

Unpredictable chronic mild stress induced behavioral deficits: A comparative study in male and female rats

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Abstract: Stress is an important precipitant factor for depression. Changes in various body systems that occur in depression are similar to those observed in response to stress. Chronic stress may alter behavioral, neurochemical and physiological responses to drug challenges and novel stressors. Unpredictable chronic mild stress (UCMS) also produces alteration in the serotonergic (5-HT; 5-hydroxytryptamine) neurotransmission. Unpredictable chronic mild stress (UCMS) could be used as an animal model of depression. Neurochemical and behavioral effects of UCMS can be reversed by antidepressant agents, suggesting an important role of serotonin. In rodents, UCMS can elicit depression-like symptoms. The objective of the present study was to evaluate and compare the behavioral deficits induced by chronic mild stress in male and female rats and finding out the vulnerability of the two groups. Male and female rats exposed to UCMS exhibited a significant decrease in cumulative food intake as well as in growth rate. Locomotor activity in home cage and open field was also decreased. Results may contribute to our understanding of the interaction between stress and behavioral functions have to depressive disorders.

Keywords: Unpredictable chronic mild stress, depression, sex difference, Locomotor activity.

INTRODUCTION

Stressful life events may play an important role in the etiology of depression. It is assumed that exposure to uncontrollable stressors induces a feeling of loss of control which may result in a depressive behavioral state. Chronic stress potentiates behavioral, neurochemical, and physiological responses to drug challenges and novel stressor. A single session of uncontrollable (inescapable tail shock), but not controllable (escapable tail shock) stress is known to selectively potentiate subsequent morphine-conditioned place preference (Bland *et al.*, 2004).

Chronic stress models are comparatively more suitable than acute stress models for investigating depression in experimental models (Katz *et al.*, 1981; Willner *et al.*, 1997). Exposure to unpredictable chronic mild stress (UCMS) can elicit depression-like symptoms such as lack of sucrose preference (Pothion *et al.*, 2004), increased REM sleep (Gronli *et al.*, 2004), altered sympathetic cardiac regulations (Grippeo *et al.* 2002; Grippeo *et al.* 2003) and decreased levels of cytokines (Grippeo *et al.* 2003; Li *et al.* 2003; Grippeo *et al.* 2005). Several researchers have reported important role of dopamine, serotonin and adrenergic receptors in the pathophysiology of depression (Gamaro *et al.*, 2003; Harro *et al.*, 2001; Papp *et al.*, 2002). Others have reported decreased serotonergic function in animal model of depression such as chronic mild stress (Kang *et al.*, 2005) and learned helplessness (Sherman *et al.*, 1982). Luo *et al.*, (2008) have reported that reduced 5-HT and neuropeptide Y

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neurotransmission is important in mediating the depression induced by chronic unpredicted mild stress. Joca *et al.* (2003) has reported an important role of decreased serotonergic function in hippocampus in the development of depressive symptoms. Others have reported altered gene and protein expressions in chronic unpredicted mild stress induced anhedonia (Bergström *et al.*, 2008; Bisgaard *et al.*, 2007). Hippocampus also plays an important role in regulating the functions of nucleus accumbens (French and Totterdell, 2003), suggesting its important role in mediating the reinforcing effects of drugs of abuse.

Since hippocampus is important in cognitive processes and memory consolidation (Witgen *et al.*, 2005). It is therefore suggested that anhedonia resulted after withdrawal from drugs of abuse as well as after exposure to chronic unpredicted mild stress, shares several key features and could be context specific like the sensitization. The present study was context specific comparative study in male and female rats; designed to monitor the effects of unpredictable chronic mild stress on activities in familiar and novel environments.

MATERIALS AND METHODS

Animals

Locally bred male Albino-Wister rats weighing 180-220g were purchased from The Aga Khan University, Karachi, Pakistan, and housed individually under 12hr light-dark cycle and controlled room temperature (25±2) with free access to cubes of standard rodent diet and water, a week before experimentation.

