Original Article

Preliminary Study: Effects of Social Instability Stress on Depressive Behaviours in Ovariectomised Rats

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Abstract

Background: **Depression is one of the common post-menopausal symptoms. In addition to estrogen deficiency, social instability stress may contribute as an additional underlying factor in the development of depressive behaviour in females. Therefore, this study was aimed at examining the influence of social instability stress on depressive behaviour in ovariectomized rats.**

Methods: **The rats were divided into four groups (n = 5 per group); (i) sham-operated control without stress, (ii) sham-operated control with stress, (iii) ovariectomized without stress, and (iv) ovariectomized with stress. These rats were subjected to social instability stress procedures for 15 days prior to an enforced swimming test. Struggling, immobility, and swimming times were recorded promptly.**

Results: **The results were analysed using the one-way analysis of variance (ANOVA) and a P value of < 0.05 was considered as significant. The mean durations of struggling, immobility, and swimming behaviour were significantly distinct among the four groups. Ovariectomized rats exhibited a substantial decrease in struggling and swimming behaviour, and an increase in immobility behaviour in comparison with the sham-operated controls (P < 0.05). Ovariectomized rats with stress displayed a supplementary decrease in struggling and swimming behaviour as well as an advanced increase in immobility behaviour, compared to sham-operated controls with or without stress (P < 0.05).**

Conclusion: **In summary, these findings suggest that ovariectomized rats encountered an augmented amount of depressive behaviour following social instability stress.**

Keywords: *depressive behaviour, forced swimming test, ovariectomy, stress*

Introduction

There is growing evidence to support the hypothesis that estrogen loss plays a crucial role in the development of depressive symptoms in post-menopausal women (1) as well as ovariectomized animal (2,3). Thus, the estrogen administration to ovariectomized animals resulted in a decreased immobility during the length of time in the forced swimming test (4–8).

Stressful experiences may also intensify and cause the proliferation of the risk of affective disorders (9–13). These findings imply that additional factors, besides the loss of ovarian hormones may further increase the chance of developing depressive symptoms in post-menopausal women (14). Due to obvious human analogies, social stress appears particularly appropriate for evaluating additional factors besides the loss of estrogen in the development of post-menopausal memory deficits and depressive symptoms.

The social instability model refers to the distinction between the more dominant and subsequently subordinate, or conquering and defeated animals, as all animals in such groups are likely to experience defeat when being moved from one group to another (15). Agonistic behaviour may be manipulated, however, by setting up social groups and later mixing them (social instability models) or by introducing a carefully selected highly aggressive male into a stable social group (social disruption model) (15). In female rodents, stress-induced changes in social instability and social disruption models were more pronounced (16) compared to other forms of social stress, i.e. social defeat and subordination. In addition, many studies have reported higher degrees of anxiety-like behaviour and higher corticosterone levels when females are housed individually as opposed to when kept in smaller groups. In contrast, males maintained

within an individual mode of isolation do not display such patterns, thereby suggesting that males and females perceive housing conditions differently (17,18). Therefore, this comparative cross-sectional study was aimed at investigating the effects of social instability stress on the development of depression-like behaviors in ovariectomized rats.

Materials and Methods

Animals

Adult female Sprague-Dawley rats (n = 20) of eight weeks old and a body weight of 200 ± 20 g were obtained from the Laboratory Animal Research Unit (LARU), USM, Kelantan. All rats were housed in polypropylene cages (40 cm × 25 cm × 16 cm) and were exposed to 12 hours of light-dark cycles, they were also maintained at a room temperature of 23 °C and were fed with a pellet diet and water ad libitum. The experimental protocol was approved by the Research & Ethics Committee, Universiti Sains Malaysia. The rats were randomly divided into four groups; (i) sham-operated control without stress, (ii) sham-operated control with stress, (iii) ovariectomized without stress and group (iv) ovariectomized with stress.

