RESEARCH ARTICLE / *Araştırma Makalesi*

Comparison of Effects of Three Distinct Stress Models on Anxiety- and Depression-Related Behaviors in Female Rats

Dişi Sıçanlarda Üç Farklı Stres Modelinin Anksiyete ve Depresyon Benzeri Davranışlara Etkilerinin Karşılaştırılması

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Abstract

Objective The aim of the present study was to compare the effects of three distinct stress protocols on anxiety/depression-related behaviors in adult female rats.
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Materials and Methods Adult Wistar rats were randomly divided into four groups (n=8/ group) as control, immobilization stress-1 (daily 45 minutes) immobilization stress-2 (daily twice 45 minutes) and social isolation (rats were housed in a metabolic cage). Stress protocols were performed for a period of 10 days. When the animals were in diestrus, anxiety and depression-like behaviors were evaluated by the open field test and forced swimming test, respectively. Anxiety and depression tests were repeated after a 10-day rest period.

Results In the open field test, a percentage of time spent in the central area was lower in the immobilization stress-2 and social isolation ($p<0.05$) groups and total distance was lower in the immobilization stress-1 ($p<0.01$) and the social isolation groups ($p<0.05$). Rearing score was lower in the social isolation group ($p<0.05$). Swimming behaviors were lower ($p<0.01$), and immobility durations were higher ($p<0.05$) in the immobilization stress-1 and social isolation groups. In the second tests, time spent in the central area was lower in the immobilization stress-1 ($p<0.05$) and the immobilization stress-2 ($p<0.01$) groups. Swimming behaviors were lower and immobility behaviors were higher in the immobilization stress-1 group ($p<0.001$) and the social isolation group ($p<0.01$ and $p<0.001$, respectively).

Conclusion We suggest that depression-like behaviors are more dominant in the immobilization stress-1 and social isolation groups of adult female rats because the depression-related results of these two groups are valid both after the stress period and after the rest period.

Keywords immobilization stress; social isolation; depression; anxiety; female rat.

Öz

Amaç *Mevcut çalışmada yetişkin dişi sıçanlarda üç farklı stres protokolünün anksiyete/depresyon benzeri davranışlara etkisinin kıyaslanması amaçlanmıştır.*
(Sakarya Tip Dergisi 2019, 9(1):131-140)*.*

Gereç ve Yöntemler *Yetişkin Wistar sıçanlar kontrol, immobilizasyon stresi-1 (günlük 45 dakika) immobilizasyon stresi-2 (günlük iki kez 45 dakika) ve sosyal izolasyon (metabolik kafeste) olarak rastgele dört gruba ayrıldı (n=8/grup). Stres protokolleri 10 gün boyunca uygulandı ve anksiyete ve depresyonla ilişkili davranışlar, hayvanlar diöstrüs dönemindeyken, açık alan ve zorunlu yüzme testi ile değerlendirildi. Anksiyete ve depresyon testleri 10 günlük dinlenme süresinden sonra tekrarlandı.*

Bulgular *Açık alan testinde, merkez alanda geçirilen zaman immobilizasyon stresi-2 ve sosyal izolasyon gruplarında azaldı ($p<0.05$). Toplam kat edilen mesafe immobilizasyon stresi-1 ($p<0.01$) ve sosyal izolasyon ($p<0.05$) gruplarında daha düşüktü. Sosyal izolasyon grubunun şahlanma davranışı ($p < 0.05$) kontrol grubuna göre düşüktü. İmmobilizasyon stresi-1 ve sosyal izolasyon gruplarında yüzme davranışı azalırken ($p < 0.01$), immobilizasyon davranışı kontrol grubuna göre artış göstermişti ($p <0.05$). İkinci testlerde, merkezi alanda geçirilen süre immobilizasyon stresi-1 ve immobilizasyon stresi-2 gruplarında daha düşüktü ($p <0.05$ ve $p <0.01$). İmmobilizasyon stresi-1 ($p <0.001$) ve sosyal izolasyon gruplarında ($p <0.01$) yüzme davranışı azalırken, immobilizasyon süresi kontrole kıyasla artmıştı ($p <0.001$).*