Experimental protocol

Twenty four animals (12 male and 12 female rats) were randomly divided into two groups; each containing 6 female and 6 male rats: (i) Unstressed and (ii) Unpredictable chronic mild stress (UCMS) groups resulting in four groups: (i). Unstressed-female, (ii) Unstressed-male, (iii) UCMS-female and (iv) UCMS-male rats. Stressed group animals were exposed to unpredictable mild stress treatments over a period of 10 days while animals of unstressed groups remained in their home cages. Cumulative food intake and body weight changes were monitored. Open field- and home cage activities were monitored 24hr after last restrained stress.

Chronic mild stress

Stressed group rats were exposed to chronic mild stress (unpredictable) for 10 days: Day1 at 11:00am 50 min cold room (4°C), Day 2 at 12:00pm 60 min cage agitation, Day 3 at 1:00pm 60 min immobilization stress (Haleem et al., 2013), Day 4 12 hrs food and water deprivation, Day 5 at 11:00am lights off for 3hr, Day 6 at 11:00am 50 min cold room (4°C), Day 7 at 12:00pm 60 min cage agitation, Day 8 at 1:00pm 60 min restrained stress, Day 9 12 hrs food and water deprivation, Day 10 at 11:00am lights off for 3hr.

Behavioral assessment

Home cage activity

The assessment of locomotor activity in a familiar environment was done in a home cage. Apparatus used in this study was made up of transparent perspex (26x26x26 cm) with a sawdust covered floor. Testing was done in a quiet room under white light as described by Ikram *et al.*, (2011). 15 minutes before monitoring the activity animals were placed in the home cage for habituation. Number of crossing across the box and grooming behavior were monitored for 10 minutes.

Open field activity

The assessment of locomotor activity and exploration in a novel environment was done in open field apparatus. Open field apparatus used in present investigation consisted of a square area (76 x 76 cm) with walls 42 cm high. The floor was divided by lines into 25 equal squares. Procedure was same as described earlier (Ikram *et al.*, 2007; Ikram and Haleem, 2010, Mirza *et al.*, 2013). To determine the activity rats were placed in the center square of the open field. Latency to move (in sec) and numbers of square crossed with all four paws were recorded for 5 minutes.

STATISTICAL ANALYSIS

Values are represented as means $\pm$ SD. Data was analyzed by two-way ANOVA. Post hoc analysis was done by Newman-Keuls test. Values of $p < 0.01$ were considered as significant.

RESULTS

Fig. 1 shows effects of chronic mild stress on % growth rate and cumulative food intake in rats exposed to chronic mild stress (10 days). Data was analyzed by two-way ANOVA. Analysis of the data on % growth rate (fig. 1a) showed significant effects of stress ($df=1, 23$; $F=16.69$; $p<0.01$) but not that of repeated monitoring ($df=1, 23$; $F=0.327$) and interaction between the two ($df=1, 23$; $F=1.52$). Post hoc analysis of the data by Newman-Keuls test showed decreased growth rate in stressed male rats as compared to unstressed male controls.

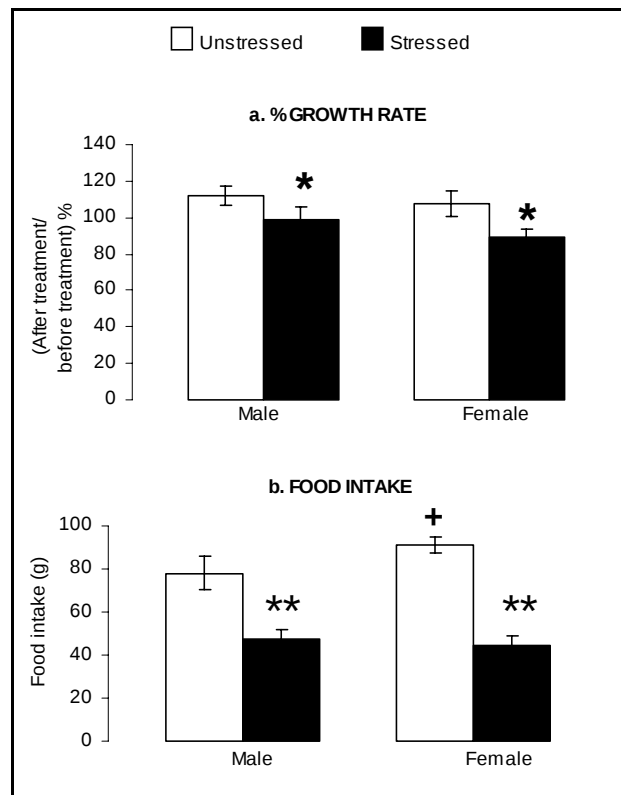


Fig. 1: Effects of chronic mild stress on cumulative body weight and food intake in male & female rats. Values are means $\pm$ SD ($n=6$) as monitored 24hr after last stress. Significant differences by Newman-Keuls test: * $p<0.05$; ** $p<0.01$ from unstressed controls of same gender; + $p<0.05$ from unstressed controls of opposite gender following two-way ANOVA.

