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Comparison of the Effects of Immobilization Stress and Chronic Mild Stress Models on Depression-Related Behaviors in Female Rats: An Assessment of a 10-Day Stress Period

Objective: This study was designed to compare the depression-related behavior-inducing potentials of 10-day immobilization stress and chronic mild stress in adult female rats.

Materials and Methods: Adult Wistar rats were randomly divided into three groups (n=10) as control, immobilization stress (45 minutes per day) and chronic mild stress. Both stress protocols were applied for a period of 10 days. Behavioral tests were performed when the rats were in diestrus.

Results: In the forced swimming test, immobility behavior in the immobilization stress group and chronic mild stress group was significantly higher than the control group (P<0.05 and P<0.001, respectively). Additionally, there was a significant decrease in swimming behavior in the immobilization stress and chronic mild stress groups compared to the control group (P<0.05 and P<0.01). In the open field test, the percentage of time spent in the central area was lower, and latency in the central area was longer in the immobilization stress group (P<0.05). The frequency of the rearing behavior in the immobilization stress group was lower than the control group (P<0.01). The percentage of body weight change was significantly higher in the chronic mild stress group compared to the control group (P<0.001).

Conclusion: We suggest that the depression-related behavior-inducing potential of the chronic mild stress model is more prominent than the immobilization stress model by means of 10-day stress protocol in female rats. However, our chronic mild stress protocol might have some risks in animals during the pregnancy and postpartum period due to significant weight loss.

Key words: immobilization stress, chronic mild stress, depression, female, rat

İmmobilizasyon Stresi ve Kronik Hafif Stres Modellerinin Dişi Sıçanlarda Depresyon Benzeri Davranışlar Üzerine Etkilerinin Kıyaslanması: 10 Günlük Stres Süresinin Değerlendirmesi

Amaç: Bu çalışmada 10 gün süresince uygulanan immobilizasyon stresi ve kronik hafif stres modellerinin erişkin dişi sıçanlarda depresyonla ilişkili davranışları indüklemeye potansiyellerinin kıyaslanması amaçlanmıştır.

Gereç ve Yöntem: Yetişkin Wistar albino ırkı sıçanlar kontrol, immobilizasyon stresi (günlük 45 dakika) ve kronik hafif stres olarak rastgele üç gruba (n = 10) ayrılmıştır. Her iki stres protokolü de 10 gün süresince uygulanmıştır. Davranış testleri sıçanlar döstrüs dönemindeyken gerçekleştirilmiştir.

Bulgular: Zorunlu yüzme testinde, immobilizasyon stres grubundaki ve kronik hafif stres grubundaki hayvanlarda hareketsiz kalma/kendini bırakma davranış süreleri kontrol grubundan anlamlı olarak yüksekti (sırasıyla P<0.05 ve P<0.001). Ayrıca, immobilizasyon stresi ve kronik hafif stres grubunda yüzme sürelerinde kontrol grubuna göre anlamlı bir azalma söz konusuydu (P<0.05 ve P<0.01). Açık alan testinde, immobilizasyon stresi grubunda merkez bölgede geçirilen zaman yüzdesinin daha düşük ve merkez alana ilk girişteki gecikme süresinin daha uzun olduğu belirlendi (P<0.05). İmmobilizasyon stres grubunda şaşlanma davranışı sayısının kontrol grubuna göre daha düşük olduğu görüldü (P<0.01). Kronik hafif stres grubunda, vücut ağırlığı değişim yüzdesinin kontrol grubuna göre anlamlı derecede yüksekti (P<0.001).

Sonuç: Dişi sıçanlarda 10 gün süresince uyguladığımız kronik hafif stres protokolünün depresyon benzeri davranışları indüklemeye potansiyelinin immobilizasyon stresinden daha belirgin olduğunu söyleyebiliriz. Bununla birlikte, kullandığımız kronik hafif stres protokolü önemli derecede kilo kaybına yol açtığından dolayı hamilelik ve emzirme dönemindeki dişi sıçanlarda bu protokolün uygulanması riskli olabilir.

