Effects of age and sex differences on conditioned and unconditioned fear in anxiety developed animals

Anksiyete oluşturulan deney hayvanlarında cinsiyetin ve yaşın şartlı ve şartsız korku üzerine etkisi

Ayşegül Küçük,
Dr., PhD.
Department of Physiology,
Hospital of Dumlupınar University,
kucukaysegul@hotmail.com

Asuman Gölgeli,
Prof. Dr., PhD.
Department of Physiology,
Erciyes University School of Medicine,
golgeli@erciyes.edu.tr

Abstract
Purpose: The aim of this research was to assess the effects of age and sex differences on conditioned and unconditioned fear in elevated T-maze both in mice (Balb-c) and rats (Wistar Albino).
Material and Methods: For this purpose, 40 mice and 40 rats (10 young male, 10 young female, 10 aged male and 10 aged female) were used in this study. Elevated T-maze, an animal model of anxiety, consists of 3 arms of equal dimensions, elevated from the floor. This test allows the measurement of two kinds of aversively motivated behaviors in the same animal by using the time taken to leave the closed (conditioned response) and open arms (unconditioned response).
The avoidance latencies were analyzed by Repeated Measures and Mann Whitney-U test, the escape latencies were analyzed by One–way ANOVA (Scheffe).
Results: Mice exhibited high latencies in avoidance responses (avoidance 1, avoidance 2) compared with baseline latencies in the 4 groups. However, only young male and aged female mice were significantly different ($p<0.05$). Avoidance 1 latencies were sgnificantly different in the aged and young male mice ($p<0.05$). While escape latencies representing unconditioed fear were decreased in the aged mice, no statistically significant difference was found in respect to sex distribution. In rats, avoidance responses also exhibited high latencies in all groups when compaired with baseline latencies. However, there were no statistically significant difference in age and sex distributions in conditioned and unconditioned responses ($p>0.05$).
Conclusion: The fact that mice were effected more than rats suggests that in animal models of anxiety the differences in age and sex may be changed according to the gender and the model which is used.

Key Words: **Conditioning, classical; Fear; Learning, maze; Mice; Rats.**

Özet
Amaç: Çalışmada cinsiyet ve yaş farklılıklarının hem fare (Balb-c) hem de sıçanlarda (Wistar Albino) şartlı ve şartsız korku üzerine etkisi yükseltilmiş T-labirent parametreleri incelenerek araştırılmak istenildi.
Gereç ve Yöntem: Bu amaçla çalışmada 40 adet fare ve 40 adet sıçan (10 genç erkek, 10 genç dişi, 10 yaşlı erkek, 10 yaşlı dişi) kullanıldı. Yükseltilmiş T-labirent anksiyete modeli oluşturmada kullanılan, eşit boylarda üç kollu, yerden belli bir yükseklikte hazırlanmış bir düzenektir. Kapalı kola konulan hayvanın açık kola çıkması için geçen süre (şartlı korku cevapları) ve açık kola konulan hayvanın kapalı kola girmesi için geçen süre (şartsız korku cevapları) bulunarak aynı hayvanda iki farklı korku türü değerlendirildi. Şartlı korkuya cevapları Tekrarlı Ölçümler ve Mann Whitney-U, şartsız korku cevabı ise tek yönlü ANOVA (Scheffe) testi ile değerlendirildi.
Bulgular: Farelerde, sakınma cevapları (sakınma 1 ve sakınma 2), bazal süreye göre 4 grup için de uzadı. Ancak istatistiksel anlamlılık sadece genç erkek ve yaşlı dişi farelerde gözlendi ($p<0,05$). Genç ve yaşlı erkek farelerin sakınma 1 süreleri arasında istatistiksel olarak anlamlı fark bulundu ($p<0,05$). Şartsız korku cevaplarını gösteren kaçma süreleri yaşlı farelerde gençlere göre kısaldı, ancak aralarında cinsiyete bağlı fark bulunmadı ($p>0,05$).Sıçanlarda da sakınma cevapları bazal süreye göre bütün gruplarda uzadı, ancak yaş ve cinsiyete bağlı farklılık göstermedi. Şartsız korku cevapları da yaş ve cinsiyetten etkilenmedi ($p>0,05$).
Sonuç: Şartlı ve şartsız korkularını değerlendirdiğimiz fare ve sıçanlarda, farelerin yaş ve cinsiyet farklılığından daha fazla etkilenmiş olması nedeniyle, yaş ve cinsiyet farklılıklarının cinse ve kullanılan modele göre önemli olabileceği sonucuna varıldı.