Analysis of the data on cumulative food intake (fig. 1a) showed that effects of repeated monitoring on food intake was not significant ($df=1, 23$; $F=3.382$). However, effects of stress ($df=1, 23$; $F=205.65$; $p<0.01$) and interaction between stress and repeated monitoring were significant ($df=1, 23$; $F=9.492$; $p<0.01$). Post hoc analysis of the data by Newman-Keuls test showed decreased ($p<0.01$) food intake in stressed animals of both male and female rats as compared to their respective unstressed controls. However, food intake in unstressed female rats was more ($p<0.01$) than the unstressed male rats.

Fig. 2 shows effects of chronic mild stress on water intake in rats exposed to chronic mild stress (10 days). Analysis of the data by two-way ANOVA showed significant effects of stress ($df=1, 23; F=36.79; p<0.01$) and repeated monitoring ($df=1, 23; F=119.69; p<0.01$) but not the interaction between the two ($df=1, 23; F=0.01$). Post hoc analysis of the data by Newman-Keuls test showed decreased ($p<0.01$) water intake in stressed animals of both male and female rats as compared to their respective unstressed controls. However, water intake in unstressed female rats was more ($p<0.01$) than the unstressed male rats.

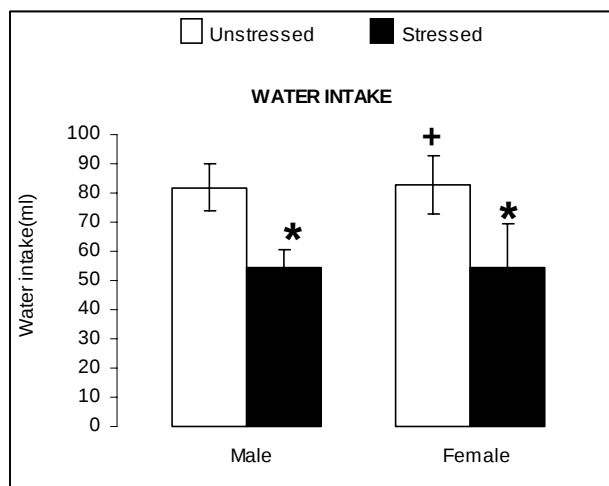


Fig. 2: Effects of chronic mild stress on water intake in male & female rats. Values are means $\pm$ SD ($n=6$) as monitored 24hr after last stress. Significant differences by Newman-Keuls test: * $p<0.05$ from unstressed controls of same gender; + $p<0.01$ from unstressed controls of opposite gender following two-way ANOVA.

Fig. 3 shows effects of chronic mild stress on home cage activity of rats exposed to chronic mild stress (10 days). Analysis of the data on cage crossings (fig. 3a) showed significant effects of stress ($df=1, 23; F=128.48; p<0.01$) and repeated monitoring ($df=1, 23; F=5.507; p<0.05$), but not the interaction between the two ($df=1, 23; F=0.062$). Post hoc analysis of the data by Newman-Keuls test showed decreased ($p<0.01$) cage crossings in stressed animals of both male and female rats as compared to their respective unstressed controls. However, cage crossings in unstressed female rats was more ($p<0.01$) than the unstressed male rats.

Analysis of the data on grooming (fig. 3b) showed significant effects of stress ($df=1, 23; F=38.98; p<0.01$) and repeated monitoring ($df=1, 23; F=8.95; p<0.05$) but not the interaction between the two ($df=1, 23; F=1.08$). Post hoc analysis of the data by Newman-Keuls test showed decreased ($p<0.01$) cage crossings in stressed animals of both male and female rats as compared to their respective unstressed controls.

