Effects of Age, Sex and Anxiety on Spatial Learning and Memory in Rats

M. Belviranlı[1], K.E. Nurullahoğlu Atalık[2], N. Okudan[1], H. Gökbel[1]

[1]*Department of Physiology Faculty of Medicine Selcuk University, Konya, Turkey.* [1]*mbelviranli@yahoo.com*

[2]*Department of Pharmacology Meram Faculty of Medicine Konya University, Konya, Turkey*

Abstract

The main objective of study was to investigate the effects of age, sex and anxiety differences on learning and memory in rats. We also investigate whether repeated elevated plus maze (EPM) tests induce anxiety in rats. Eighty Wistar rats were divided into the eight groups according to their sex, age and anxiety status. Spatial learning and memory were evaluated with Morris Water Maze (MWM). All behavioral tests were recorded online and analyzed offline with analytical software. In conclusion, age, sex and anxiety affect learning and memory in different aspects.

Keywords. Behavioral measurement; anxiety; aging; sex difference.

Introduction

Aging is a physiological process which adversely affects many vital functions, including cognitive functions [1]. Aging induced cognitive dysfunctions are well documented in humans [2,3] and several animal species including rats and mice [4]. Behavioral sex differences are observed in many examples of anxiety and memory tests. These differences depend on the sex hormones and other mediators in the brain [5]. However, there is no consensus about the effects of sex differences on spatial learning and memory and possible effects of aging and anxiety. In the present study we aimed to investigate the effect of aging and sex differences on learning and memory in anxiety induced rats.

Materials and methods

Animals

The study protocol was approved by the Ethics Committee of the Selcuk University Experimental Medicine Research and Application Center. A total of 80 adult male (n: 40) and female (n: 40) Wistar rats were obtained from the same center. Animals were divided into eight groups (ten animals in each group) as follows:

- Group 1: 7-month-old control young males (CYM).
- Group 2: 7-month-old control young females (CYF).
- Group 3: 15-month-old control aged males (CAM).
- Group 4: 15-month-old control aged females (CAF).
- Group 5: 7-month-old experimental young males (EYM).
- Group 6: 7-month-old experimental young females (EYF).
- Group 7: 15-month-old experimental aged males (EAM).
- Group 8: 15-month-old experimental aged females (EAF).

The principle of laboratory animal care of the National Institute of Health (NIH) guideline was followed in all these experiments.

Behavioral Tests

All behavioral test devices were placed in an isolated room and test protocols were performed here to eliminate the factors that affect animal's behavior. MWM was carried out followed by EPM (5 min) for four consecutive days. During the training period, four starting points were randomized in everyday to avoid the horizontal navigation to the platform. Each trial was terminated in 90 s, if the rats were unable to locate platform in this time interval and then rats were placed on the platform for 30 s. Following the four days of training period, 120 s of probe trial was conducted after the hidden platform was removed. Control groups were performed same protocols with the experimental groups, except the anxiety protocols.

Tracking System

All tests were recorded online using a video camera. The videos of the behavioral tests were analyzed offline using analytical software (Noldus Information Technology, Ethovision XT 8.0, Wageningen, The Netherlands).

Statistical Analysis

All data are presented as mean ± SD. Changes of the MWM variables through the experiment were analyzed using three-way analysis of variance (ANOVA) with repeated measures. EPM values were analyzed using two-way ANOVA with repeated measures. Probe trial data were analyzed by three-way ANOVA. A p value less than 0.05 was considered to be statistically significant.

Results and discussion

During the repeated EPM tests, total distance traveled, numbers of entries in center, open arms and closed arms significantly affected by time interaction (F=22.907, P=0.000; F=6.093, P=0.001; F=4.033, P=0.009 and F=7.157, P=0.000, respectively). These variables were decreased by repeated measurements in all groups.

The latency to find platform and total distance traveled significantly affected by time interaction (see Table 1). In all groups latency to reach platform and total distance traveled significantly decreased with time. Average swimming speed was affected by both time and time x age interactions (see Table 1). Average swimming speed was decreased in all groups with time and young rats were faster than aged rats.

In probe trial, total distance traveled and average swimming speed affected by anxiety and age factors (see Table 2). Total distance and swimming speed were higher in experimental and aged groups compared to control and young groups, respectively. Although number of platform crossing was not affected by anxiety, age and sex factors, time spent in platform was significantly affected by age (see Table 2). Time spent in platform zone was higher in young groups than aged groups. During the probe trial, the latency to reach the platform was not different among the groups.

Table 1. Morris water maze. Summary of the three-way repeated measures ANOVA results in all groups.

	Time		Time x Anxiety		Time x Age		Time x Sex	
	F	P	F	P	F	P	F	P
Latency (s)	35.087	0.000	1.514	0.130	0.862	0.608	0.826	0.645
Total distance traveled (cm)	35.438	0.000	1.466	0.149	1.127	0.354	0.966	0.501
Average swimming speed (cm/s)	16.909	0.000	1.486	0.141	2.278	0.013	1.153	0.334

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Table 2. Morris water maze probe trial. Summary of the two-way ANOVA results in all groups.

	Anxiety		Age		Sex	
	F	P	F	P	F	P
Distance traveled (cm)	5.476	0.022	11.116	0.001	2.139	0.148
Average swimming speed (cm/s)	5.141	0.026	11.023	0.001	2.246	0.138
Number of platform crossing	2.747	0.102	1.112	0.295	1.112	0.295
Time spent in platform (s)	0.016	0.889	4.997	0.029	1.241	0.269

Conclusions

In conclusion, age, sex and anxiety differentially affect spatial and emotional memory in rats. More detailed researches are needed about the cognitive functions to clarify the exact role of estrogen in sex difference and exact roles of neurotransmitters and oxidative stress in aging process.

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