Sonuç *İmmobilizasyon stresi-1 ve metabolik kafeste sosyal izolasyon protokolü uygulanan erişkin dişi sıçanlarda depresyon benzeri davranışların daha baskın olduğu söylenebilir çünkü her iki grupta da depresyonla ilişkili bulgular hem stres sonrası hem de dinlenme periyodu sonrası geçerlidir.*

Anahtar Kelimeler *immobilizasyon stresi; sosyal izolasyon; depresyon; anksiyete; dişi sıçan*

Introduction

Although stress is an integral part of life, constantly or intensive stress has a major influence on our sense of well-being or mood, as well as physiological functions.[1] Stressful events, acute or chronic, can induce an increase in cortisol (corticosterone in rodents) in the body. This elevation in plasma cortisol (or corticosterone) level is a result of activation of the hypothalamic-pituitary-adrenal (HPA) axis.[2] The HPA activation is started with the release of corticotropin-releasing hormone from the paraventricular nucleus in the hypothalamus, causing the secretion of adrenocorticotropic hormone from the anterior pituitary gland, which in turn stimulates the release of cortisol/corticosterone from the adrenal glands.[3,4] If the stress remains heightened, it can cause increased anxiety or mood disorders such as major depression, bipolar depression. Therefore, constant or repeated stress is accepted to quicken or worsen the mood disorders and anxiety disorders.[5,6]

World Health Organization estimates that 4.4% of the global population suffers from depression, and 3.6% from anxiety disorder based on data for 2015. Moreover, both diseases are more common among females than males.[7] The socio-economic costs of these disorders are relevant; for instance, their prevalence in the prime of life for reproductive-aged women in developed countries results in combined mortality and morbidity estimates much greater than any other health problems.[8] All of these facts require that notable efforts be made to understand anxiety and depression pathophysiology for treatment. For this purpose, animal experiments are an essential method for improving our knowledge of these disorders, as well as their pharmacological treatment.[9] The fact is taken into account that men and women not only respond in opposite directions to the same stressful event but also respond differently to controllability and antidepressant treatments.[10] As related to anxiety and depression, although gender differences in these disorders have been recognized in humans, the great majority of studies on the antidepressant and anxiolytic drugs have focused on male rodents as experimental models.[7,11,12] For this reason, it may be beneficial to use female rats in experiments to assess the effects of stress on depression and anxiety-like behaviors.[13]

Many animal stress models for triggering of depression or anxiety-like behavior have been developed and used frequently to evaluate the anti-stress activity of compounds. Immobilization, restraint, electric shock, and social isolation stress are commonly employed models for inducing stress in rodents.[14] Immobilization/restraint stress is one of the most popular experimental models used to assess the stress-induced physiological alterations and anti-stress activity of pharmacological agents in animals.[15,16] Although immobilization and restraint stress procedures are considered as similar or equivalent, the immobilization stress model may be a more intense stressor than the restraint stress model.[14,17] There are various time periods ranging from 5 to 30 days to induce chronic immobilization stress of varying degree in rats and mice.[17] Another important stress model is social isolation in the rodents because mice and especially rats are social animals.[18] Therefore, the long-term single housing of rats causes a social isolation stress with some physiological and behavioral abnormalities. There are many reports on the relationship between metabolic cage housing and social isolation stress in rodents.[18-21] Metabolic cages are frequently used for measurement of total intake of food and water as well as excretion of urine and feces in small rodents.[18] Therefore, these cages are widely used in metabolism and pharmacological studies. However, metabolic cages and the studies performed with them require the use of grid flooring, an absence of bedding substrate, single housing, and a smaller-than-usual living space area.[22] Recently, we reported that long-term metabolic cage housing causes a social isolation stress with increased anxiety and depression-related behaviors in male rats.[18]

To the best of our knowledge, there is currently no study on the comparison of the effects of immobilization stress and social isolation stress in female rats. We hypothesized

that the chronic immobilization stress and social isolation stress may cause behavioral abnormalities and these effects may continue after stress application. Therefore, we aimed to compare the effects of immobilization stress-1 (45 minutes for a period of 10 days), immobilization stress-2 (45 minutes twice a day for a period of 10 days), and social isolation stress (single housing in metabolic cages for 10 days), as well as 10-day rest period after stress conditions on anxiety and/or depression-like behaviors in adult female rats.

