

# Age, Sex and Anxiety Affect Locomotor Activity in Rats

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## Abstract

The main objective of study was to investigate the effects of age, sex and anxiety differences on locomotor activity in rats. Locomotor activity was assessed in open field (OF). Repeated anxiety tests were performed in elevated plus maze (EPM). All behavioral tests were recorded online and analyzed offline with analytical software. In conclusion, age, sex and anxiety affect locomotor activity 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]. It has been reported that there may be many different reasons for this impairment such as impairment in long-lasting, synaptic plasticity processes, like long-term potentiation [2]. Changes in neurotransmitter systems might also reason of the loss of memory [3]. Behavioral sex differences depend on the sex hormones and other mediators in the brain [4]. However, there is no consensus about the effects of sex differences on locomotor activity and possible effects of aging and anxiety. In the present study, we aimed to investigate the effect of aging and sex differences on locomotor activity and exploratory behavior in anxiety induced rats.

## Materials and methods

### Animals

A total of 80 adult male (n: 40) and female (n: 40) Wistar rats were obtained from Selçuk University Experimental Medicine Research and Application Center (Konya, Turkey). Animals were divided into eight groups (ten animals in each group) as follows:

- Group 1: 7-month-old control young males (CYM, n:10).
- Group 2: 7-month-old control young females (CYF, n:10).
- Group 3: 15-month-old control aged males (CAM, n:10).
- Group 4: 15-month-old control aged females (CAF, n:10).
- Group 5: 7-month-old experimental (anxiety-induced) young males (EYM, n:10).
- Group 6: 7-month-old experimental (anxiety-induced) young females (EYF, n:10).
- Group 7: 15-month-old experimental (anxiety-induced) aged males (EAM, n:10).
- Group 8: 15-month-old experimental (anxiety-induced) aged females (EAF, n:10).

The study protocol was approved by the Ethics Committee of the Selçuk University Experimental Medicine Research and Application Center. The principle of laboratory animal care of the National Institute of Health (NIH) guideline was followed in all these experiments.

## **Behavioral Tests**

On day 1, an evaluation test in OF was performed. On the following days (2-5) EPM was carried out. On day 6, second evaluation test in OF was performed. After each animal's behavioral tests, EPM and OF were cleaned with an alcohol and dried to prevent the olfactory cues.

## **Tracking System**

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

## **Open Field**

Spontaneous locomotor activity of the rats was assessed in OF. The animals individually placed in the centre of the box and allowed to freely explore for 5 min.

## **Elevated Plus Maze**

The EPM consisted of a plus shaped platform with two open and two enclosed arms. 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 testing period.

## **Statistical Analysis**

Statistical analyses of data were carried out by using SPSS 15.0 for Windows. All data are presented as mean  $\pm$  SD. Changes of the OF 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.

## **Results and discussion**

### **Open Field**

The total distance traveled and average speed were significantly affected by the time and time x sex interactions (see Table 1). They were significantly decreased in second trial compared to first trial in all groups; also total distance traveled and average speed were higher in females than males. Time spent in center and walls were affected by time x sex interaction (see Table 1). Time spent in center increased in males and decreased in females however, time spent in walls decreased in males and increased in females in second trial compared to first trial. Time spent as a mobile and immobile were significantly affected by time and time x sex interactions (Table 1). Time spent as a mobile significantly lower and time spent as an immobile significantly higher in second trial than first trial in all groups. Furthermore, decrease in time spent as a mobile and increase in time spent as an immobile were higher in females compared to males. The number of defecation was significantly affected by time, time x anxiety and time x sex interactions (see Table 1). Changes of number of defecation were higher in experimental groups and males compared to controls and females, respectively. Grooming behavior was significantly affected by time, time x anxiety and time x age interactions (see Table 1). Changes of number of grooming were significantly decreased in second trial compared to first trial in all groups. Grooming behavior was lower in experimental groups and aged rats compared to controls and young rats, respectively. Rearing behavior was significantly affected by time, time x anxiety and time x sex interactions (see Table 1). The number of rearing events was significantly decreased in the second trial compared to first trial for all groups. Incidence of rearing was lower in experimental groups and males compared to control groups and females.

### **Elevated Plus Maze**

Total distance traveled, numbers of entries in center, open arms and closed arms significantly affected by time interaction (see Table 2). These variables were decreased by repeated measurements in all groups.

Table 1. Open Field. 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
Total distance traveled (cm)	170.889	0.000	0.095	0.759	0.243	0.624	11.618	0.001
Average speed (cm/s)	170.855	0.000	0.099	0.754	0.225	0.636	11.617	0.001
Time spent in center (s)	0.128	0.722	0.617	0.435	0.242	0.625	4.863	0.031
Time spent in walls (s)	0.128	0.722	0.617	0.435	0.242	0.625	4.863	0.031
Number of rearing	179.530	0.000	5.691	0.020	3.000	0.088	7.007	0.010
Number of defecation	0.330	0.014	4.308	0.042	0.479	0.491	6.330	0.014
Number of grooming	11.903	0.001	8.561	0.005	4.579	0.036	0.154	0.695
Time spent as a mobile (s)	86.657	0.000	0.669	0.416	0.328	0.569	36.595	0.000
Time spent as an immobile (s)	86.447	0.000	0.605	0.439	0.247	0.621	36.350	0.000

Table 2. Elevated plus maze. Summary of the two-way repeated measures ANOVA results in all groups.

	Time		Time x Age		Time x Sex	
	F	P	F	P	F	P
Total distance traveled (cm)	22.907	0.000	1.321	0.283	1.923	0.130
Number of entries in center	6.093	0.001	0.506	0.732	0.460	0.764
Number of entries in open arms	4.033	0.009	1.659	0.183	0.874	0.490
Number of entries in closed arms	7.157	0.000	0.657	0.626	1.872	0.139
Time spent in center (s)	1.380	0.262	0.161	0.956	0.186	0.944
Time spent in open arms (s)	2.046	0.111	0.427	0.788	0.717	0.587
Time spent in closed arms (s)	0.293	0.881	0.201	0.936	0.322	0.861
Time spent as a mobile (s)	0.887	0.482	0.772	0.551	0.547	0.702
Time spent as an immobile (s)	0.180	0.947	1.106	0.370	0.119	0.975

## Conclusions

In conclusion, age, sex and anxiety affect locomotor activity and exploratory behavior. 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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