Anahtar Kelimeler: **Fare; Klasik şartlanma; Korku; Öğrenme, labirent; Sıçan.**

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Corresponding Author:
Ayşegül Küçük
Department of Physiology,
Hospital of Dumlupınar University,
Central Campus, Tavsanlı Road 10. km,
43270, Kütahya/Turkey

Telephone : +90 274 2652031 - 2424
E-mail : kucukaysegul@hotmail.com

Introduction

Behavioural differences between male and female animals have been studied in a number of different experimental procedures (1). In the social interaction test, the social interaction scores of female rats were lower and did not increase as readily following familiarization to the apparatus as those of the male rats. In the elevated plus maze test, female rats showed a reduced aversion to the open arms compared to male rats. In a modified Vogel conflict test, the punished licking rates of the female rats were lower than those of the male rats. It is concluded that the behaviors of male and female rats differ in these tests (1-7).

There are many reports, in which it was reported that the anxiety levels were greater in female than those in male rats (8-11). However, there are also other studies, which stated that males showed greater levels of spontaneous activity than those of females on measures of activity (12). Furthermore, there are studies in which locomotor activity did not differ from male to female rats (13).

Sprott et al. explained that senescent organisms are less active, less responsive to stress, less emotional and also perhaps have lower motivation levels than younger organisms. Furthermore, it has beeen suggested that the results obtained with senescent organisms are also influenced by the stress history and by the genetic composition of the organism. These observations make the interpretation of extent learning data extremely difficult since all of the variables described could have an effect upon the performance in learning situations (14). Aging introduces functional as well as structural changes in systems. Although there may be individual differences, learning and short-term memory especially, may be effected dramatically due to aging.

The elevated T-maze test is used to investigate the effects of new anxiolytic drugs on memory as well as to investigate brain mechanisms underlying anxiety (5-7). Elevated T-maze is derived from the elevated plus maze, developed as an animal model of anxiety to separate, in the same animal, conditioned from unconditioned fear (2-5). Conditioned fear (passive avoidance, inhibitory avoidance) is related to anticipatory anxiety. Anticipatory anxiety is caused by expectation of anxiety or panic in a particular situation. Unconditioned fear is related to panic disorders. Animals learn actively to avoid places or items that are harmful (2-7, 15-18).

Mice differ from rats in defense repertoire, mice resembling wild rather than labaratory rats. Therefore, mice may be more suitable than labaratory rats for studying behavioural reactions to proximal threat, which may relate to panic disorder (2). Conde et al. (4) declared that individual emotional differences could affect inhibitory avoidance performance.

The purpose of the present study was to investigate whether age and sex differences effect conditioned and unconditioned responses in the anxiety model of the elevated T-maze both in aged, young, male, female mice and rats.

Materials and Methods

Animals were paired within their sexes on the basis of weight. Rats and mice were kept 10 per cage in an animal house at 203 ºC. All experiments were performed during the light phase of the light-dark cycle. Forty mice and 40 rats were included in the study. Each group was comprised of 10 animals; young groups (20-25g, 3-4 month for mice and 250-300g, 3-4 month for rats), aged groups (30-34g, 18-20 month for mice and 400-450g, 18-20 month for rats). The Erciyes University Guide For Care and Use of Laboratory Animals was adhered to follow throughout the experiments described.