MATERIAL and METHODS

All experimental procedures in the present study were approved by the Local Ethical Committee of Necmettin Erbakan University and animals were treated in accordance with the national and international laws and policies on the care and use of experimental animals.

Animals

Thirty-two adult female Wistar albino rats weighing 280-320 g (KONÜDAM Experimental Medicine Application and Research Center of Necmettin Erbakan University, Konya) were used in this study. The rats were housed under standard light/darkness schedule (12-h light/12-h dark cycle), at constant temperature (21±1°C) and humidity (55±5%) with free access to pelleted food and tap water.

Experimental protocols

Rats were randomly divided into four groups (n=8 for each group) as control, immobilization stress-1 (45 minutes daily for a period of 10 days), immobilization stress-2 (45 minutes twice a day for a period of 10 days), and social isolation (single housing in metabolic cages for 10 days). Anxiety and depression tests were performed after the stress protocols. Subsequently, the animals were taken for 10-day of the rest period, and during this period, the animals were housed in their normal groups as at the beginning of the experiments. That is, nothing has been done in the immobilization groups and the rats in the metabolic cage are housed in groups in the normal cages. Anxiety and depression tests were repeated after this term.

All behavioral tests and sacrification were performed when the rats were in diestrus. Sexual cycle period of the animals was determined by vaginal smear.

By the end of the last open field test and the forced swimming test, the rats were fasted overnight but the water was given ad libitum. Trunk blood samples were obtained by decapitation for analysis of serum corticosterone levels. The serum samples were stored at 20° C until the analysis.

Immobilization stress and social isolation procedures

For immobilization stress, our own method that was modified from previous studies[23,24] was used. The rats were placed in cylinder apparatuses in two different sizes, which were suitable for their body volumes: 5 cm × 22 cm, and 5.5 cm × 22 cm dimensions (Figure 1). These cylinders were made from a transparent Plexiglas. There were ventilation holes in the walls of these apparatuses in the parts surrounding the animal body. The same holes were also present at the front of the head section. This plastic front section was designed to prevent head movement and was adjustable to the length of the animal body. At the back of the cylinder was lockable and perforated sliders for allowing the tail of rats to stay out. After the animals were placed in the apparatus, the head section could be moved backward from 22 cm down to 8 cm with the aid of a slider, according to the length of the animal. Thus, the cylinder-shaped collapsed area ensured the immobilization of both the animal limbs and head movements.

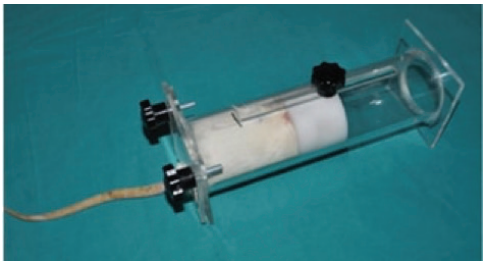

Figure 1. An overview of the application of immobilization stress by the transparent plexiglass apparatus.

For social isolation stress, the rats were individually housed in metabolic cages for 10 days (Figure 2). This procedure was performed by using commercial metabolic cages (220 mm in diameter × 120 mm tall, Tecniplast, Buguggiate VA, Italy).

