were treated as statistically significant.

Results

Racine scoring system according to PTZ kindling model

All seizures were scored after each intraperitoneal PTZ injection. Scoring of all animals was recorded according to the Racine scoring system. Afterwards, phase values were analyzed and data were obtained after 13 injections. Expected kindled seizure coded as phase 5 "generalized tonic-clonic seizures" were observed in almost all animals especially at the last dose. Kindling was observed to occur slowly as the number of injections increased. Racine scoring analysis was shown in Figure 2 for female rats and Figure 3 for male rats, respectively.

For female rats, phase 5 seizures were observed and the mean value of last injection score was 5.2 (± 0.47) while it was around 5.31 (± 0.58) in male rats. Mass weights were measured during the whole PTZ injection process and it was not significantly changed (data not shown).

Suggested scoring system via PTZ kindling model

All seizures explained above were scored after each intraperitoneal PTZ injection by a different observer. Scoring of all animals was recorded according to suggested Racine scoring system. Afterwards, phase values were analyzed and data were obtained after 13 injections. Expected kindled seizures coded as phase 5 "Severe recurrent generalized tonic-clonic seizures" and "Prolonged generalized tonic-clonic seizures and unconsciousness" were observed in almost all animals especially at the last dose (50 mg/kg). Comparative analysis of original Racine and suggested Racine scorings was shown in Figure 2 and 3 for female and male rats, respectively. For female rats, phase 5 seizures were observed and the mean value of last injection score was 5.63 (± 0.36) while it was around 5.43 (± 0.39) in male rats.

Discussion

The PTZ epilepsy model is one of the most prominent experimental epilepsy animal models that have been used frequently over the past two decades (11-13). Also, Racine scoring system is one of the mainly used methods to determine the degree of seizure in experimental epilepsy models. This scale, published by Racine in 1972, has not been revised, although it is currently used as a method for determining different seizure phases (14). Therefore, in this study, a current approach to Racine scoring system used with experimental epilepsy model is introduced.

Lüttjohan et al. introduced a new perspective (7) on the Racine scoring system in the PTZ epilepsy model. In this perspective, the first four stages remain the same while stage 5 and after are re-categorized [5a-6c]. In this categorization by using the same electroencephalographic (EEG) recordings, animal behaviors observed during seizures are shown in more details. In that new approach,

however, only the tonic-clonic seizure (Phase 4) and subsequent stages are elaborated while the previous stages are ignored (7). In 2019, another approach to the Racine scoring system was introduced in the epilepsy model created in mice by PTZ. In this approach, instead of the known Racine scoring system, a scale consisting of eight stages was designed (8). Both studies support the need to update the Racine scoring system. In the literature, there are few studies on the development of the Racine scale, especially the lack of the first four phases. In recent experiments, staging of tonic-clonic seizures is an indication that this scale needs a wider spectrum.

In our study the suggested new system, it was planned to design each phases of original Racine scoring system divided into two new phases. Accordingly, the behaviors observed in the epileptic levels of the animals were examined in more detail by placing intermediate phases. The fact that the phases identified by Racine are actually divided into two parts in terms of behaviors appeared in short and long term emphasizes the importance of this research. For example, ear and face twitching of animals is observed for a short time in some, while in others it is seen repeatedly for a long time. Here, taking note as phase 1 for both animals makes the validity of the model questionable. Similarly, for phase 2, some of the animals showed short-term and relatively calm myoclonic reflexes, while another animal showed severe myoclonic reflexes. In this case, instead of taking notes because exemplified animals both had phase 2, the score of 2 for the short-term and 2.5 for the long-term and severe ones were considered appropriate. Racine scoring of phase-3, in which animal rearing-up is considered, is a separate topic of discussion. Because some of the animals show too short-term exacerbation, while others out of the seizure, soon after the transition to phase 4 is very severe. Accordingly, it was determined that the shorter ones were suggested phase 3, the more severe ones were phase 3.5.

Racine scoring of phase-4 has two separate states. The first was proposed that animals had short-term tonic-clonic seizures and were immediately normalized (suggested phase 4), and the other was planned to be the normalization of animals after a long-term tonic-clonic seizure (suggested phase 4.5). Finally, it was essential that Racine scoring of phase-5 be determined as two separate phases. As the animals were normalized from severe recurrent generalized tonic-clonic seizures (suggested phase 5), seizures that lasted long and animals remained unconscious (approximately 30 min; status epilepticus mimic) were observed at phase 5.5. Animals that could not return to normal life ended in Racine scoring of phase-6. In the light of all these regulations, it was aimed to increase the reliability of Racine scoring in the studies conducted with PTZ model and to provide the results to be obtained with clear data.

Like the traditional Racine scoring system, this scoring system is valid only for kindling model of epilepsy. Behavior investigations and scoring systems are not using in epilepsy created with single dose chemical insult. Racine scoring and the main purpose of this scoring are to confirm animals with chronic epilepsy as a result of multiple injections of PTZ. In addition, the scoring system is valid for mice with PTZ model.

In summary, it could be concluded that original Racine scoring system is insufficient to scale behaviors observed in PTZ-induced epilepsy model. However, better scaling might be achieved by adding intermediate phases. Therefore, this new approach proposes a revision that includes intermediate phases that may detailed scale the PTZ model.

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Ethical Approval:

The study was approved by the ethical committee of the Kayseri Erciyes University. (Ethics Committee Decision Number: 19/027, 13.02.2019).

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