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ORIGINAL ARTICLE

Effects of chronic stress and antidepressant treatment on behavioral, physiological and neurochemical aspects in male and female rats

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Abstract: *Background:* Although the etiology of clinical depression is unknown, women are more likely to suffer from major depressive disorder than men. The biological basis of gender differences in stress response and recovery still remain poorly understood. *Objectives:* The aim of the present study was to investigate the gender specific behavioral, physiological and neurochemical aspects of rats exposed to chronic stress paradigm along with the recovery using antidepressant drug treatment in rats using Learned Helplessness (LH) model of depression. *Methods:* Stress induction was applied on rats through inescapable footshocks and subsequent shuttle-box escape test. Fluoxetine hydrochloride as antidepressant drug was used for recovery. Body weight and adrenal gland weight were measured. BDNF (Brain derived neurotrophic factor) level in hippocampus was measured by sandwich ELISA. Estrous stages in female rats were also examined. *Results:* Rats of both sexes exhibited marked alteration in BDNF level, body weight and adrenal weight along with their escape latency. After antidepressant drug treatment restoration of normal behavior was also observed. In estrous cycle analysis the depressed female rats exhibited significant variations. *Conclusion:* Depressogenic effect favors females with prolonged persistence of estrus phase, significant enlargement of adrenal glands and significant reduction of BDNF levels in hippocampus.

Keywords: Brain derived neurotrophic factor (BDNF); Depression; Escape-test; Estrous cycle; Gender; Inescapable footshock (IS); Learned-helplessness(LH).

Introduction

Depression is a devastating illness that affects ~17% of the population at some point of life, resulting the major social and economic consequences [1]. Depression is a wide spread, incapacitating psychiatric ailment, with 10-30% of women and 7-15% of men in a population being afflicted with this disorder at any given time [2]. At its worst, depression can lead to suicide, a tragic fatality associated with the loss of 10.50 lives per 100,000 person in every year in India. Depression, a common public health problem, occurs twice as frequently in women as in men [3-5]. Such a gender difference in depression may occur for a number of reasons, including the influence of particular sex hormones [6], dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) axis [7], anatomical differences of the brain studied through magnetic resonance imaging [8] and fluctuation of neurotrophins expression levels in brain due to depressogenic effect of the stress [9-10].

There are several stress induced models of depression in animal. Among them Learned helplessness (LH) is a valid and well established model of stress-induced behavioral depression in which prior exposure to inescapable stress produces deficits in escape testing [11-12]. The learned helplessness model offers an opportunity to understand the behavioral and neurochemical correlates of clinical depression, and has been investigated for over 30 years.

The cellular, molecular and the psychosocial mechanism underlying stress responses and depression may differ between males and females [13]. However many experimental studies focusing on the pathophysiology of depression have examined the effects of stress and/ antidepressant in male subjects [14], and the gender differences in pathophysiology of depression remain poorly understood.

Despite studies of sex differences and depressive behavior, few studies have investigated the influence of the estrous cycle on animal behavioral analogs of depression [15]. Briefly female rats have multiple cycles throughout a year (once every 4 to 5 days). For clarity, the term 'estrous' defines the cycle whereas the term 'estrus' defines a stage within the cycle. The estrous cycle of rodents consists of: (1) proestrus in which luteinizing hormone, follicle stimulating hormone, estrogen and progesterone levels peak, (2) estrus in which estrogen levels are falling, progesterone levels are moderate, and luteinizing hormone, follicle stimulating hormone levels are high, (3) metestrus in which luteinizing hormone levels fall, and (4) dioestrus in which estrogen levels begin to rise [16].

Exposure to a stressful events and elevated level of stress hormones are associated with impaired spatial memory and neuronal damage in the hippocampus and prefrontal cortex regions of the brain leading to the cognitive impairment in the behavioral attributes. The neurons of these regions are considered to be maintained by various neurotrophins such as BDNF (brain derived neurotrophic factor), NGF (nerve growth factor), NT-3 (neurotrophin 3), NT4/5 (Neurotrophin 4/5) etc. through their receptor mediated signaling pathways [17-18]. It is evident that quantitative analysis of neurotrophins shows a marked alteration of their expression levels in prefrontal cortex and hippocampus in stressed condition [19]. Among them BDNF is the most widespread growth factor in the brain. BDNF has diverse function in the adult brain as a regulator of neuronal survival, fast synaptic transmission, and activity-dependent synaptic plasticity [20]. Several studies support the hypothesis of BDNF involvement in depression and suggest that depressive disorders induce a marked decrease in hippocampus BDNF levels [21-22].