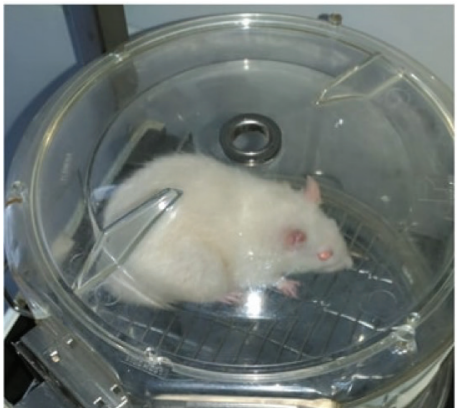

Figure 2. An overview of the social isolation conditions in the metabolic cage.

Anxiety and depression tests

Anxiety and depression-related behaviors were evaluated by the open field test and forced swimming test after stress protocols and rest term. The open field test is generally used to determine anxiety-related behavior in rodents.25 The test was performed between 9.00 and 11.00 am. The rats were placed in the center of a square-box test apparatus (80×80×30 cm, black Plexiglas) and tracked by a video tracking system (Ethovision 11, Netherlands) for a period of 300 seconds. On the software screen, the platform surface was divided into the center and edge regions. Scores of distance traveled (cm), mean velocity (cm/s), time spent in the central area (s), and mobility frequency were analyzed by the Ethovision software. Rearing and grooming behaviors were manually scored by using the video records in the software.[18]

The forced swimming test, first described by Porsolt et al.[26], is widely used to analyze depression-like behaviors of rodents. This test was performed according to our previously developed protocols.[18,27] For the pretest session, animals were individually placed for 15 min into Plexiglas cylinders (49 cm height, 25 cm diameter) containing 39 cm of water (27±1 °C). 24 h after the pretest session, the forced swimming test was performed. The total duration of the swimming, climbing and immobility behaviors was analyzed by the Ethovision XT11 video tracking system for a period of 300 seconds.

Corticosterone analysis

Serum corticosterone levels were analyzed by enzyme- linked immunosorbent assay (ELISA) according to previous methods with some modifications.[18] Briefly, corticosterone-bovine serum albumin (1 µg/mL), as the stock solution, was diluted with carbonate buffer (pH 9.6), and this solution (200 µl/well) was transferred into a 96-well microtiter plate (Nunc, Roskilde, Denmark). After overnight incubation at +4 °C, the plate was washed with washing buffer and blocking buffer (200 µl/well) was added for 120 min at 37 °C. The plate was washed, and serum samples or standards (50 µl/well) were preincubated with primary antibodies (50 µL/well) for 45 min at 37°C and then were transferred into coated plates for competition with antigens on the solid phase for 30 min at 37 °C. After washing, biotinylated goat anti-rabbit IgG (100 µl/well) coated wells were incubated for 30 min at 37 °C. The plate was washed again, 100 µl/well streptavidin-peroxidase solution (Sigma-Aldrich, Taufkirchen, Germany) coated wells were incubated for 15 min at 37 °C. After washing, tetramethylbenzidine substrate (150 µl/well) was added, and the plate was incubated in the dark for 10 min. Stop solution (50 µl/well, sulfuric acid 10%) was added, and the absorbance was measured at 450 nm using a microplate reader (Biotek, Synergy HT, USA). A dynamic range of the assays was between 10-2000 ng/ml. Intra- and inter-assay coefficients of variations were below 10 %.

Statistical analyses

All results were expressed as mean ±SEM. The data were analyzed with the SPSS statistical program (version 23.0 for Windows, licensed for Karadeniz Technical Univer-

sity, Turkey). The Shapiro-Wilk test was used in all cases to test for normality of the data set, the homogeneity of variances was evaluated using Levene test and the results were found to be nonparametric. Therefore, differences between groups were assessed using the Kruskal-Wallis test followed by the Mann-Whitney U test. P < 0.05 was considered to be statistically significant.

