

AGE AND SEX AFFECT SPATIAL AND EMOTIONAL BEHAVIORS IN RATS: THE ROLE OF REPEATED ELEVATED PLUS MAZE TEST

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Abstract—The main objective of the study was to investigate the effects of age and sex differences on locomotor activity, learning and memory in rats. Another objective was to investigate whether repeated elevated plus maze tests induce anxiety in rats. Eighty Wistar rats were divided into eight groups according to their sex, age and anxiety status. Locomotor activity was assessed in open field. Repeated anxiety tests were performed in elevated plus maze. Spatial learning and memory were evaluated with the Morris water maze. All behavioral tests were recorded online and analyzed offline with an analytical software. Exploratory behavior was lower in anxiety-induced rats. Male rats had lower anxiety levels, locomotor activity and exploratory behavior compared to females. During the training period of Morris water maze latency to find platform, total distance traveled and average swimming speed decreased in all groups with repeated tests and young rats generally were faster than aged rats. During the probe trial, although the number of platform crossings was not affected, time spent in the platform zone was higher in the young groups compared to the aged groups. In conclusion, age and sex affect locomotor activity, learning and memory in different aspects. © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: behavioral measurement, anxiety, aging, sex difference.

INTRODUCTION

Aging is a physiological process which adversely affects many vital functions, including the cognitive functions (Gottfries, 1990). Aging-induced cognitive dysfunctions are well documented in humans (Wetherell et al., 2002) and several animal species including rats and mice

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Abbreviations: ANOVA, analysis of variance; CAF, control aged females; CAM, control aged males; CYF, control young females; CYM, control young males; EAF, experimental aged females; EAM, experimental aged males; EPM, elevated plus maze; EYF, experimental young females; EYM, experimental young males; MWM, Morris water maze; OF, open field.

(Almaguer et al., 2002; Bowman et al., 2006; Küçük et al., 2008; Bergado et al., 2011). Aging-induced cognitive dysfunction might be due to impairment in long-lasting, synaptic plasticity processes, like long-term potentiation (Bergado et al., 2011). Changes in the levels of neurotransmitters such as acetylcholine, catecholamines or glutamate (McEntee and Crook, 1993; Richter-Levin and Segal, 1993; Nitta et al., 1995) might also be associated with memory loss. In addition to changes in cognitive performance, baseline anxiety increases in the aged rats (Baxter and Gallagher, 1996; Küçük et al., 2008).

Behavioral sex differences are observed in many anxiety and memory tests, depending on the sex hormones and some mediators in the brain (Simpson and Kelly, 2012). However, there is no consensus about the effects of sex on locomotor activity, spatial learning and memory and possible effects of aging and anxiety.

Morris water maze (MWM) was first described 30 years ago to investigate spatial learning and memory in laboratory animals (Morris, 1981). Open-field (OF) and elevated plus maze (EPM) tests are used to examine the locomotor activity and anxiety, respectively (Grace et al., 2009). In a review of Carobrez and Bertoglio (2005), EPM is classified as an unconditioned type of model and therefore, implying that the task is aversive per se and repeated test situation leads to a gradual increase in avoidance behavior and decision to stay inside a safer place in the maze. Therefore repeated EPM tests may induce anxiety-like situation in rats.

In the present study we investigated the effects of aging and sex differences on locomotor activity, exploratory behavior, learning and memory in rats after repeated EPM test. Anxiety levels and locomotor activities were tested by EPM and OF, learning and memory activities were tested by MWM. These parameters were analyzed by using complex video-based devices and software.

EXPERIMENTAL PROCEDURES

Animals

A total of 80 adult male ($n = 40$) and female ($n = 40$) Wistar rats weighing between 250 and 500 g were obtained from the Selcuk University Experimental Medicine Research and Application Center (Konya, Turkey). Animals were divided into eight groups (10 animals in each group) as follows:

Group 1: 7-month-old control young males (CYM) (391.3 ± 48.2 g).

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| Group 2: | 7-month-old control young females (CYF) | (248.8 ± 17.7 g). |
| Group 3: | 15-month-old control aged males (CAM) | (439.1 ± 60.9 g). |
| Group 4: | 15-month-old control aged females (CAF) | (312.0 ± 39.1 g). |
| Group 5: | 7-month-old experimental young males (EYM) | (418.6 ± 55.5 g). |
| Group 6: | 7-month-old experimental young females (EYF) | (267.2 ± 22.4 g). |
| Group 7: | 15-month-old experimental aged males (EAM) | (402.8 ± 52.9 g). |
| Group 8: | 15-month-old experimental aged females (EAF) | (339.7 ± 29.4 g). |

The study protocol was approved by the Ethics Committee of the Experimental Medicine Research and the Application Center of the Selcuk University. The principles of laboratory animal care of the National Institute of Health (NIH) guideline were followed in all the experiments. All animals were housed in polycarbonate cages (five rats/cage) with a 12-h light–dark cycle and environmentally controlled facilities (temperature: $21 \pm 2^\circ\text{C}$, relative humidity: 50%). The rats were fed by a standard rat chow and tap water *ad libitum*.