The effect of stress in different brain regions has been investigated several times [22-24]. There is no clear information regarding the depressogenic effect on gender vulnerability. In this context, the present study investigated the effects of chronic unpredictable stress paradigm on behavioral, physiological and neurochemical parameters to investigate the differential gender specific vulnerability to depression induction and converging antidepressant responses in rats.

Material and Methods

Animals: Male (n=32) and female (n=40) Sprague-Dawley rats were used in present experiment. At the start of the experiment rats were of the same age (approximately 2 months) weighing 224±1.5 gm and 246±1gm respectively. All rats were individually housed in temperature controlled (22-24°C) room for at least 1 week prior to the experimentation, with ad libitum access to food and water. Rats were maintained on a 12h light / dark cycle (lights on at 7am). All experimental protocols were designed to minimize the number of animals and sufferings were approved by the Institutional Animal Ethics Committee (IAEC) of the Raja Peary Mohan College, Uttarpara, Hooghly, West Bengal.

The stress protocol was utilized conform the literature [5, 22, 25]. Individually housed male rats and female rats were randomly assigned to 3 experimental groups: 1) Control group: subjected to no footshock throughout the experiment; 2) Chronic stress group received 60 footshocks daily for first 20 days followed by next 20 days with alternating exposure to the footshocks; 3) Recovery group: exposed to footshocks daily for 20 days and received daily injections of either saline (SAL) or fluoxetine hydrochloride (FLX) for consecutive 20 days and on day 42 exposures to the footshock box with only the light signal to all of the rat groups (Fig.1).

Figure-1: Schematic overview of the 42-day experimental protocol. Control: rats were subjected to no footshocks. Recovery: rats received footshocks daily for 20 days followed by a 20-day chronic antidepressant treatment instead of footshocks. Chronic: rats received footshocks daily for 20 days followed by 20 days of alternating exposure to the footshocks. On day 42 rats of chronic and recovery groups were exposed to the footshock box with the light only.

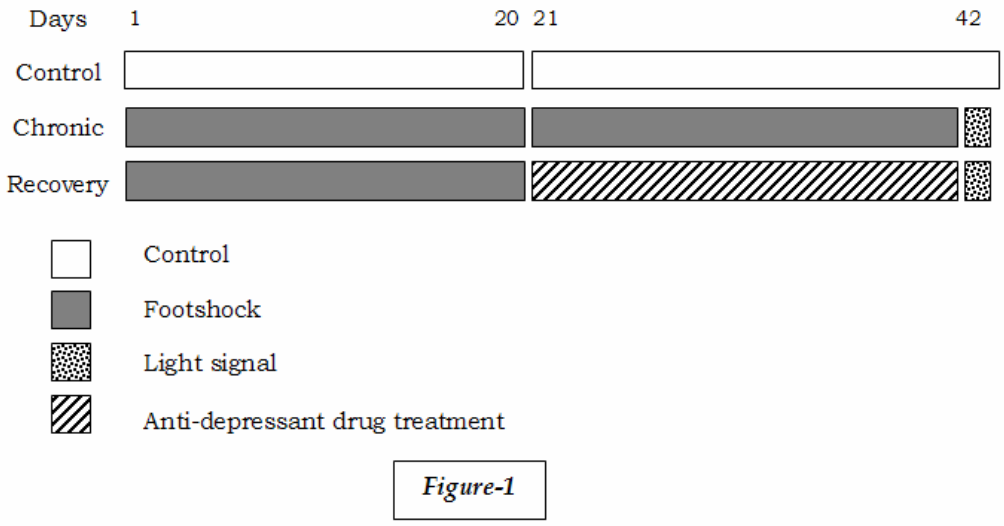


Figure-1

Stress Procedure: The footshock chamber consists of a box containing an animal space positioned on a metallic grid floor connected to a shock generator and scrambler. Rats in stress group were placed in a box and received 60 inescapable footshocks (0.8mA intensity and 15 sec duration with interval of 45 sec.) with randomized starting time (between 9:00 and 17:00h) and intervals during a 30 to 120 min session to make the procedure as unpredictable as possible. A light signal (15 sec) preceded each footshock adding a "psychological" component to the stressor. On the last day the stress exposed rats were subjected to the light stimulus only, which was crucial as it provided a way to create a stress condition without the unwanted side effects of direct physical or painful stimuli [5, 22]. On day 42 rats were sacrificed using isoflurane anesthesia.

