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Turning bias in Morris water maze as an index of susceptibility to chemical kindling in male but not in female rats

Narges-al-sadat Haeri, Mohammad Reza Palizvan*

Department of Physiology, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran

Abstract

Background and Objective: Epilepsy is a prevalent neurological condition marked by recurring seizures. Additionally, individuals affected by this disorder may experience cognitive deficits, as well as difficulties with learning and memory. Rodents generally exhibit rotational bias associated with asymmetry in the striatum. The role of the striatum in the genesis of different types of seizures has been mainly addressed in animal models. In this study, the correlation between rotational bias of animals in the Morris Water Maze (MWM) and seizure parameters in a chemical kindling model has been studied in male and female rats.

Materials and Methods: Animals were trained in the Morris Water Maze for four trials per day for 4 days. We measured the left and right turning of the animals that were placed into the water facing the pool wall at the moment of entry into the maze. Forty-eight hours after learning in the Morris Water Maze, animals were kindled by pentylenetetrazol (PTZ, 37.5 mg/kg, i.p) injections, repeated every 48 hours for 12 days. Finally, the relationship between seizure parameters and left and right turning bias of animals in MWM was analyzed.

Results: The results showed a significant association between turning bias and susceptibility to PTZ kindling in males but not in female rats. Male rats with a left turning bias had higher resistance to seizure induction and lower resistance to seizure propagation compared to right-turning rats.

Conclusion: The results suggest that susceptibility to seizures induced by PTZ kindling could be anticipated by rotational bias in males, but not in female rats.

Keywords: Chemical kindling, Rotational bias, Handedness, Pentylenetetrazole, Hippocampus

1. Introduction

Epilepsy is a chronic disorder of the brain characterized by recurrent seizures. Kindling is a chronic animal model of epilepsy in which regularly applied of an initially subconvulsive electrical or chemical (Pentylenetetrazole, PTZ) stimulus eventually leads to a generalized tonic/clonic seizure (1). PTZ is a central nervous system convulsant which acts as a competitive antagonist of GABAA receptor (2). PTZ kindling is a suitable model and can be used to find useful treatments for cognitive impairment in human epilepsy (3). Different regions in the brain seem to have a different tendency to generate seizure activity. It has been shown that the hippocampus has the lowest seizure threshold (4).

Behavioral lateralization eventuating from the functional asymmetry of the central nervous system is known in all classes of vertebrates (5, 6). Handedness is one of the most studied human asymmetry and laterality (7). Over 90% of the human population shows right hand superiority in the world (8) this predilection is in lined with the asymmetry in the underlying motor system. In addition to handedness, laterality also includes rotation direction preference (e.g., turning and circling when swimming) (9).
Experimental findings demonstrate that genetic factors, environmental factors, such as infections, toxins, or stress and sex hormones, play important roles in the development of cerebral asymmetry (10). Testosterone secretion during the middle and late

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***Corresponding Author:** Mohammad Reza Palizvan **E-mail:** palizvan@yahoo.com

trimester of pregnancy is one of the most important environmental factors in cerebral asymmetry (11). The testosterones delays the development of the temporal lobe of the left hemisphere and respectively promote the development of the corresponding part in the right hemisphere and culminate in changes in the development of brain lateralization and a shift to the left in handedness (12). For example the structure of the rat hippocampus displays responsiveness to sex hormones, with male rats exhibiting a higher number of pyramidal neuron dendritic spines compared to female rats. This disparity is further accentuated in males through environmental stimulation, whereas females do not show a similar increase in dendritic spines under such conditions (13).

Available data suggest that gender differences exist in the epidemiology, and pathophysiology of epilepsy. According to a large epidemiological report (14) and a subsequent meta-analysis (15), the prevalence of epilepsy is lower in females compared to males.

Considering the important role of hippocampal formation in the kindling phenomenon, and its role in the cerebral lateralization and effects of sex hormones on hippocampal formation, it is possible that animals that differ in the brain laterality had different susceptibility to seizure. The aim of this study was to find the correlation between susceptibility to PTZ induced kindling and rotational bias in male and female rats.

2. Materials and Methods

2.1. Subjects

In the experiment conducted, a total of 34 male and 19 female Wistar rats were used, with an initial weight ranging from 200 to 250 grams. These animals were housed in groups of four per cage under controlled environmental conditions at the Arak University of Medical Sciences animal facility.

