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Chronic mild stress (CMS)-induced behavioral deficits were attenuated by fluoxetine

Muhammad Farhan[1], Huma Ikram[1] and Darakshan Jabeen Haleem[2]*
[1]Neurochemistry and Biochemical Neuropharmacology Research Unit, Department of Biochemistry, University of Karachi, Karachi, Pakistan
[2]Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

Abstract: Fluoxetine, a selective serotonin re-uptake inhibitor (SSRI), has been proposed to be more effective as an antidepressive drug as compared to other SSRIs. After chronic SSRI administration, the increase in synaptic levels of 5-HT leads to desensitization of somatodentritic 5-HT autoreceptors in the raphe nuclei. Chronic stress may alter behavioral, neurochemical and physiological responses to drug challenges and novel stressors. Depression is a serious disorder often manifested with symptoms at the psychological, behavioral and physiological level. Chronic mild stress (CMS) model could be used as an animal model of depression. The objective of the present study was to evaluate that treatment with fluoxetine for two weeks could attenuate CMS-induced behavioral deficits. CMS-induced hypophagia were significant in water administrated animals but not in fluoxetine administrated animals. Repeated administration of fluoxetine increased exploratory activity in novel environment. Results may help to understand the interaction between stress and behavioral functions of depressive disorders.

Keywords: Chronic mild stress (CMS), selective serotonin re-uptake inhibitors (SSRIs), depression, exploratory activity.
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***Author for Correspondence:** farhankamali@uok.edu.pk

INTRODUCTION

Fluoxetine, a selective serotonin re-uptake inhibitor (SSRI), has been reported to be more effective for the treatment of depression as compared to other SSRIs[1,2]. A number of studies have reported that fluoxetine as well effective in treating a wide spectrum of mood disorders including depression, panic disorder and anxiety[3,4]**.** After chronic SSRI administration, the increase in synaptic levels of 5-HT leads to desensitization of somatodentritic 5-HT autoreceptors in the raphe nuclei. However, this desensitization occurs within 3 days of drug administration, a time-course that is shorter than the delayed onset of therapeutic improvement and may correlate with an initial aggravation of anxiety[5-7].

Stress is an important predisposing and precipitating factor in depression and the changes in various body systems that occur in depression are similar to those observed in response to stress[8]. The first chronic mild stress (CMS) model of depression was developed by Kaltz in 1981. It is a rodent model of depression that was developed to induce the decreased responsiveness to reward (anhedonia) as observed in human depression[9]**.** Exposure to unpredictable chronic mild stress results in significant behavioral changes in a wide range of animal models[10]**.**

Chronic mild stress (CMS) model is argued to possess a high degree of validity and utility[9] and has been used to study behaviors associated with depression and mood disorders such as anxiety[11], mechanisms of antidepressant treatments[12]**,** neurotransmitter changes[13,14], hypothalamic pituitary adrenal (HPA) function[15] and immune system mechanisms[16]. It has been reported that chronic mild stress models are comparatively more suitable than acute stress models for investigating depression in experimental models[17,18].

A previous study has reported that exposure to unpredictable stressors induces significant changes in behavioral parameters, such as altered locomotive and explorative behavior, a decline in food intake, water intake and sexual activity[10] . It has also been suggested that chronic mild stress-induced behavioral deficits in experimental animals could be used effectively as an animal model of depression[19]. In addition to anhedonia, CMS has shown to decrease aggressive and male sexual behavior in rats[20]. The present study was designed to evaluate the ability of fluoxetine to reverse CMS-induced depression-like behavior in rats.

MATERIALS AND METHODS

Animals

Locally bred male (180-220gm) albino-Wister rats purchased from Aga Khan University, Karachi, Pakistan were housed individually under 12 hours light and dark cycle and controlled room temperature (25±2ºC) with free access to cubes of standard rodent diet and water, for a period of three days before experimentation.