Anahtar Kelimeler: immobilizasyon stresi, kronik hafif stres, depresyon, dişi, sıçan

Introduction

Depression is one of the major health problems in general population due to long-term morbidity and its socioeconomic impact (1, 2). Moreover, women have a stronger predisposition to depressive disorders (3-5). Prevalence of major depression during the pregnancy and postpartum period is also common in women (6). From a general perspective, there is a clear need for an improvement in treatment of depression because it is suggested that up to 45% of depressive patients do not show improved mood after therapy, and 15% of patients do not respond to antidepressant treatments

(7). For this reason, new and more powerful treatment approaches are needed for depression (8). There is a limited possibility to experiment directly on people in relation to the mechanism of depression neurobiology and antidepressant activity. Therefore, experimental studies conducted in small rodents may contribute to development of new goals in improving treatment efficacy by understanding the changes in the brain during depression and anxiety (9). Although gender differences in this disorder have been recognized in humans (3, 4), the great majority of studies on the antidepressant drugs have focused on male rodents as experimental models (10). For this reason, it may be beneficial to use female rats in depression experiments (11). Many animal stress models such as immobilization stress and chronic mild stress for triggering depression have been developed and used frequently to understand the pathophysiology of this disease and evaluate the efficacy of the antidepressants. The immobilization stress model is based on exposure to movement restriction, and this procedure is usually performed in two ways: immobilization-induced stress with a restrainer and immobilization-induced stress without a restrainer (12). In our previous experiment, we used an adjustable transparent Plexiglas apparatus as a restrainer for immobilization stress in rats. We determined that 45 minutes of immobilization stress per day for 10 days causes an increase in depression-like behaviors in female rats (13). Another procedure is chronic mild stress that is a commonly employed model for inducing depression in small rodents (14). In our previous experiment, we observed that chronic mild stress induces depression-related behaviors in female rats (15).

The main aim of this study was to compare the effects of 10-day immobilization stress (45 minutes per day) and 10-day chronic mild stress on depression-related behaviors in adult female Wistar rats. For this purpose, depression-like behaviors were evaluated by the forced swimming test (16). We also used the open field test for analyzing anxiety-related behaviors (17)

and determining a possible comorbidity between depression and anxiety in female rats.

Materials and Methods

Adult female (aged 2-5 months) Wistar albino rats (Experimental Medicine Application and Research Center of Necmettin Erbakan University, Konya) were used in this study. The animals were randomly divided into three groups as the control, immobilization stress and chronic mild stress group, each consisting of 10 rats. In the control group, the rats were housed in standard conditions. In the immobilization stress group, the animals were kept in cylinder apparatuses for 45 minutes per day for a period of 10 days. The rats were placed in cylinder apparatuses, which were suitable for their body volumes with dimensions of 5 cm × 22 cm. There were ventilation holes in the walls of these apparatuses in the parts surrounding the rat's body. The same holes were also present in 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's body with the aid of a slider. Thus, the cylinder-shaped collapsed area ensured the immobilization of both limbs and head of the rats (13, 18).

In the chronic mild stress group, the animals were exposed to different stress factors for a period of 10 days (Table 1). For the chronic mild stress model, stress factors and time protocols were modified by utilizing those that were used in previous studies (19, 20).

Depression- and anxiety-related behaviors were evaluated by the forced swimming test and the open field test after the stress protocols were implemented. For the pretest session in the forced swimming test, the animals were individually placed for 15 min into Plexiglas cylinders (49 cm height, 25 cm diameter) containing 39-cm-high water (27±1 °C). 24 h after the pretest session, the forced swimming test was performed. The total durations of the swimming, climbing and immobility behaviors were analyzed by the Ethovision XT11 video tracking system for a period of 300 seconds (21).