The housing conditions included a 12-hour light/dark cycle, with lights on from 7:00 AM to 7:00 PM and lights off from 7:00 PM to 7:00 AM. The temperature was maintained at 22±2°C to ensure a stable and comfortable environment for the rats.

The animals had access to food and water ad libitum, allowing them to eat and drink freely as needed. All experimental procedures were conducted in compliance with the regulations outlined in the EU Directive 2010/63/EU and adhered to the ethical standards set by the local ethics committee (Arak University of Medical Sciences Research Ethics Committee #92-159-11).

2.2. Assessment of swimming rotation

In the Morris water maze test, swimming rotation was evaluated. The setup included a cylindrical pool with a diameter of 140 cm, filled with water maintained at a temperature of 25 ± 3°C and a height of 32.5 cm. The water in the pool was made opaque by adding black non-toxic paint to prevent the animals from seeing through it.

During the test, the animals were placed into the water while facing the wall of the pool. As the animals entered the pool, their left and right turning movements were observed and recorded. The direction of the animals' initial turning was used to determine if they exhibited right or left rotations:

- Clockwise rotation was considered as right rotation.
- Counter-clockwise rotation was considered as left rotation.

2.3. Kindling

The process for inducing kindling involved administering a sub-convulsive dose of PTZ (37.5 mg/kg, i.p. Sigma, USA) every other day for a total of 26 days, equivalent to 13 injections. Following each PTZ injection, the animals were placed in a Plexiglas chamber (30 × 30 × 30 cm), and their convulsive behavior was observed and recorded for a period of 30 minutes.

The convulsive responses were classified into different stages according to a predefined scale as described in previous literature (16):

- Stage 0: No response
- Stage 1: Ear and facial twitching
- Stage 2: Myoclonic jerks without upright position
- Stage 3: Myoclonic jerks with upright position and bilateral forelimb clonus
- Stage 4: Clonic-tonic seizures
- Stage 5: Generalized clonic-tonic seizures with loss of postural control

Rats were deemed fully kindled when they exhibited stage 5 seizure stage following each PTZ injection for three consecutive injections.

Various parameters were recorded during the experiments:

1. Seizure stage (SS)
2. Latency to the onset of stage two (S2L) seizures
3. Latency to the onset of stage five (S5L) seizures
4. Duration of stage five seizures (S5D)

2.4. Statistical analysis

In this study, statistical analyses were conducted using GraphPad Prism (Version 6). The normal distribution of the data was confirmed using the Kolmogorov-Smirnov test. To compare two groups, unpaired Student’s t-tests were utilized when appropriate. The threshold for statistical significance was set at $p < 0.05$. Results are presented as mean ± standard error of the mean (SEM).

3. Results

3.1. Correlation between chemical kindling and rotational bias in male rats

In chemical kindling experiments, various seizure parameters such as severity, latency, and duration are commonly evaluated to assess the development of epileptic seizures. Latency, which reflects the time taken the PTZ to reach different seizure stages, such as stage two (S2 L), stage four (S4 L), and stage five (S5 L), serves as an indicator of seizure onset and progression in animal models.

It is observed in many studies that a faster initiation of seizures in animals often leads to a quicker propagation of seizures as well, resulting in a positive correlation between seizure parameters like S2L, S4L, and S5L. Initial analysis of the results has indicated a direct correlation among these parameters.

Upon further exploration by dividing the animals based on gender, it was found that while female animals exhibited a positive correlation between seizure parameters (S2L, S4L, S5L) (Figure 1A), this relationship was not present in male rats. Additionally, investigating turning bias by categorizing male animals into right and left-turning groups revealed interesting findings.