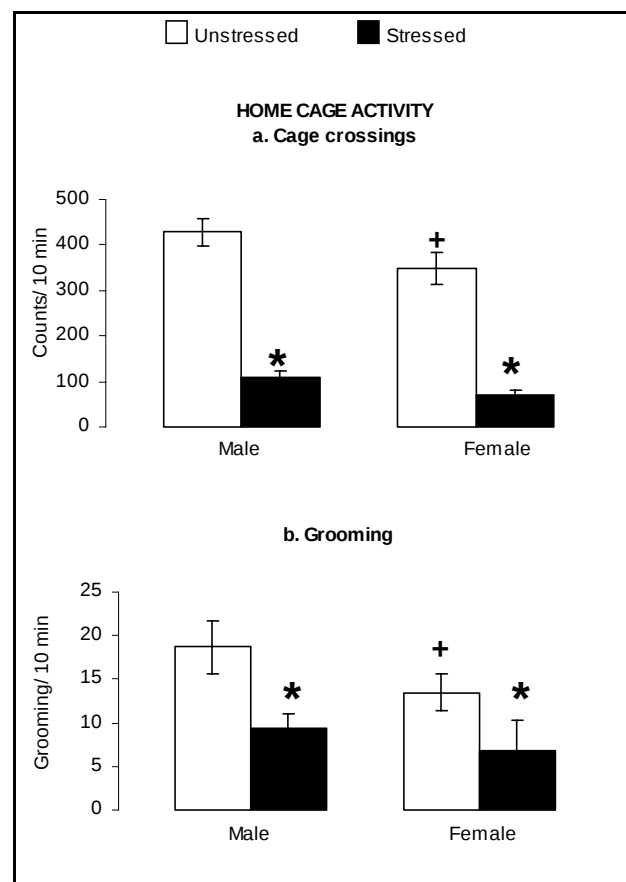


Fig. 3: Effects of chronic mild stress on home cage activity in male & female rats. Values are means $\pm$ SD ($n=6$) as monitored 24hr after last stress. Significant differences by Newman-Keuls test: * $p<0.01$ from unstressed controls of same gender; + $p<0.05$ from unstressed controls of opposite gender following two-way ANOVA.

Fig. 4 shows effects of chronic mild stress on open field activity in rats exposed to chronic mild stress (10 days). Analysis of the data on squares crossed (fig. 4) showed significant effects of stress ($df=1, 23; F=25.89; p<0.01$) but not that of repeated monitoring ($df=1, 23; F=0.24$) and interaction between the two ($df=1, 23; F=0.052$). Post hoc analysis of the data by Newman-Keuls test showed decreased squares crossed by stressed male ($p<0.05$) and stressed ($p<0.01$) rats as compared to unstressed male and unstressed female controls respectively. Squares crossed by the stressed female rats were greater ($p<0.05$) than stressed male rats.

Analysis of the data on latency to move showed significant effects of stress ($df=1, 23; F=4.95; p<0.05$), repeated monitoring ($df=1, 23; F=18.46; p<0.01$) as well as interaction between the two ($df=1, 23; F=5.46; p<0.01$). Post hoc analysis of the data by Newman-Keuls test showed increase latency to move in both unstressed female and stressed female rats as compared to unstressed

male and stressed male controls respectively. Stressed female rats showed increased latency as compared to stressed male rats.