Table 1. 10-day chronic mild stress protocol

Days	Morning			Afternoon			Night		
	Time	Stressor	Duration	Time	Stressor	Duration	Time	Stressor	Duration
1	09.00	Immobilization	45 min	12.00	Noise	4 hr	-----	-----	-----
2	09.00	Wet cage				7 hr	-----	-----	-----
3	09.00	Immobilization	45 min	12.00	Swimming	10min	16.00	Food deprivation	overnight
4	09.00	Noise	4 hr	14.00	Immobilization	45 min	-----	-----	-----
5	09.00	Swimming	10 min	-----	-----	-----	16.00	60° cage tilting	overnight
6	09.00	Noise	4 hr	-----	-----	-----	19.00	light on	overnight
7	09.00	Wet cage	7 hr						
8	09.00	Immobilization	45 min				-----	-----	-----
9	09.00	Swimming	10 min	16.00	60° cage tilting	overnight	-----	-----	-----
10	09.00	Noise	4 hr	14.00	Immobilization	45 min	-----	-----	-----

In the open field test, 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. The scores of the 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 recordings in the software (21). Additionally, the body weight changes were determined by weighing the rats before and after the 10-day stress period in all groups.

The data were analyzed with the SPSS software (version 23.0 for Windows, licensed for Karadeniz Technical University, Turkey). Shapiro-Wilk test was used in all cases to test the normal distribution of the data, the homogeneity of the variances was evaluated using Levene's test, and our data were found to require nonparametric tests. Therefore, the differences among the groups were assessed using Kruskal-Wallis test followed by Mann-Whitney U test. All results are expressed as median (minimum-maximum). $P < 0.05$ was considered to be statistically significant.

Results

As shown in Table 2, the duration of the swimming behavior in the forced swimming test was lower in the immobilization stress and chronic mild

stress groups in comparison to the control group ($P=0.012$ and $P=0.002$, respectively). There was no significant difference in the climbing durations of the immobilization stress and chronic mild stress groups in comparison to the control group. The immobility scores were found to be significantly higher in the immobilization stress and chronic mild stress groups in comparison to the control group ($P=0.035$ and $P=0.001$, respectively).

Table 3 shows results obtained from the open field test after inducing 10 days of stress. There was no significant alteration in the total distance and mean velocity between the control group and the stress groups. The percentage of time spent in the central area was found to be significantly lower in the immobilization stress group ($P=0.033$). Additionally, the score of latency in the central area was longer in the immobilization stress group in comparison to the control group ($P=0.049$). The rearing behaviors were found to be significantly lower only in the immobilization stress group ($P=0.009$). There was no significant alteration in the grooming behaviors between the control group and the stress groups.

The results of initial weight (g), final weight (g) and body weight change (%) of the stress groups and the control group are presented in Table 4. The percentage of body weight change was significantly higher in the chronic mild stress group in comparison to the control group ($P=0.001$).

Table 2. Durations of the forced swimming test

	Control	Immobilization stress	Chronic mild stress
Swimming (s)	125 (80-170)	85 (45-95) ^a	60 (20-110) ^b
Climbing (s)	62.5 (45-105)	90 (65-120)	75 (55-115)
Immobility (s)	97.5 (75-125)	130 (105-150) ^a	150 (115-225) ^c

The data represent median (minimum-maximum) (n=10). a: $P < 0.05$, b: $P < 0.01$, c: $P < 0.001$ compared to the control group.

Table 3. Results of the open field test

	Control	Immobilization stress	Chronic mild stress
Total distance (cm)	1234.06 (894.54-1468.05)	1191.74 (705.65-1382.83)	1165.42 (723.72-1291.79)
Velocity (cm/s)	6.17 (4.47-7.34)	5.96 (3.53-6.91)	5.82 (3.62-6.46)
Time spent in the central area (%)	4.07 (2.20-8.63)	1.45 (1-2) ^a	2.4 (1.03-3.37)
Latency in the central area	33.95 (15.07-71.33)	112.46 (41.63-261.57) ^a	42.23 (28-71.50)
Rearing frequency	18 (6-29)	10 (4-16) ^a	16 (10-20)
Grooming frequency	3 (2-4)	3.5 (2-5)	3 (1-5)

The data represent median (minimum-maximum) (n=10). a: $P < 0.05$ compared to the control group.

Table 4. Values of the body weight change

	Control	Immobilization stress	Chronic mild stress
Initial weight (g)	255 (222-310)	265 (220-282)	262 (237-274)
Final weight (g)	263 (223-306)	262 (218-286)	248 (239-269)
Body weight change (%)	1,33 (-3.79-3.54)	-1.72 (-5.28-2)	-4.19 (-10.37-0.84) ^c

The data represent median (minimum-maximum) (n=10). c: $P < 0.001$ compared to the control group.