Surgical procedures

The animals received surgical operations to remove both ovaries (ovariectomized) under general anaesthesia using a combination of ketamine (Sigma, USA, 60 mg/kg) and xylazine (Sigma, USA, 5 mg/kg) intra-peritoneally. Following anaesthesia, the dorsal lumbar fur was shaved and cleaned with a chlorhexidine scrub and 70% ethanol. Immediately, a small (2 cm) midline incision was made at the dorsal area at the level of L3–L5 vertebrae. The ovarian fat pad was gently grasped using forceps until the ovary was exposed and removed. A high degree of aseptic procedures were maintained throughout the operation. After the completion of the operation, the animals were left for one hour under an overhead light source in order to avoid hypothermia. The sham-operated rats underwent the same procedure as the ovariectomized rat but without the removal of the ovaries.

Post-operative care

Following the surgical procedure, the animals were given post-operative care by isolating each animal in a clean cage for 10 days in order to avoid any fighting which could otherwise cause bleeding or poor healing. After 10 days, they were housed in groups of three per cage and kept for a further two months for surgical recovery.

After the recovery period, the rats were exposed to social instability stress procedures which consisted of alternating isolation and crowding phases for 15 days based on the previously described model (16). The experiment commenced and ended with an isolation phase, and each phase lasted for approximately 24 hours. During the crowding phase, eight rats cohabited in one cage and each group consisted of three males and five females. Social contact between the group members was video-taped for 30 minutes at the beginning of each crowding phase. Biting attacks and dominant posture fighting for water or food were video-recorded (19).

Body weight

To compare the mean weight between pre-surgery and post-surgery, all the rats were weighed one day before the surgery (ovx/sham), and also eight weeks post-surgery.

Forced swim test

Following the 15 days stress procedure, the bodily weight of the rats was taken. Subsequently, the animals were first trained for 15 minutes in each training session for the duration of two days followed by a test session 24 hours later. All rats were tested in the same brightly-lit room. The time of the tests were between 10:00 and 17:00 hours. All rats were individually placed into glass cylinders (40 cm in height, 18 cm in diameter) filled with water (23 °C) to a level of 30 cm for 5 minutes. The water level was purposely chosen at a higher level than in the procedure described by Porsolt et al. (20), in order to prevent the rats from supporting themselves by touching the bottom with their hind limbs or tails during the swimming sessions (21). The animals were forced to swim in the cylinders for 5 minutes during the test session. The three behaviours which were recorded and scored were immobility, swimming, and struggling. These 3 behaviours are defined as follows: (1) Immobility–floating in the water, and making those movements which are only necessary to keep their heads above the water (2) swimming–making active swimming motions (3), struggling–rats making active attempts to escape from the cylinder, including visually searching for the escape routes and diving (22). After completion of the test, the rats were dried gently with a towel and were returned to their home cages.

Statistical analyses

The data collected was expressed as mean ± SD. The effects of stress and surgery on the duration of swimming, immobility and struggling behaviours were evaluated statistically using a two-way analysis of variance (ANOVA). Differences were considered significant when the $P < 0.05$. Pearson's Correlation Coefficient was used to test the correlation between weight and immobility time. Repeated measures ANOVA were applied respectively to determine the significance of the differences between pre-operative and post-operative mean body weight.

Results

Social stress on struggling behaviour

There are extremely noteworthy and focal effects for the stress status on the total struggling duration, F (1, 16) = 29.17, $P < 0.001$. Moreover, there was a significant main effect of surgery (ovx) on the total struggling time F (1, 16) = 157.28), $P < 0.001$.

There occurred substantial interactive effects between the stress status and surgery (ovx) during the struggling duration F (3, 16) = 72.29, $P < 0.001$. The data gathered is also indicative that the animals were affected differently by both ovx surgery and stress. For example, the mean duration of struggling behaviour was suggestively shorter in ovariectomized rats with stress 50 seconds (SD 201) compared to ovariectomized rats without stress 65 seconds (SD 2.05). The ovariectomized rats with or without stress displayed shorter amounts of the duration of struggling behaviour compared with the sham-operated control with stress 80 seconds (SD 1.89), or without stress 88 seconds (SD 66) as shown in Figure 1. However, there was no significant metamorphosis in the mean duration of struggling behaviour between the sham-operated control with stress and the sham-operated control without stress.

Social stress on immobility behaviour

There was an important main effect on the stress status of the total immobility duration, F (1, 16) = 27.33, $P < 0.001$. Moreover, there was a significant main effect for surgery (ovx) on the total immobility time F (1, 16) = 132.31, $P < 0.001$.