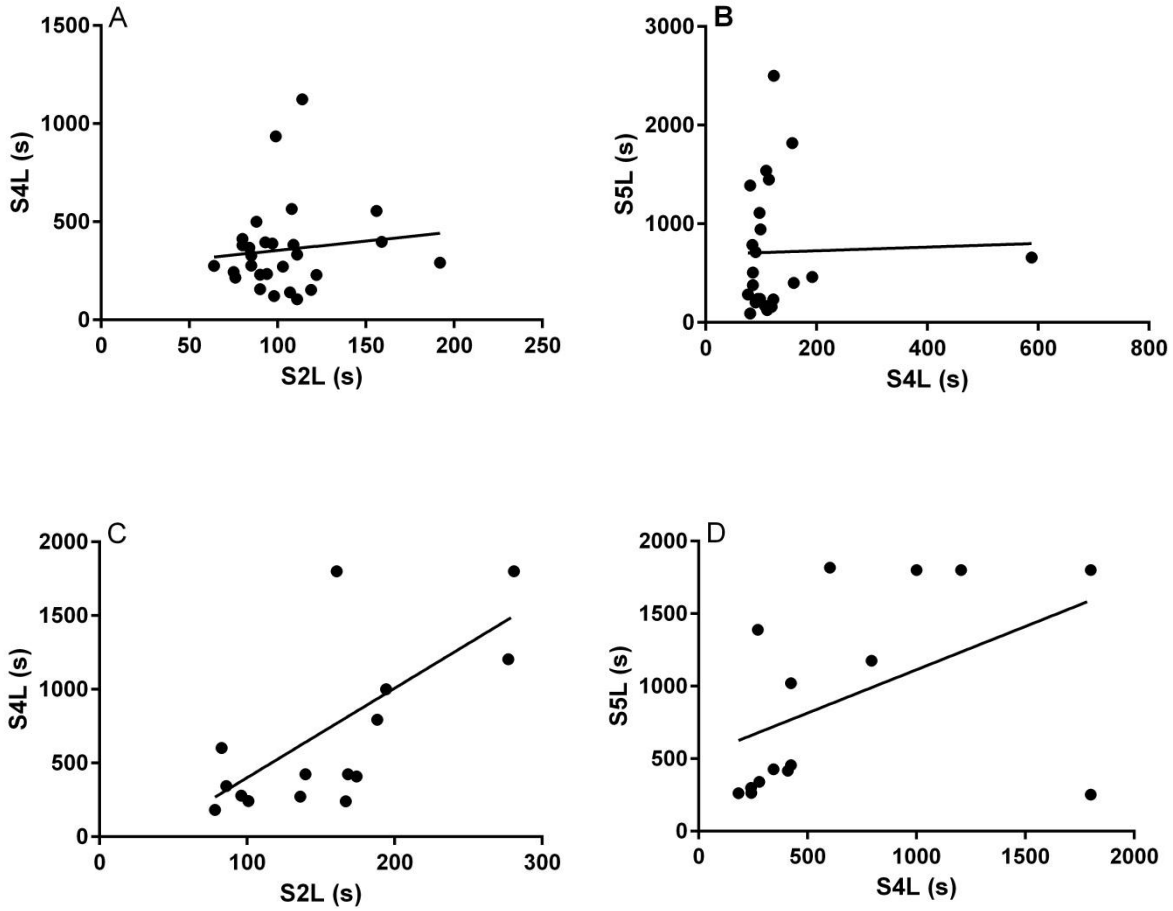

Fig.1. Correlation between S2L and S4L (p=0.556, r2 = 0.0326) (A), and S4L and S5L (p=0.89, r2=0.009) (B) in male rats; and between S2L and S4L (p=0.009, r2=.39) (C), and S4L and S5L (p=0.036, r2=0.28) (D) in female rats.

In male rats, it was noted that left-turning animals showed significantly higher S2 latency compared to right-turning animals (unpaired t-test t(32)=-2.06, p=0.047, n=34) (Figure 2A), indicating differences in seizure onset based on turning direction. Conversely, right-turning male rats exhibited significantly longer S4 [n=34, t (32) =2.159, p=0.038] and S5[n=34, t (32) =2.435, p=0.021] latencies than left-turning counterparts (Figure 2B), suggesting differences in the progression to more severe seizure stages based on turning bias.

On the other hand, in the study involving female rats, no significant association was found between seizure parameters in chemical kindling and the direction of rotation at the moment of entering the maze.