Discussion

This study was conducted to compare the depression-inducing effects of immobilization stress and chronic mild stress in female rats. We observed that both stress models caused a significant increase in immobility duration and a decrease in swimming duration. In the forced swimming test, immobility scores are used as an index of behavioral despair (22). Therefore, rendering behavioral despair in this test is one of the main approaches to depression-related behavior in experimental studies (23) because this behavior may be modulated by a wide range of antidepressants (22, 24). In a 10-day chronic stress study, the researchers used two different chronic stress models: restraint in small cages and immobilization in adaptable plastic cones, and they reported that two stress paradigms induced depressive-like behaviors in rats (25). In our previous experiments, we determined that both 45-minute daily immobilization stress for 10 days and 21 days of chronic mild stress induce depression-related behaviors in female rats (13, 15). However, we obtained different results in our previous study in male rats (18). We used two immobilization models, immobilization-1 (45 minutes daily for a period of ten days), and immobilization-2 (45 minutes twice a day for a period of ten days), both of which caused a significant increase in anxiety-like behaviors in adult male rats. These different results can be interpreted as a result of gender-related effects in such diseases (3, 4).

Comorbidity between depression and anxiety is an essential issue in humans (26). The same condition may be true for small rodents. Indeed, in our previous study, we determined that long-term social isolation stress induces both depression- and anxiety-related behaviors in rats (21). Moreover, we observed that anxiety-like behaviors predominated than depression-like behaviors in the immobilization stress-treated adult male rats (18). Therefore, we also evaluated the possible anxiety-related behavior-inducing potentials of 10 days of the chronic mild stress and 10 days of 45-minute per day immobilization stress models. In the open field test, we determined that the percentage of time spent in the central area was lower in the chronic mild stress and immobilization stress models, but it reached a statistically significant value only in the immobilization stress group. The values of latency in the central area and rearing frequency were lower in the immobilization stress group. However, there was no significant alteration in the chronic mild stress and immobilization stress groups for the scores of total distance, mean velocity and grooming behavior. These

results mean that an immobilization stress procedure can cause a partial comorbidity between depression and anxiety, but chronic mild stress induces depression-related behaviors in female rats. In a 21-day chronic immobilization stress study, researchers reported that immobilization stress caused depression in Sprague Dawley male rats (27). In mice, there are several studies that reported that 120 minutes of chronic immobilization stress per day for 10 days causes increased anxiety-related behaviors (28, 29). These different results indicate that the intensity and duration of applied stress as well as species and gender are important in modeling these disorders (30, 31).

We also evaluated the percentage of body weight change in two models after 10 days of stress. There was a significant difference in the percentage of body weight change in the chronic mild stress group. This result is an important clue for evaluating the effects of both stress models on the body weights of rats. A significant weight loss is a symptom profile of chronic mild stress (32). The same result may also be true for immobilization stress (33). However, it is useful to state that these effects are related to the length of the stress period. Especially in long-term chronic mild stress protocols, when food and water restriction are included among the stressors, weight loss can be observed in the animals due to these factors. Therefore, it is suggested that chronic mild stress models which do not include food and water restriction would be more useful models (34). Since we applied a chronic mild stress model for 10 days, we think that stress procedure, rather than stress period itself, might have had an effect on the weight loss in the animals. Food deprivation, 60-degree cage tilting and wet cage procedures have a high potential to adversely affect the feeding routine of the animals. These stress factors were important causes of weight loss in the chronic mild stress group. Importantly, this stress protocol may be associated with some disadvantages in modelling pregnancy or postpartum depression. In relation to weight loss in animals, abortion may occur in pregnant animals, or death of pups may result among lactating animals due to insufficient milk yield.

In conclusion, we suggest that the depression-related behavior-inducing potential of the chronic mild stress model is more prominent than the immobilization stress model (45 minutes per day) by means of 10-day stress protocol in female rats. However, our chronic mild stress protocol might have some risks in animals during the pregnancy and postpartum period due to significant weight loss.

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