There was a rather noteworthy interaction effect between the stress status and surgery (ovx) on the immobility duration F (1, 16) = 59.46, $P < 0.001$. The mean duration of immobility behaviour was significantly longer in the ovariectomized rats with stress 130.4 seconds (SD 0.74) in comparison with the ovariectomized rats without stress 99.8 seconds (SD 1.42). The mean duration of immobility was longer in ovariectomized rats with or without stress when paralleled to the sham-operated controls with stress or without stress 78.2 seconds (SD 2.1) as shown in Figure 2. However, there was no significant difference in the mean duration of immobility behaviour between the sham-operated controls with stress compared to the sham-operated controls without stress.

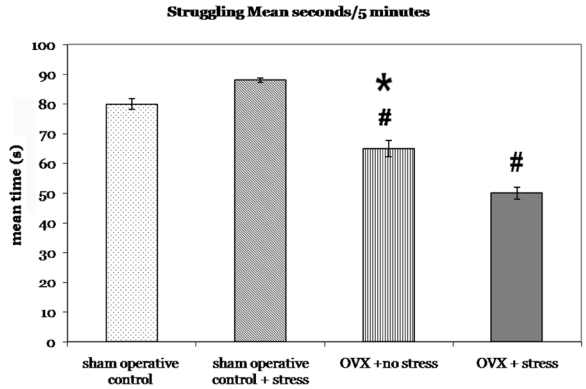

Figure 1: Mean duration of struggling behaviour (seconds) in different groups ($n = 5$ per group). # $P < 0.01$ Ovx + stress vs. sham-operated control groups, # $P < 0.01$ Ovx + no stress vs. sham-operated control groups, and * $P < 0.05$ Ovx + no stress vs. Ovx + stress.

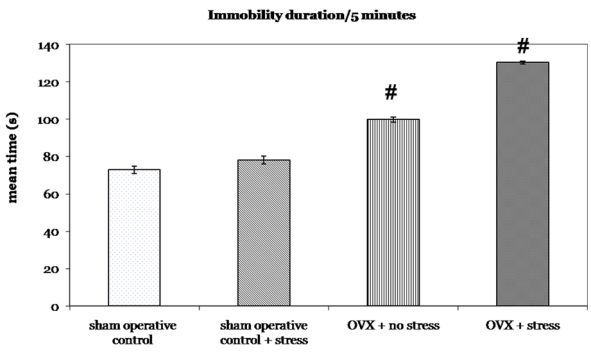

Figure 2: Mean duration of immobility behaviour (seconds) in different groups ($n = 5$ per group). # $P < 0.01$ Ovx + stress vs. sham-operated control groups and # $P < 0.01$ Ovx + no stress vs. sham-operated control groups.

Social stress on swimming behaviour

There was a significant main effect for the stress status on the total swimming duration, F (1, 16) = 109.64, $P < 0.001$. Moreover there was a significant main effect for surgery (ovx) on the total swimming time F (1, 16) = 213.21, $P < 0.001$.

There was a significant interaction effect between the stress status and surgery (ovx) on the swimming duration F (3, 16) = 110.21, $P < 0.001$. The data also indicated that the mean duration of swimming behaviour of ovariectomized rats with stress was significantly shorter compared to ovariectomized rats without stress 130 seconds (SD 1.63). However, there was no significant difference in the swimming behaviour between the sham-operated control with stress 131 seconds (SD 1.71) or without stress 147 seconds (SD 2.21) as shown in Figure 3. In addition, the data displayed no significant difference in the mean duration of swimming behaviour between the sham-operated control with stress and the sham-operated control without stress.

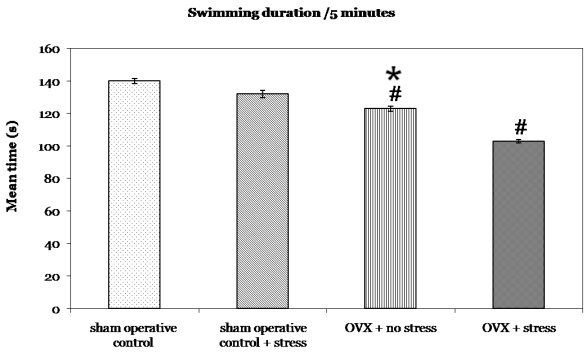

Figure 3: Mean duration of swimming behaviour (seconds) in different groups (n = 5 per group). # $P < 0.01$ Ovx + stress vs. sham-operated control groups, # $P < 0.01$ Ovx + no stress vs. sham-operated control groups, and * $P < 0.05$ Ovx + no stress vs. Ovx + stress.