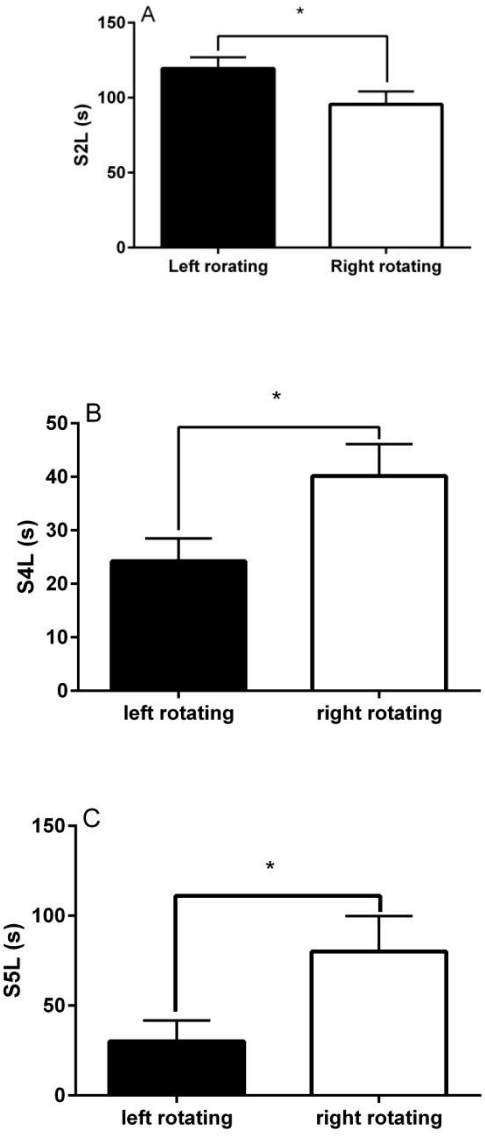

Fig.2. Comparison of stage 2 latency [*: n = 34, p = 0.047] (A); stage 4 latency [*: n = 34, p = 0.038] (B); and stage 5 latency [*: n = 34, p = 0.021] (C) between right and left rotating in male rats.

4. Discussion

The results showed that chemical kindling parameters have significant correlations with rotational bias in male rats, but not in female rats. In male rats, the stage two latency for left rotating animals was higher than for those rotating to the right, while stage 4 and stage 5 latency was lower in left rotating rats. This suggests that in left rotating rats, the brain has a higher resistance to the initiation of seizures and lower resistance to the propagation of seizures. In female rats, there was no correlation between seizure parameters and left or right rotation in the Morris water maze.

Our results for the first time showed that susceptibility to seizures induced by kindling could be anticipated by examining the rotational bias of male rats in the Morris water maze. In this study, a preference for left rotation in male rats has a significant correlation with resistance against seizure initiation in the brain. In animal studies, a left turn is considered equivalent to the superiority of the right hand. The hippocampus is one of the main brain regions involved in Kindling. Szabo et al. (2001) reported that the right hippocampus was significantly larger than the left hippocampus in left-handed persons (17). On the other hand, the relationship between handedness and epilepsy has been suggested in previous studies, and while only about 10% of the general population is developmentally left-handed, the occurrence of left-handedness is observed in up to 30% of patients with medically refractory epilepsy.

Our results showed that in male rats, there is no correlation between seizure parameters. Some of these parameters indicate seizure initiation (e.g., stage 2 latency), some reflect seizure propagation (e.g., stage 5 latency), and some depict seizure termination (e.g., stage 5 duration). The independent correlation between these parameters supports the hypothesis that the phenomena of initiation, propagation, and termination of epileptiform activity involve distinct and independent mechanisms within the underlying cortical circuit (18). According to this theory, epileptiform activity involves three stages, each with independent mechanisms: initiation, propagation, and termination. Initiation is a phenomenon that depends on both synaptic excitation and inhibition. In contrast, propagation velocity and amplitude depend only on synaptic excitation but not inhibition (above a threshold level) (18). Nevertheless, positive correlation exists between seizure parameters in female rats. It suggests potential differences in the underlying mechanisms of seizure initiation, propagation, and termination between male and female rats. Why this difference exists between male and female rats is a question that needs further research

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Sex differences in seizure susceptibility are indeed well recognized in preclinical models. In many animal models of induced seizures, such as the pentylenetetrazol test, male mice or rats are generally more prone to seizures compared to females. For example, experimental findings indicate that male adult mice have a lower seizure threshold than females within the pentylenetetrazol test (19). Similarly, sex differences have been observed in models involving pilocarpine and kainic acid-induced status epilepticus-induced status epilepticus (20, 21). There is a growing consensus supporting the notion that female rats exhibit remarkable resistance to pilocarpine or lithium-pilocarpine-induced seizures compared to their male counterparts (22, 23).

Conclusion

The study findings suggest that there are significant correlations between chemical kindling parameters and rotational bias in male rats, but not in female rats, suggesting a potential influence on seizure susceptibility and propagation. Further research is necessary to fully understand the implications of these findings and their relevance to epilepsy and seizure disorders.

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Ethical approval

Ethical approval for the study was provided by the Arak University of Medical Sciences Research Ethics Committee # 92-159-11

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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