Experimental protocol

Thirty six animals were randomly divided into two equal groups (i) Unstressed and (ii) CMS. Animals of both groups were further divided into three groups (i) Unstressed-Water (ii) Unstressed-

Fluoxetine (1.0mg/kg), (iii) Unstressed - Fluoxetine (5.0mg/kg), (iv) CMS - Water (v) CMS-Fluoxetine (1.0mg/kg) and (vi) CMS-fluoxetine (5.0mg/kg). Animals of the CMS group were exposed to a schedule of chronic mild stress shown below over a period of 14 days (Table 1) while animals of unstressed groups remained in their home cages. Water or respective dose of fluoxetine (1.0mg/kg and 5.0mg/kg) was given orally to animals each day 1 hour before exposing to daily schedule of CMS. Food intake and body weight changes were monitored on next day of the 1st, 7th and 14th stress. Exploratory activity was monitored in novel environment (open field) on next day of 1st, 7th and last stress.

Table 1: Chronic mild stress (CMS) schedule.

Day	CMS	Time
Day 1	Exposed to 4°C for 50 minutes	11:00 am
Day 2	60 min cage agitation (60 rpm)	11:00 am
Day 3	60 min restrained stress (wire grid)	11:00 am
Day 4	12 hrs water deprivation	11:00 am to 11:00 pm
Day 5	3 hrs light off day time	11:00 am to 02:00 pm
Day 6	60 min Noise Stress	11:00 am
Day 7	60 min restraint Stress (tube)	11:00 am
Day 8	Exposed to 4°C for 50 minutes	11:00 am
Day 9	60 min cage agitation (60 rpm)	11:00 am
Day 10	60 min restrained stress (wire grid)	11:00 am
Day 11	12 hrs water deprivation	11:00 am to 11:00 pm
Day 12	3 hrs light off day time	11:00 am to 02:00 pm
Day 13	60 min Noise Stress	11:00 am
Day 14	60 min restraint stress (tube)	11:00 am

Behavioral assessment

Food intake

Twenty four hours food intake was monitored. A weighed amount of food was placed in the hooper in the cage of each animal. Intake was monitored by weighing the food left in the hooper of the cage after the required time.

Growth rate

Daily body weight changes were monitored to find out the effect of treatment. Daily growth rate changes were calculated as percentage of starting day weight (experiment day body weight/starting day body weight) X 100.

Open field activity

The assessment of exploratory activity in a novel environment was done in an open field apparatus. Open field apparatus used in present investigation consisted of a square area (76x76cm) with walls 42cm high. The floor was divided by lines into 25 equal squares. Procedure was same as described earlier[21]**.** To determine the activity rats was placed in the center squarer of the open field. Numbers of square crossed with all four paws were recorded for 5 minutes.

Statistical analysis

Values are presented as means±SD. Data of unstressed and stressed rats were analyzed by three-way ANOVA. Software used for the analysis was SPSS (version 17.0). Post-hoc comparison was done by Newman-Keuls test. Values of $p<0.05$ were considered as significant.

RESULTS

Figure 1 shows effects of repeated fluoxetine administration on body weight change of rats exposed to CMS as monitored on next day of 1st, 7th and 14th stress. Data on growth rate as analyzed by three- way ANOVA (repeated measures design) showed that effect of stress ($F=83.71$; df= 1, 32; $p<0.01$) as well as the effect of fluoxetine ($F=15.16$; df= 2, 32; $p<0.01$) were significant. However, the effect of repeated monitoring ($F=0.12$; df= 3, 32) and the interaction among the stress, fluoxetine and repeated monitoring ($F=1.593$; df= 6, 64) were not significant. Post-hoc analysis by Newman-Keuls test showed CMS decreased growth rate in water treated animals after 7th and 14th day of stress. Administration of fluoxetine decreased growth rate in unstressed animals and values were significant after 14th day of administration at dose 1.0mg/kg as well as 5.0 mg/kg. Exposure of fluoxetine treated animals to CMS, attenuate decrease in food intake after 7th day of stress in 5.0 mg/kg as well as after 14th day of stress in 1.0 mg/kg fluoxetine administered animals.

Figure 2 shows effects of repeated fluoxetine administration (14 days) on activity in novel environment (open field) of rats exposed to CMS as monitored on next day of 1st, 7th and 14th stress. Data on number of square crossing as analyzed by three-way ANOVA (repeated measures design) showed that effects of repeated monitoring ($F=42.79$; df=3, 32; $p<0.01$), fluoxetine ($F=25.62$; df=2, 32; $p<0.01$) and stress ($F=92.154$; df=1, 32; $p<0.01$) were significant. Interaction among CMS, fluoxetine and repeated monitoring ($F=21.10$; df=6, 64; $p<0.01$) were also significant. Post-hoc analysis by Newman-Keuls test showed that exposure to CMS decreased

activity in water administrated animals after 7th and 14th day of stress.