Body weight

Eight weeks post-surgery the mean body weight noticeably increased in the ovx rats compared to the sham operated control groups with or without stress F (3, 16) = 16.71, $P < 0.01$, and data also indicated a significant difference between the ovx pre-surgery and the ovx post-surgery F (3, 16) = 16.87, $P < 0.001$ as shown in Table 1.

Correlation between body weight and immobility

To exclude the possibility of higher body weight associated with longer immobility time as observed in the ovariectomized rats, we evaluated the correlation between body weight and immobility time using the Pearson's correlation. There was no significant correlation between these variables on the day of behavioral testing (r = 0.172) (data not shown).

Discussion

This study hypothesized that the removal of ovaries (ovariectomy) in animals which mimicked spontaneous or surgically-induced menopause increased the risk of depressive behaviour (4,23). Stress has been shown to increase the likelihood of depressive behaviour in animals as well as in human beings (24,25). The depressive behavior in either stress or ovariectomized animal models from previous studies displayed longer immobility time and a much shorter duration of swimming and struggling (4,19,26). We therefore hypothesized

Table 1: Mean body weight pre and post-operation in different groups

Groups	One day Pre-operation (g)	Two month Post-operation (g)
Sham-operated control without stress (n = 5)	203 (2.00)	224.0 (0.3)
Sham-operated control with stress (n = 5)	210 (1.5)	230.4 (2.00)
OVX without stress (n = 5)	195 (0.7)	319.4 ± (0.4)# $
OVX with stress (n = 5)	200 (1.00)	329.4 ± (2.00)# $

Note: Data is expressed in mean body weight (g) SD.
# $P < 0.01$; Ovx + stress /without stress vs. sham-operated control groups.
$ $P < 0.001$; Ovx body weight pre-surgery vs. post-surgery.

that, if we combined ovariectomy and stress, the animals will display more depressive behavioural tendencies compared to the animals subjected to ovariectomy or stress in an isolated existence.

In this study, we found that the immobility time was longer in ovariectomized rats with stress in comparison to ovariectomized species without stress. However, immobility time was longer applicable in ovariectomized animals without stress when equated with the sham-operated control with or without stress. This finding suggests that the ovariectomized rats with stress displayed more depressive and bleak behaviour as shown by the longer immobility time compared with the ovariectomized rats without stress, sham-operated controls with or without stress. This observation is consistent with previous studies conducted focusing on intact female rats compared with ovariectomized rats (26). It was widely reported that ovariectomy results in weight gain, therefore there is a possibility that the prolongation in immobility behaviour observed in this study was due to overiectomized-induced weight gain (26). The reason why reduced estrogen is associated with less activity is not yet known. It could be argued that reduced physical activity in the ovariectomized rats is simply a result of their increased body weight; however, we are not in agreement with this case because bodily weight does not correlate with the immobility behaviour in our rats.

Focusing on the struggling and swimming time, both parameters exposed a longer duration in the sham-operated control without stress compared to the sham-operated control with stress, ovariectomized rats with and without stress. This outcome supports our earlier hypothesis that the combination of ovariectomy and stress induces more depressive behaviours compared to the ovariectomized rats without stress, sham-operated controls with and without stress.

Conclusion

In conclusion, this study demonstrates that the combination of ovariectomized and social instability stress increases depressive behaviour in female rats. We propose that the depressive behaviour observed in the ovariectomized rats subjected to social instability stress may be associated with the diminution of the anti-depressant effect of estrogen and inappropriate stress regulation. However, the exact mechanisms underlining depression behaviour in ovariectomized rats with stress have yet to be clarified.

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Conflict of interest

No conflict of interest.

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Authors' contributions

Conception and design and critical revision of the article for the important intellectual content: RZ, ZO, AH
Collection and assembly of data, provision of study materials or patient and collection and assembly of data: BAR, SM
Drafting of the article and statistical expertise: BAR
Final approval of the article: RZ
Obtaining of funding: RZ, AH

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