Behavioral tests

The experimental design is illustrated in Fig. 1. On day 1, an evaluation test in OF was performed. On the following days (days 2–5) EPM was conducted before MWM. EPM and MWM tests were performed on the same days, respectively. On day 6, a second evaluation test in OF was performed after EPM. On day 7, MWM probe trial was performed. Control groups underwent the same protocols as in the experimental groups, except EPM. Anxiety was induced by repeated EPM tests. In the previous studies (Rodgers et al., 1996; Lamprea et al., 2000) it has been demonstrated that two sessions of repeated EPM test induced anxiety-like behavior in rats.

All behavioral measures were collected by a single trained laboratory researcher in a sound-attenuated room. For all testing procedures, rats were brought into the testing room 30 min prior to behavioral testing and left undisturbed to habituate to the changes in environment. The testing room used was ventilated and maintained at a temperature of $21 \pm 2^\circ\text{C}$. After the behavioral test of each animal, EPM and OF were cleaned with 70% alcohol and then dried to prevent the olfactory cues. To minimize diurnal effects, all behavioral tests were performed at the same hours of the day (09:00–12:00 a.m.).

Tracking system

All tests were recorded online using a video camera placed vertically above the test arenas. The videos of the behavioral tests were analyzed offline using an analytical software (Noldus Information Tech. Ethovision XT 8.0, Wageningen, The Netherlands).

Open field

Spontaneous locomotor activity of the rats was assessed in OF. The OF consisted of a black wooden square box (80×80 cm) with walls 40 cm high. The animals were individually placed in the center of the box and allowed to explore freely for 5 min. The box was divided into two different parts imaginarily: center and walls. The following parameters were recorded: (a) total distance traveled during the test (cm), time spent (s) in (b) center and (c) walls, number of (d) rearings, (e) defecations, and (f) groomings, (g) average speed during the test (cm/s), time spent (s) as a (h) mobile and (i) immobile.

Elevated plus maze

The EPM consisted of a plus-shaped platform (50 cm higher than ground level) with two open (50×10 cm) and two enclosed arms (50×10 cm). Rats were placed individually on the center of the platform facing one of the open arms and were allowed to freely explore the maze for 5-min of testing period. During the test the following variables were recorded: (a) total distance traveled (cm), number of entries in (b) center, (c) open and (d) closed arms, time spent (s) in (e) center, (f) open and (g) closed arms, time spent (s) as a (h) mobile and (i) immobile.

Morris water maze

Spatial learning and memory were assessed by MWM. The MWM consisted of circular, galvanized steel tank with 150 cm diameter and 60 cm in depth. The temperature of water was adjusted to $25 \pm 2^\circ\text{C}$. The maze was imaginarily divided into four quadrants such as southeast (SE), southwest (SW), northeast (NE) and northwest (NW). A hidden square platform – served as the escape platform- placed 2 cm below the water surface. It was located into the SE region and fixed during the training period. Several identical cues were placed on the surrounding walls of MWM for the spatial orientation of rats.

The rats were trained for four consecutive days with four trials per day and 48 h after the last training session a probe trial was performed. During the training period, four starting points were randomized everyday to avoid left and right navigation to the platform. This is known as response strategy and was first described by Clements et al. (2007) where the rats remember which direction their body turned instead of using visuospatial cues to guide them. Instead of hippocampal memory used in a place strategy where the rats use spatial cues to guide them, the response strategy relies on striatal memory (Grace et al., 2009). Each trial was terminated in 90 s and if the rats were unable to locate the platform in this time interval then they were placed on the platform for 30 s. The following parameters were recorded: (a) latency to find platform, (b) total distance traveled and (c) average swimming speed. The data of four consecutive trials for each of the groups obtained were averaged and presented as a block. Blocks in graphs were numbered consecutively for the day.

Following the four days of training period, a 120-s probe trial was conducted after the hidden platform was removed. The following variables were recorded during the probe trial: (a) total distance traveled, (b) average swimming speed, (c) the number of platform crossings and (d) time spent in the platform zone.

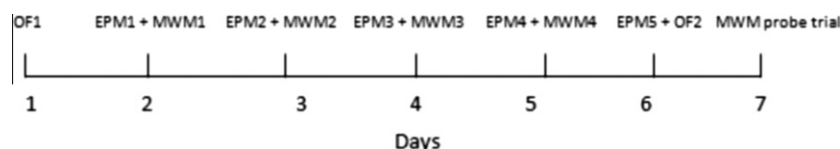


Fig. 1. Experimental design of the study. OF: open field, EPM: elevated plus maze, MWM: Morris water maze.

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