The elevated T-maze is made of white plastic, consisting of 3 arms of equal dimensions and is elevated from the floor. One arm is enclosed by walls and stands perpendicular to 2 opposite open arms.

The elevated T-maze apparatus is designed differently for mice and rats. For mice, it had three arms of equal dimensions (30x5cm), elevated 38.5cm from the floor. One arm is enclosed by walls (15cm) and stood perpendicular to the 2 open arms. To avoid falls, the open arms were surrounded by plexiglass rim 0.25cm high (2). For rats, it had 3 arms of equal dimensions (50x12cm), elevated 50cm from the floor. One arm is enclosed by walls (40cm) and stood perpendicular to the two open arms. To avoid falls, the open arms were surrounded by plexiglass rim 1cm high (3).

Inhibitory avoidance of the open arms representing learned fear was measured by recording the time taken to leave the enclosed arm in three consecutive trials. Unconditioned fear was evaluted by recording the time to escape from the open arm.

The animal was placed at the distal end of the enclosed arm of the elevated T-maze and the baseline latency is recorded by the time taken to withdraw from this arm with all 4 paws. In order to evaluate conditioned fear, which represents avoidance 1 and avoidance 2, the same measurement was repeated in two subsequent trials performed at 30-s intervals. Thirty seconds after the completion of the avoidance task, the animal was placed at the distal end of the right open arm and the escape latency is recorded by the time to withdraw from this arm with all 4 paws. This one-way escape response represented unconditioned fear. Latency cut-off time was 300s. The maze was cleaned with 20 % alcohol after removal of each animal. Each animal was tested only once.

Statistical analysis: The avoidance latencies were analyzed by ANOVA Repeated Measures and the differences between the groups were analyzed by Mann Whitney-U test and the escape latencies were analyzed by one-way analyses of variance (ANOVA-Scheffe). Values are expressed as means (SEM) and significance is defined as $p< 0.05$ for all tests.

Results

Animals were matched within their sexes on the basis of weight, so that there were no significant differences between the groups ($p>0.05$). Inhibitory avoidance latencies (passive avoidance) and one-way escape latency (active avoidance) were evaluated with this test. As illustrated in Figure 1, avoidance 1 and avoidance 2 was greater than baseline latency for the 4 groups in mice. However, significant differences were observed only in young male mice (baseline latency: 18.50±2.98s, avoidance 1 latency: 188.50±36.55s) and aged female mice (baseline latency: 17.80±4.06s, avoidance 1 latency: 124.20±40.28s). Avoidance 1 was 168.00±33.05s in the aged male mice and 249.50±30.57s in the young male mice ($p<0.05$). Avoidance 2 was significantly different from baseline latency for 4 groups ($p<0.05$). Avoidance 2 latency is longer than avoidance 1 and this difference was significant except in the aged females ($p<0.05$). Escape latency was 157.20±17.51s in the young male mice, 73.70±18.71s in the aged male mice, 126.00±27.17s in the young female mice, 40.60±13.10s in the aged female mice. When the groups were compaired, escape latency was found to be reduced with aging ($p<0.05$), although this was not affected by sex ($p>0.05$).

As shown in Figure 2, avoidance 1 was longer than baseline latency and avoidance 2 was longer than avoidance 1 in rats in the elevated T-maze. The differences in avoidance 2 were statistically significant for the 4 groups except the young female rats ($p<0.05$). There were no differences between the groups in conditioned fears ($p>0.05$). Age and sex did not affect the escape latencies ($p>0.05$).

