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Prediction of seizure incidence probability in PTZ model of kindling through spatial learning ability in male and female rats

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HIGHLIGHTS

- Male rats learn water maze more effectively than females in egocentric learning.
- There are no differences in allocentric learning between male and female rats.
- Male rats have higher swimming speed in dark environment than females.
- A negative correlation between working memory and seizure scores was observed.
- The reference memory and seizure scores were inversely correlated in females.

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ABSTRACT

Epilepsy is a common neurological disease characterized by periodic seizures. Cognitive deficits and impairments in learning and memory are also associated with epilepsy. Neuronal changes and synaptic modifications in kindling model of epilepsy are similar to those occur during the learning procedure and memory formation. Herein we investigated whether seizure susceptibility in pentylenetetrazol (PTZ) model of kindling is predictable based on the learning ability in the Morris water maze (MWM) task in male and female rats. Allocentric learning was tested using MWM in present of light while egocentric learning was evaluated by MWM in dark room. The results indicated no significant differences in allocentric learning abilities between male and female rats. However, male rats were able to memorize the location of the platform more effectively compared to females in egocentric test. In addition, a statistically significant negative correlation between learning abilities (working memory) and seizure susceptibility in male rats was found while this correlation was positive in female rats. On the other hand, although there was no significant correlation between retrieval (reference memory) of spatial memories and seizure parameters in male rats, female rats showed a significant negative correlation. These findings may provide some evidences for prediction of seizure susceptibility according to learning ability and memory retention.

1. Introduction

Epilepsy is a common neurological disease that is characterized by recurrent epileptic seizures. Epilepsy is associated with cognitive deficits, emotional perturbations and psychosocial problems [1–4]. Impairment in cognitive functions has been reported for both children and adult patients with epilepsy [1,5–7]. In addition, deficits in short term memory and reference memory have been found in patients with temporal lobe epilepsy [8,9]. Allocentric memory in the MWM can be directed by memory of the target coordinates relative to remote landmarks (in present of light). Egocentric memory also can be tested when the start and goal positions remain constant and visual cues are eliminated by darkness, so memory of the route may become decisive.

Experimental animal models of epilepsy, such as electrical and chemical kindling, have played a fundamental role in improvement our understanding of basic mechanisms underlying epileptogenesis. In kindling model of seizure, repetitive application of an initially subconvulsive electrical or chemical stimulations induce stable neuronal and synaptic changes at different areas of the brain [10–12]. Pentylenetetrazol (PTZ) kindling is a procedure of chemical kindling in which the repeated administration of PTZ causes a chronic and progressive increase in the excitability of the central nervous system which is accompanied with a reduction in the threshold for seizure incidence [13]. Standard PTZ chemical kindling is elicited by intraperitoneal (i.p.) injections of a subconvulsant dose of PTZ once every 48 h until the animal achieves the fully kindled [14].

Seizure susceptibility is different among animals partly because of difference in their neural network properties [15,16]. Individual differences in learning abilities among rats also referred to differences in

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the neural networks. In fact, the neuronal properties and synaptic ability of the brain areas achieve some changes during kindling, which are similar to those occurring during the learning procedure and memory formation [17]. Previous studies have also shown the similarity between kindling-induced seizure and memory formation [18]. Accordingly, in the current study, we investigated whether learning ability of spatial memory in MWM task could be an appropriate index for seizure susceptibility in chemical kindling model of epilepsy and if it is different between sexes.

2. Materials and methods

Forty-six male and thirty two female Wistar rats weighing 200 to 250 g were divided into four groups including: 1—male allocentric learning in Morris water maze ($n = 34$), 2—male egocentric learning in Morris water maze ($n = 12$), 3—female allocentric learning in Morris water maze ($n = 20$), and 4—female egocentric learning in Morris water maze ($n = 12$). The animals were housed under environmentally controlled conditions (12 h light/dark cycles, 7:00–19:00 light and 19:00–7:00 dark, temperature between 22 ± 2 °C) at the Arak University of Medical Sciences animal facility. Food and water were supplied ad libitum. All procedures carried out in accordance with EU Directive 2010/63/EU as well as all norms established by the local ethical committee (Arak University of Medical Sciences Research Ethics Committee # 92–159-11).

2.1. Morris water maze test

The water maze (WSI, Iran) was a circular pool with a diameter of 140 cm and a height of 60 cm with walls and bottom painted black. The pool was filled with water (temperature 22 °C) to 32.5 cm. A black circular platform (10 cm in diameter), and 30 cm height, hidden from rat's view, was placed in it 30 cm from the wall. The testing room was maintained at 22 °C and contained several distal extra-visual cues on the wall. The movements of the rat in the water maze were recorded by a video camera placed directly over the center of the pool monitoring the whole pool. Video camera was coupled to a computer with analytical software. This software was able to measure the distance traveled by the animal, the average speed of animal, and the time animal spent in each part of the maze.