RESULTS

Table-1 shows results obtained from the open field test after 10-day stress application. Total distance and velocity scores were generally lower in the stress groups than the control group. However, values of the total distance were found to be statistically lower in the immobilization stress-1 and social isolation groups compared with the control group (p=0,008 and p=0,042, respectively). In addition, the velocity score of the immobilization stress-1 group was statistically lower than the control group (p=0,036). A percentage of time spent in the central area was significantly lower in the immobilization stress-2 and social isolation groups compared to the control group (p=0,040 and p=0,046, respectively). A total mobility of the immobilization stress-1 group (p=0,043), and rearing score of the social isolation group (p=0,034) were lower than the control group. Grooming frequency was higher in the immobilization stress-1 group, but it did not reach to statistical significance.

Scores of the swimming, climbing and immobility behaviors in the forced swimming test after 10-day stress application are presented in Figure 3. Duration of the swimming behavior was lower in the immobilization stress-1 (36,66±5,65 s) and social isolation (45,71±6,85 s) groups compared with the control group (70,83±8,50 s, p=0,004 and p=0,009, respectively). There was no significant difference in the climbing durations between groups. Duration of the immobility behavior was generally higher in the stress groups than the control group. However, immobility scores were found to be significantly higher in the immobilization stress-1 (182,77±13,96 s) and social isolation (179,28±10,54 s) groups compared with the control group

Table 1: Results of the open field test after 10-day stress application

	Control	Immobilization stress-1	Immobilization stress-2	Social isolation
Total distance (cm)	2125,06±46,67	1564,53±91,34 b	1902,96±140,11	2066,74±47,49 a
Velocity (cm/s)	7,09±0,15	5,21±0,30 a	6,34±0,46	6,88±0,15
Time spent in the central area (%)	13,28±1,42	11,82±2,26	7,42±0,94 a	7,32±3,44 a
Mobility frequency	272,00±20,61	163,01±18,64 a	266,03±26,06	282,80±24,38
Rearing frequency	20,81±2,33	17,80±3,46	18,51±2,18	13,40±2,94 a
Grooming frequency	2,27±0,86	4,11±0,89 a	2,83±0,47	2,42±1,24

Values plotted are mean±S.E.M (n= 8 for each group).a; P < 0.05, and b; P < 0.01 compared to the control group.

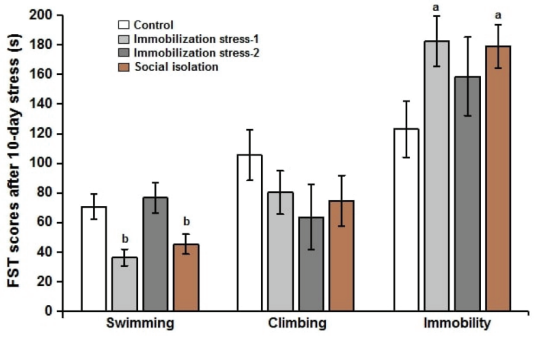

Figure 3. Scores of the forced swimming test after 10-day stress application.
Results are plotted as mean ± S.E.M. a: p < 0.05, b:p < 0.01 vs. control group. n=8 for each group. FST: Forced Swimming Test

(123,33±11,94 s, p=0,019 and p=0,025, respectively).

Table-2 shows results obtained from the open field test after 10-day of the rest period. Total distance and velocity scores were found to be statistically lower in the immobilization stress-2 group compared with the control group (p=0,045 and p=0,043, respectively). A percentage of time spent in the central area was significantly lower in the immobilization stress-1 and the immobilization stress-2 compared to the control group (p=0,022 and p=0,008, respectively). A total mobility of the immobilization stress-1 group was lower than the control group (p=0,036). There was no significant alteration in the frequencies of rearing and grooming behaviors between the stress groups and the control group.

Scores of the swimming, climbing and immobility behaviors in the forced swimming test after 10-day rest period are presented in Figure 4. In the immobilization stress-1 (53,88±7,25 s) and social isolation (65,21±6,11 s) groups, duration of the swimming behavior was lower than the control group (101,71±11,56 s, p=0,000 and p=0,006, respectively). There was no significant alteration in the scores of climbing behavior between the stress groups and the control group.