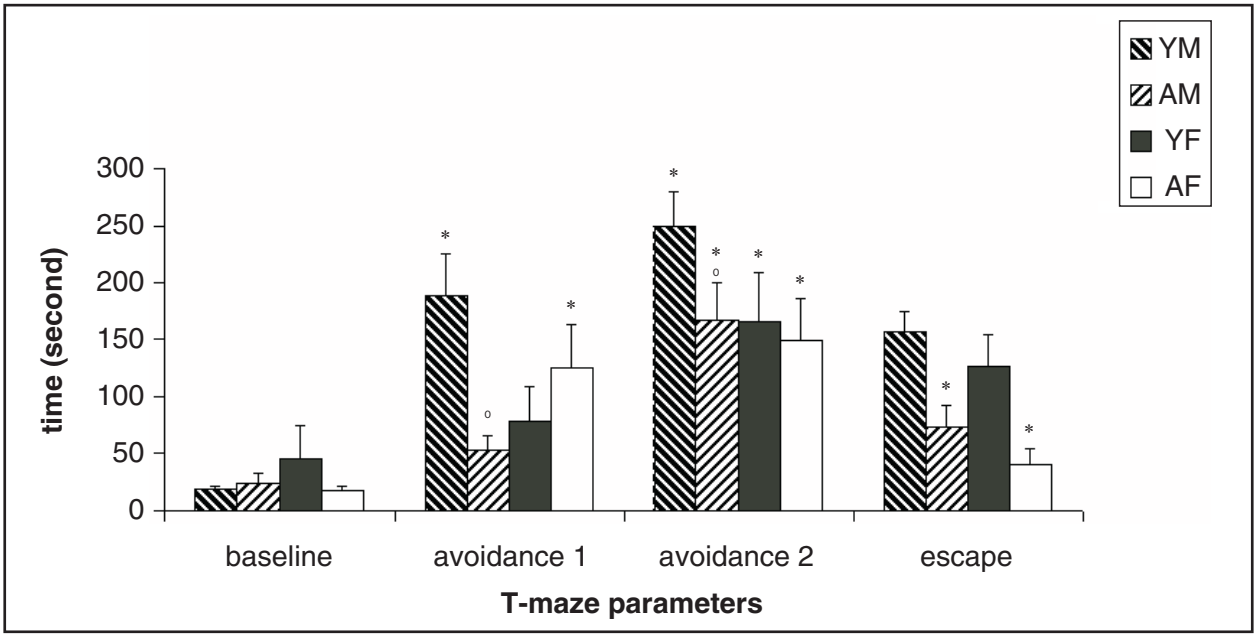

Figure 1. Response of avoidance and escape latencies in mice in elevated T-maze (n=10).
(YM; young male, AM; aged male, YF; young female, AF; aged female)
(***:** different from baseline, °: different from young male, #: different from young group, $p<0.05$).