2.1.1. Allocentric learning in Morris water maze

The rats were given four trials per day using four different starting positions (assigned in a pseudo-random order between days) for four consecutive days. In each trial, the rats were placed into the water facing the wall and they released from a different starting point were permitted to swim until they reached to the escape platform. If a rat found the platform, it was left there for 20 s before being removed for an inter-trial interval of 10 min. If a rat did not find the platform within 60 s, it was gently guided and allowed to stay on the platform for 20 s. To assess the spatial memory retention following a period of learning, probe trials were conducted 24 h after the last hidden platform training. The hidden platform was removed from the pool and each animal was allowed to swim for 60 s, starting from the same position.

2.1.2. Egocentric learning in Morris water maze

For egocentric learning in MWM, the same apparatus were used except that the learning paradigm took place in complete darkness environment. A trial began by turning the light off and placing the rat to the fixed starting point into the water while facing the wall of the pool. The rat was given a maximum of 60 s to find the hidden platform and a dim light was switched on either after 60s or after the animal found and remained on the platform. The animal was always allowed to rest there for 20 s before returning to a waiting cage. The light was switched off and 10 min later the next trial was started (4 trials per session per day) [19].

2.2. Kindling

Forty-eight hours after learning in Morris water maze, a subconvulsive dose of pentylenetetrazol (PTZ) (37.5 mg/kg, i. p. Sigma, USA) was administered every other day for a period of 26 days (13 injections). However, the data for six injections were presented here because after day 12 the data were not statistically significant. After each PTZ injection, animals were kept in a Plexiglas chamber (30 × 30 × 30 cm), and seizure intensity was observed over a cutoff period of 30 min. Convulsive responses were classified as described previously [20]. Briefly, stage 0, no response; stage 1, ear and facial twitching; stage 2, myoclonic jerks without upright position; stage 3, myoclonic jerks, upright position with bilateral; forelimb clonus; stage 4, clonic–tonic seizures; stage 5, generalized clonic-tonic seizures, loss of postural control [21]. Rats were considered kindled when seizure attacks (stage 5) occurred after each PTZ injection for three consecutive days. The recording parameters included seizure stage (SS), the latency to the onset of stage two (S2 L), stage four (S4 L), stage five (S5 L) seizure and stage five duration (S5D) time (the time that animal remains in stage five of seizure), and the number of injections required for onset of stage five seizures. Because some of the animals did not show stage 2, 4 or 5 of seizures we used 1/S2L, 1/S4L and 1/S5D to add their data to the sample.

2.3. Statistical analyses

SPSS Version 20 was used for all statistical analyses. One-way and two-way analysis of variance (repeated measures for time rows), followed by Tukey's test for multiple comparisons, were used when comparing three or more groups. To compare two groups, unpaired Student's *t*-tests were used when appropriated, as indicated in the legends of the figures. Slope of learning was determined by plotting the learning latencies and obtaining the slope of the linear regression, and slope of learning was correlated with seizure parameters. Pearson correlation coefficient was used to assess the significance of correlations. The threshold for statistical significance was set at $p < 0.05$. Results are expressed as mean ± standard error of the mean (SEM).

3. Results

3.1. Allocentric spatial memory

The average latencies to reach the submerged platform in present of light decreased significantly in male [$F(3, 132) = 15.79$, $p < 0.001$] and female [$F(3, 76) = 10.73$, $p < 0.001$] groups during four days of training in MWM test (Fig. 1A); revealed most of the animals were able to learn the location of the platform. Subsequent comparisons via two-way repeated measure ANOVA and Tukey's post-test revealed no significant difference in escape latency between male and female groups [$F(4, 49) = 1.89$, $p = 0.127$] (Fig. 1A). In addition, the assessment of reference memory in probe trial showed no significant differences in percentage time spent in the platform quadrant between male and female groups.

3.2. Egocentric spatial learning

Comparing the learning abilities of male and female rats in the dark environment showed that escape latency was significantly shorter in male than in female rats [$F(4, 19) = 4.57$, $p = 0.009$, two-way repeated measure ANOVA] (Fig. 1B). However, comparing the escape latencies of male rats in light and dark environments showed that there is no significant difference in escape latency, and male rats learned to find platform in water maze in darkness as well as in light environment (Fig. 2A). On the contrary, escape latency of female rats was significantly longer in darkness than in light environment [$F(4, 27) = 6.482$, $p = 0.001$, two-way repeated measure ANOVA] (Fig. 2B). The assessment of working memory ability in probe trial showed that there was no significant