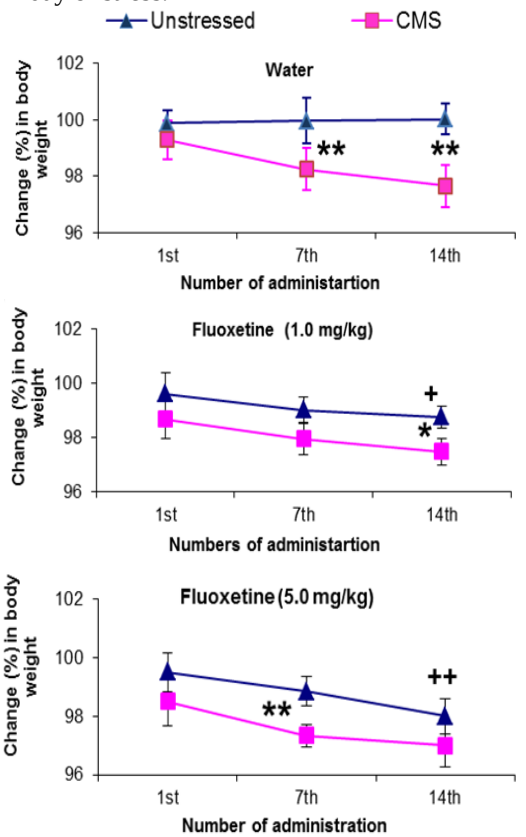

Figure 1: Effects of administration of fluoxetine (1.0 mg/kg and 5.0 mg/kg) on growth rate in unstressed and CMS rats. Values are means±SD (n=6) as monitored on next day of the administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from respective unstressed animals; +p<0.05, ++p<0.01 from respective water treated animals. Following three-way ANOVA (repeated measure design).

Administration of fluoxetine increased activity in unstressed animals and values were significant after 14th day of administration at dose 1.0 mg/kg as well as after 7th and 14th day of administration at dose 5.0mg/kg. Exposure of fluoxetine administrated animals (1.0mg/kg as well as 5.0mg/kg) to CMS decreased activity after 7th and 14th day of stress.

Figure 3 shows effects of repeated fluoxetine administration on food intake of rats exposed to CMS as monitored on next day of 1st, 7th and 14th stress. Data on food intake as analyzed by three-way ANOVA (repeated measures design) showed that effect of stress (F=49.34; df= 1, 32; p<0.01) was significant. Whereas, the effects of fluoxetine (F=2.439; df= 2, 32), repeated monitoring (F=0.87; df= 3, 32) and the interaction among the stress, fluoxetine and repeated monitoring (F=2.67; df= 6, 64) were not significant Post-hoc analysis by Newman-Keuls test showed that exposure to CMS decreased food intake in water treated animals and difference were significant after 7th and 14th day of stress. Fluoxetine administration for 14th days at dose 5.0 mg/kg decreased food intake in unstressed animals.

DISCUSSION

The aim of the present study was to investigate that whether fluoxetine administration could reverse the behavioral deficits induced by CMS. In this experiment we used CMS to produce behavioral deficits which are considered to be a valid and useful experimental model of depression[22, 23]. Results from the present study show that exposure to CMS reduces food intake, growth rate and locomotor activity as compared to unstressed animals indicating a behavioral consequence of CMS as predicted for an animal model of depression. It has been reported that exposure to stressors induced significant changes in behavioral parameters, such as decreased locomotive and explorative activity, a decline in food intake, water intake and sexual activity[24].