Values of the immobility behavior were generally higher in the stress groups than the control group. However, immobility behavior durations were found to be significantly higher in the immobilization stress-1 (199,44±9,05) and social isolation (191,42±10,36) groups compared with the control group (136,14±10,36, p=0,000 and p=0,000, respectively).

Table 2: Results of the open field test after 10-day of rest period

	Control	Immobilization stress-1	Immobilization stress-2	Social isolation
Total distance (cm)	1840,15±105,32	1580,38±166,76	1364,93±170,56 a	1805,27±142,96
Velocity (cm/s)	6,13±0,35	5,26±0,55	4,55±0,56 a	6,01±0,47
Time spent in the central area (%)	12,10±3,26	5,48±1,05 a	3,63±1,84 b	9,46±2,49
Mobility frequency	200,42±18,51	160,22±20,33	126,57±23,38 a	229,25±26,19
Rearing frequency	13,28±2,32	13,88±2,33	15,28±3,42	20,62±2,69
Grooming frequency	2,01±0,65	3,20±0,33	2,26±0,43	1,50±0,32
Values plotted are mean±S.E.M (n= 8 for each group).a; P < 0.05, and b; P < 0.01 compared to the control group.				

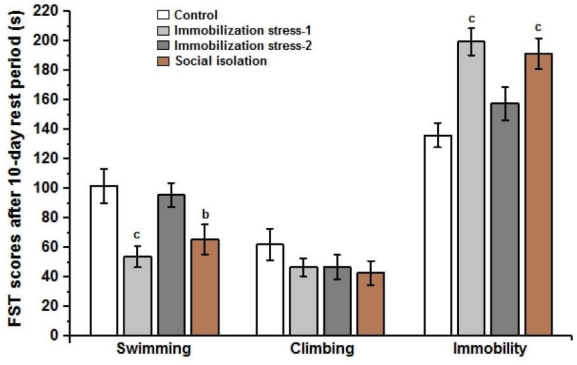

Figure 4. Scores of the forced swimming test after 10-day stress application.
Results are plotted as mean ± S.E.M. b: p < 0.01, c:p < 0.001 vs. control group. n=8 for each group. FST: Forced Swimming Test

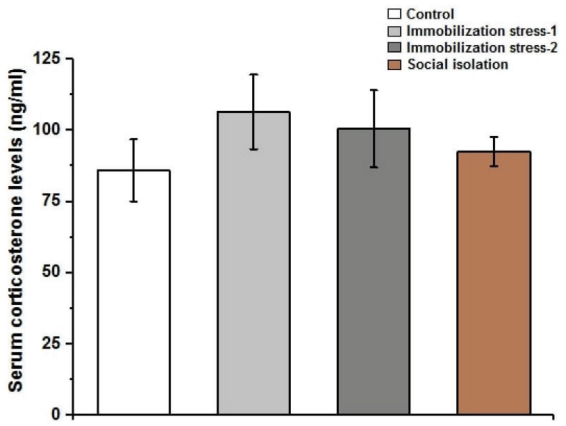

Figure 5. Serum corticosterone levels (ng/ml).
Results are plotted as mean ± S.E.M. (n=8 for each group).

Serum corticosterone levels of the stress groups and the control group are presented in Figure 5. The serum corticosterone concentrations were generally higher in the stress groups, but these elevations did not reach to statistical significance.