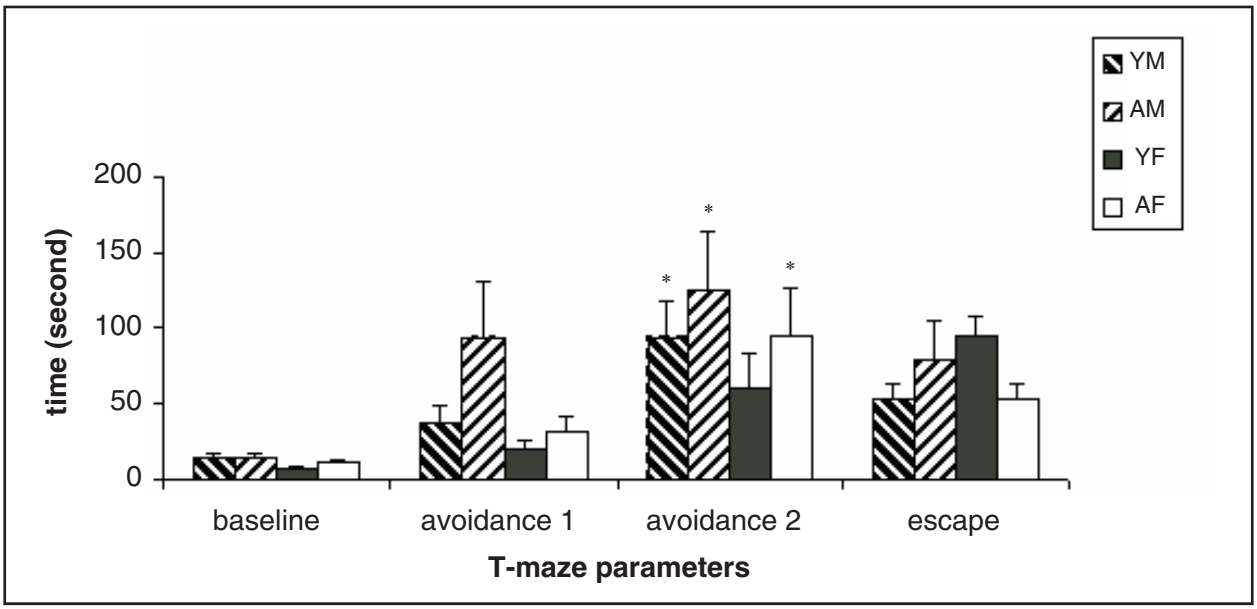

Figure 2. Response of avoidance and escape latencies in rats in elevated T-maze (n=10).
(YM; young male, AM; aged male, YF; young female, AF; aged female)
(***:** different from baseline, $p<0.05$).

Discussion

Recently, Graeff et al. developed an animal model of anxiety to separate conditioned and unconditioned fear in the same rat and mice (5). This test, called the elevated T-maze, is derived from the elevated plus maze, a widely used animal model of anxiety. This experimental model allows the parallel measurements of responses related to both innate and learned fear in the same subject and permits the simultaneous assessment of memory for these behaviors (4). Therefore, this test apparatus is fundamental in the investigation of anxiolytic and antipanic effects in 2 types of fear and for the understanding of the brain mechanisms underlying anxiety (3-7). Inhibitory avoidance, the time taken to leave the enclosed arm, is related to short-term memory (6).

Rats, which were given diazepam, showed impaired inhibitory avoidance 72 hours after injection. Therefore, diazepam had both anxiolytic and amnestic effects on inhibitory avoidance (6). Shorter avoidance responses seen in aged male mice in the elevated T-maze may have arisen from difficulty in learning fear and decrease in memory due to aging. However, no effects could be seen in the avoidance responses in female mice due to age. Shorter avoidance responses in aged female and aged male mice showed that mice readily exhibit panic disorders due to aging. The reason why young females showed prolonged avoidance latency was that they were not affected by height and openness. As there were no statistically significant differences in avoidance and escape latencies between male and female mice, it is possible to conclude that sex does not affect these two types of fear. However, extended avoidance responses seen in both male and female rats in elevated T-maze were statistically not significant. Such responses prove that aged rats learn conditional fear more quickly than mice. We can interprete that short-term memory is better in aged rats than the young ones. Avoidance latencies in male and female rats were not statistically different, which showed that sex is not an important factor in short-term memory. As the avoidance latencies in both male and female rats were similar, this parameter was not affected by age and sex. Sex differences in the open field appeared around puberty with the males showing a postpubertal decrease in activity, however the females retained their prepubertal levels (19,20). The neuroendocrine responses to stress were higher in females (21).

Jardim and co-workers have hypothesized that mice differ from rats in defense repertoire, mice resembling wild rather than labaratory rats. Thus, mice may be more suitable than labaratory rats for studying behavioural reactions to proximal threat, which may relate to panic disorder (2). However, our findings suggest that elevated T-maze can be applied to rats (Wistar Albino) as a model of anxiety. In addition, individual differentations should be taken into account (14). The aim of this study was to assess the effects of age and sex differences in Balb-c mice and Wistar Albino rats. In the present study, we proved that the elevated T-maze, an anxiety model, is a suitable experimental model for rats (Wistar Albino) as well as for mice (Balb-c).

In conclusion, our results showed that sexis not important both in mice and rats, but age is an important factor in mice. It is concluded that in behavioural studies species, genus, age and sex differences can influence the results, and thereby each factor should be taken into account by researchers.

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