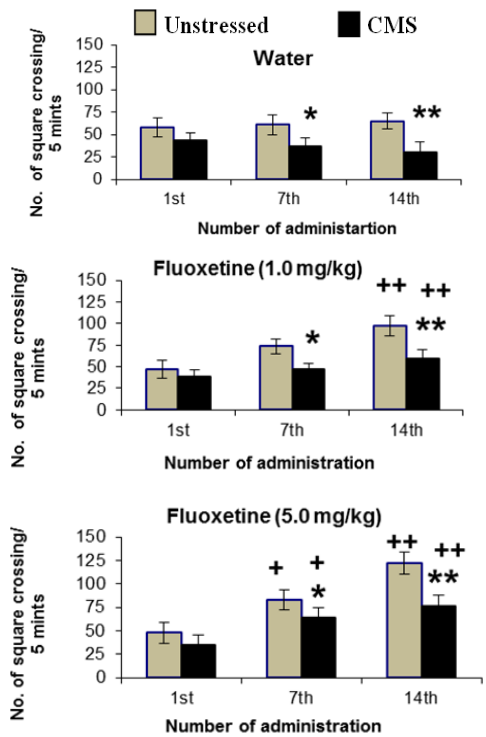

Figure 2: Effects of administration of fluoxetine (1.0 mg/kg and 5.0 mg/kg) on activity in open field in unstressed and CMS rat. Values are means±SD (n=6) as monitored on next day of the administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from respective unstressed animals; +p<0.05, ++p<0.01 from respective water treated unstressed or CMS animals; # p<0.01 from respective day 1.0 mg/kg fluoxetine treated unstressed or CMS animals; following three-way ANOVA (repeated measure design).

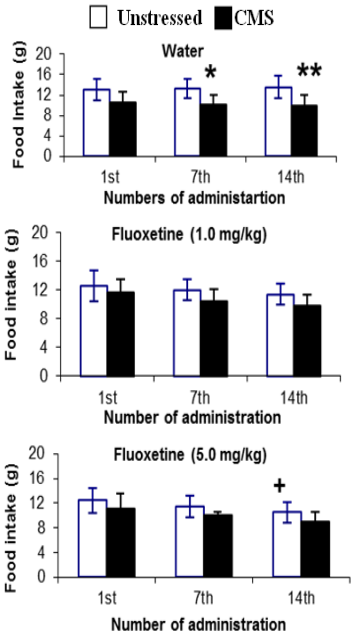

Figure 3: Effects of administration of fluoxetine (1.0mg/kg and 5.0mg/kg) on food intake in unstressed and CMS rats. Values are means±SD (n=6) as monitored on next day of the administration. Significant differences by Newman-Keuls test:*p<0.05, **p<0.01 from respective unstressed animals; +p<0.05, ++p<0.01 from respective water treated animals. Following three-way ANOVA (repeated measure design).

It has been reported that fluoxetine an SSRI, decreased appetite and food intake in rats' results from the present study showed a significant decrease in food intake and body weight after one week and persist till second week of drug administration [26]. Fluoxetine-induced hypophagia was smaller in CMS than unstressed animals. A number of studies have reported that fluoxetine and other selective serotonin reuptake inhibitors (SSRIs) produce anorexia in human and experimental animals [27, 28, 29, 30, 31, 32]. SSRI-induced anorexia in thought to result, at least in part, from blockage of the reuptake of serotonin (5-HT) into nerve terminals and a subsequent elevation of extracellular 5-HT in the somatodentritic region which desensitizes somatodentritic receptors to increase 5-HT avaibility in terminal region3[1,33-39].

Serotonergic mechanisms play an important role in the modulation of locomotor activity at a number of levels in the neuroaxis including the spinal cord, the basal ganglia, limbic structures, and in the frontal cortex[40-42]. Results from the present study showed that fluoxetine induced higher activity were more significant in novel environment at both doses that is low (1.0mg/kg) as well as high (5.0mg/kg) in unstressed than CMS animals.

SSRIs administered acutely or sub-chronically are known to produce limited beneficial effects or even adverse effects on anxiety and depression[43,44]. However, chronic SSRIs treatments are effective in depressed or anxious patients[45,46] as well as in highly emotional animal models[44,47].

In conclusion, the present study demonstrates that CMS exposure for 14 days resulted into behavioral deficits and produced depressive-like symptoms. Fluoxetine, an SSRI, administration attenuated behavioral deficits induced by CMS. Therapeutic / antidepressant effects of fluoxetine were produced at least after one week. Therefore, it is suggested that during the first week fluoxetine should be administered with some other antidepressant (probably first generation) and then subsequently that drug should be ceased and after one week, fluoxetine could be continued alone. Results may be beneficial for the treatment of multiple mood disorders including depression.

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