DISCUSSION

The present study emphasizes the results of three different stress protocols on anxiety and depression behaviors in adult female rats. Regarding the anxiety-like behavior, a percentage of time spent in the central area was found to be lower in the immobilization stress-2 and social isolation groups in the open field test. When animals leave an open space, they may prefer edge areas as a result of increased anxiety due to the new environment. This is a behavioral reflection of a vague uneasiness in the animals. Therefore, a decrease in the time spent in the central area in the open field test is accepted as a sign of increased anxiety.[28,29] However, after 10-day of the rest period, scores of the time spent in the central area was lower in immobilization stress-1 and immobilization stress-2 groups. This result may indicate that this effect is weakened in the social isolation group after a 10-day rest period following social isolation in the metabolic cage. However, it can be said that this effect continues in the immobilization stress-2 group. In the immobilization stress-1 group, the score of the time spent in the central area did not differ from the control group after 10-day stress application, but this score was significantly lower than the control group after 10-day rest period. This interesting result may be a reflection of the psychophysiological changes that occur after severe stress or traumatic conditions in the body. One of them is post-traumatic stress disorder, which can be triggered by some physical stress models such as immobilization/restraint stress, underwater/forced swim paradigms, or a combination of multiple stressors.[30,31] Therefore, both the effect of immobilization stress and the forced swimming test (the pretest session and test steps) after stress period may be related to this finding. We also determined locomotor activity or exploratory behavior-like parameters such as total distance, mobility frequency, mean velocity, and rearing frequency. Analysis of these behaviors may give some clues on anxiety or depression, because the emotional state may affect locomotor and related behaviors.[32] Anxiolytic agents help to overcome the fear-caused inhibition of exploratory behaviors, while anxiogenics reduce these parameters.[33] In our experiment, scores of the total distance, mobility frequency, mean velocity, and mobility frequency were lower in the immobilization stress-1 group, and scores of the total distance and rearing frequency were lower in the social isolation group. In the second test, the values of the total distance and mean velocity in the social isolation group were at the same level as the control group. It is known that housing on the grid floor can cause hypersensitivity and pain in the feet of rats.[18] Therefore, these two different results can be explained that 10-day housing on the grid floor in the metabolic cage causes foot hypersensitivity or pain, and 10-day rest period eliminates this hypersensitivity. Scores of the total distance, mobility frequency, and mean velocity were found to be lower in the immobilization stress-2 group. However, rearing frequency of this group did not differ from the control rats. The rearing behavior is accepted as an exploratory behavior type in the rodents.[34] A reduction in this behavior is accepted as a result of the high anxiety or fear because this case also

means a decrease in the self-control of coping with dangers or novel stimuli. Therefore, it is expected to be accompanied by a decrease in the percentage of time spent in the central area and a decrease in the rearing frequency in an anxious rodent.[29] For this reason, we conclude that scores of the rearing frequency and other exploratory locomotor activities in the immobilization stress-2 group are not compatible with the increased anxiety-induced behaviors. This is also true for the immobilization stress-1 group. Since behavioral tests are performed in the diestrus period, the differences in the time interval until the determination of this period may also lead to such nonspecific findings. We must emphasize that the difficulties in standardizing the variables related to female animals as well as the experimental protocol are among the limitations of our study.

A reduction in the exploratory locomotor activities may reflect an emotional disturbance in rats. However, it is an interesting result that these reductions in the second open field test occurred only in the immobilization stress-2 group. The rats in this group were exposed to immobilization stress 45 minutes twice a day for a period of 10 days. This condition may have resulted in the formation of adaptation to repeated stress in animals. Therefore, stress adaptation in this group may also be valid for the second tests. In a study was reported that another important consideration in behavioral research is the number of test repetitions performed in an animal, because these repeats may lead to reduced exploratory locomotor activities in the open field test due to habituation.[35] The habituation or adaptation phenomenon is considered as a form of simple non-associative learning, in which the volume of the response to a specific stimulus decreases with repeated exposure to this stimulant.[36] Unfortunately, we used only the open field test to evaluate the anxiety-like behaviors in this study. Using other anxiety tests, such as elevated plus maze test or light-dark box test, could be more useful to eliminate such possibilities.