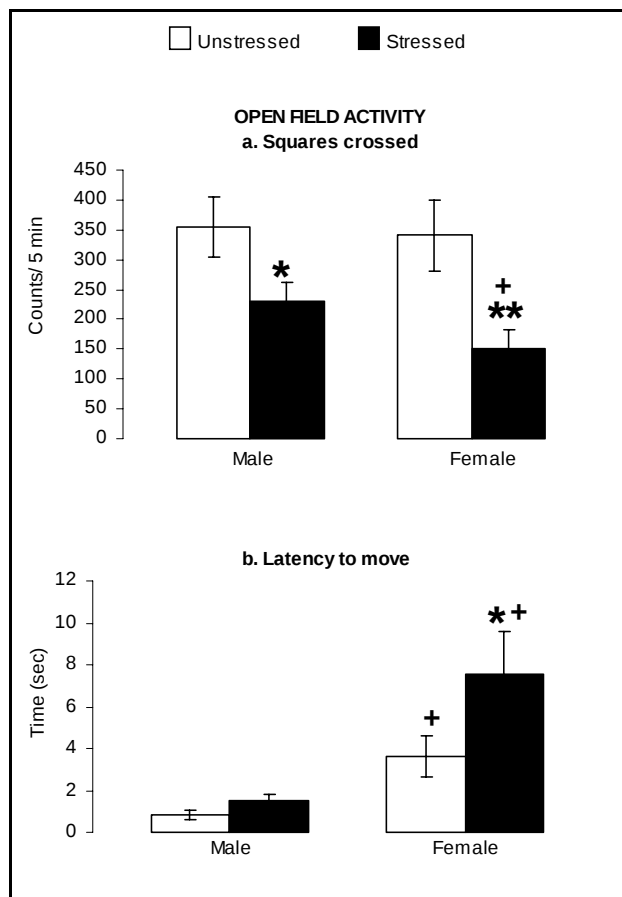


Fig. 4: Effects of chronic mild stress on open field activity in male & female rats. Values are means $\pm$ SD (n=6) as monitored 24hr after last stress. Significant differences by Newman-Keuls test: * $p < 0.05$; ** $p < 0.01$ from unstressed controls of same gender; + $p < 0.01$ from unstressed controls of opposite gender following two-way ANOVA.

DISCUSSION

Exposure to unpredictable chronic mild stress results in significant behavioral changes in a wide range of animal models (Willner, 1991). Results from present study revealed that exposure to UCMS over a period of 10 days induced a significant decrease in the growth rate of both male and female rats. This decreased food intake could be due to the anhedonia produced by UCMS. Luo *et al.*, (2008) has also reported reduction in bodyweight and sucrose preference in rats following UCMS. In present study we found decreased food intake which could also be due to the anhedonia produced by UCMS.

Exposure to unpredictable stressors have been resulted to induce significant changes in behavioral parameters, such

as altered locomotive and explorative behavior, a decline in food intake, water intake and sexual activity (Willner, 1991). D'Aquila *et al.*, (2000) have also suggested that chronic unpredictable mild stress-induced behavioral deficits in experimental animals could be used effectively as animal model of depression. UCMS-induced decreased water intake in animals suggests: (i) decreased food intake, as observed after repeated exposure to mild stress was not due to increased fluid intake and (ii) decreased fluid intake could also be suggested as part of anhedonia. Studies are not available regarding water intake in animals exposed to UCMS. However, it is very important to monitor water intake besides food intake, as increased water intake could be the reason of decreased food intake sometimes.

UCMS decreased loco motor activity as monitored in familiar environment in both female and male rats. This could be due to the decrease in serotonergic function resulting in the development of depressive symptoms (Joca *et al.*, 2003). In present study we found a decrease in locomotive activity of UCMS group animals as monitored in activity box as compared to unstressed group animals. Both male and female rats of UCMS group showed a significant decrease in locomotive activity in open field, which could be due to decreased serotonergic function. Gronli *et al* (2005) have shown that when stressed groups of animals were placed in a novel environment it results in an increased activity in the first few minutes of the test.

Similarly, in present study, UCMS exposed rats had shown an increased in after first stress but it was decreased significantly afterwards. In previous studies, repeated restrained and chronic mild stress has been reported, to increase and to decrease the loco motor response to dopamine agonists, respectively (Cabib & Allegra, 1996; Papp *et al.*, 1993; Willner *et al.*, 1992). Unpredictable chronic mild stress (UCMS) has been used as an animal model of depression and these effects of UCMS can be altered by antidepressants agents (Willner *et al.*, 1997, 1996, 1987), illustrating a strong predictive validity. Many studies have reported that systematic injections of dopaminergic drugs results in a profound changes in the behaviors of the animals (Nasello *et al.*, 2003).

CONCLUSION

In conclusion, the present study confirmed the effects of exposure to UCMS in rats. This study gives a comparative mode of discussion in both male and female rats. It will also help to understand the role of serotonergic system and about the deficits created by UCMS exposure. Our current protocol of a 10 day UCMS is able to induce depression-like behavioral changes characterized with anhedonia, loss of appetite, lost weight and decreased

loco motor & exploratory activities and these UCMS-induced deficits were higher in female than male rats.

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