Regarding our results associated with depression-related behaviors, durations of the swimming behavior were lower and scores of immobility behavior were higher in the immobilization stress-1 and social isolation groups. The same findings for these groups were valid for the second forced swimming test after 10-day rest period following immobilization and social isolation stress procedures. The forced swimming test is used to determine depressive-like behavior and is based on the assumption that immobility behavior.[37] Therefore, increased immobility duration may be interpreted as reflecting a state of behavioral despair.[27,37] It is known that swimming and immobility behaviors are affected by a wide range of antidepressant treatments.[27,38,39] We also evaluated the corticosterone levels at the end of the experiments. The serum corticosterone concentrations were generally higher in the immobilization stress-1, immobilization stress-2 and social isolation groups than the control group, but these elevations did not reach to statistical significance. Moreover, there are many reports on the high corticosterone levels and social isolation stress in metabolic cages.[18,40] In our current experiment, we performed 10-day social isolation stress using the metabolic cage in female rats. Following the behavioral tests, the rats were subjected to a 10-day rest period. After this term, the behavioral tests were repeated and the animals were decapitated. Unfortunately, the serum corticosterone was only measured at the end of the experiments. On the other hand, it would be more useful to measure corticosterone level after each application step. In a 14-day immobilization stress study was reported that plasma corticosterone was elevated at 1 and 7 days but not at 14 days.[40] This data indicates that the corticosterone level may change in a time-dependent manner even under stress conditions.

Our main focus is the definition of an effective depression and/or anxiety-model in the female rats. Therefore, a protocol including stress and rest period was performed in this study because we consider that the rest period is important to determine the effectiveness of the applied stress in animals. In the second test after 10-day the rest period, we observed that essential anxiety-related behaviors such

as decreased time spent in the central area and reduced rearing frequency did not continue in a stable manner in any of the groups. In a study was reported that 10-day of chronic immobilization stress increase anxiety-like behavior in the open field test in rats.[41] This report is compatible with our results of the open field test after 10-day stress application. However, we determined that this effect weakened in both the immobilization stress groups and social isolation group after 10-day rest period.

Regarding our results on the depression-related behaviors, the scores of the swimming behavior and immobility behavior significantly altered in the immobilization stress-1 and social isolation groups in the forced swimming test both after 10-day stress application and after 10-day rest period following immobilization and social isolation stress procedures. These results suggest that immobilization stress, [45] minutes daily for a period of ten days, and social isolation stress, single housing in metabolic cages for ten days, causes an increase in the depression-related behaviors in adult female rats. In 2015, Ampuero et al.[42] performed two different chronic stress models including restraint in small cages and immobilization in adaptable plastic cones for 10 days. Researchers reported that both stress models caused depression-like behaviors in rats. Moreover, in a study was suggested that acute (1 h) or chronic (1 h a day for 14 days) immobilization stress significantly altered the depression-like behaviors and anxiety-like behaviors in mice.43 However, there are some reports that after restraint or immobilization stress, male rats exhibit higher levels of anxiety-like behaviors.[44,45] The common feature of these studies is that the experiments were carried out in male rodents. However, for female rats, the data on depression-like behavior and 10-day chronic immobilization stress or social isolation stress in the metabolic cage are limited, and this fact increases the importance of our results. Moreover, we performed a rest period as applied stress period, and then we repeated the same behavioral tests. We consider that this protocol is important in determining the permanent effects of immobilization or social isolation stress in female rats.

In conclusion, anxiety-related behaviors of the immobilization stress -1, the immobilization stress -2 and the social isolation stress groups were inconsistent after the stress period and after the rest period. This result may be related to the open field habituation after repeated administration. Unfortunately, we did not use any other tests (elevated plus maze test or light-dark box test) to evaluate anxiety-like behavior in this study. However, depression-related behaviors of the immobilization stress -1 and the social isolation stress groups were consistent after the stress period and after the rest period. This result was not valid for the immobilization-2 group. We applied the same stress of the immobilization-1 to the immobilization-2 group twice. Therefore, we believe that this application may have resulted in the formation of adaptation to repeated stress in the immobilization-2 stress group. Therefore, we suggest that depression-like behaviors are more dominant in the immobilization stress-1 and social isolation groups of adult female rats.

Conflicts of interest

The authors declared that there is no conflict of interest.

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