Chronic Antidepressant Treatment: Fluoxetine hydrochloride (Sigma Aldrich, St. Louis, MI, USA) was dissolved in 0.9% physiological saline and injected intra-peritoneally (i.p) at the dose of 10 mg/Kg body weight of the rats. The dosage of FLX was based on studies demonstrating a reversal of shuttle box escape deficits, after injections of FLX [26] or exposure to chronic unpredictable shock [27]. Saline was used as a control vehicle for this experiment. Antidepressant drug was administered chronically from day 21 to day 41 once per day.

Shuttle box Testing: Shuttle box sessions were run by PC computer with custom software developed for the system (TSE Active Avoidance Systems GmbH, Bad Hamburg, Germany). At the start of each shuttle box session, animals were exposed to a 5 min habituation period in the same chamber where Inescapable shock (IS) or Escapable shock (ES) was applied. This was followed by 30 escape trials in which the gate separating the two halves of the shuttle box opened 5 sec prior to shock onset followed by randomized footshocks delivered at a intensity of 0.6 mA for 30 sec duration of escape latency. The test consisted of five fixed-ratio 1 (FR-1) trials during which one shuttle-crossing terminated shock. FR-1 trials were used to determine the normal motor function of the rats. For escape testing, FR-1 trials were followed by 25 trials during which the rat had to cross from one side of the shuttle-box to the other, and then return, to terminate shock (fixed-ratio 2 or FR-2 trials). Shock terminated automatically if the response requirement was not met within 30 seconds of the shock onset. A mean latency for the 25 FR-2 trials of $\geq$20 seconds defined rats as learned helpless (LH) while mean latency of <20 seconds classified rats as non learned helpless (NLH). Both FR-1 and FR-2 trials were presented with an average inter-trial interval of 60 sec.

Crosses were automatically stored by the PC whenever a micro-switch was activated by tilting of the pivoted grid floor after crossing event. Shuttle box escape test was performed under red light conditions between 9:00 and 13:00h during the active period of the animals at least 16 hour after the last stress session and before the stress procedure of that day. The test was repeated 3 times on day1, day22 and day41. Animals of recovery group were exposed to shuttle box escape testing every third days starting after 6 days of drug treatment for a total of five shuttle box test session (day28, 31, 35, 38, 41). On testing days injections were given immediately after each shuttle box session.

Neurochemical Analysis: Hippocampus were immediately isolated after anesthesia is over. Hippocampus was stored at-80˚C for posterior analyses of BDNF protein levels. BDNF levels were measured by anti-BDNF sandwich-ELISA, according to the manufacturer instructions (Chemicon,USA). Briefly, hippocampus was homogenized in phosphate buffer solution (PBS) with 1 mM phenylmethylsulfonyl fluoride and 1mM EGTA. Microtiter plates (96-well flat-bottom) were coated for 24h with the sample diluted 1:2 in sample diluent. The plates were then washed four times with sample diluent, and a monoclonal anti-BDNF rabbit antibody diluted to 1:1000 sample diluent was added to each well and incubated for 3h at room temperature. After washing, a peroxidase conjugated anti-rabbit antibody (diluted 1:1000) was added to each well and incubated at room temperature for 2 h. After addition of streptavidin-enzyme, substrate was added followed by stop solution. The amount of BDNF was determined by absorbance in 450 nm. A standard curve was produced and it ranged from 7.8 to 500pg/ml of BDNF. This curve was obtained from a direct relationship between Optional Density and BDNF concentration. Total protein was measured by Lowry's method using bovine serum albumin as a standard.

Vaginal Cytology: During the entire stress paradigm, every morning between 8:00 and 9:00 a.m. each animal cage of female rats was carried to the experimental room. Vaginal secretion was collected with a plastic pipette filled with 10µL of normal saline (0.9% NaCl) by inserting the tip into the vagina of rats, but not deeply. Vaginal fluid was placed on glass slides. A different glass slide was used for each cage of animals. One drop was collected with a clean tip from each rat. Unstained material was observed under a light microscope, with 10X and 40X objective lenses. Three types of cells could be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes. The proportion among them was used for the determination of the estrous cycle phases [28]. For the collection of vaginal secretion, each rat was grasped with proper care.

Statistical Analysis: The Statistical Package for the Social Science (SPSS) 15.0 was utilized for statistical analyses. All data are expressed as mean ± standard error of the mean of n animals, and have been statistically analyzed with the student's t- test. *P* values less than 0.05 were considered statistically significant. Average escape latencies of FR-1 and FR-2 trials, weight changes, for each gender was analyzed with a repeated measured ANOVA.

Results

Effect of inescapable shocks on escape latency: The mean FR-1 escape latencies were significantly differ (male: $F_{2,27}$=10.19; p<0.001 and female:$F_{2,27}$=15.96; p< 0.001; Fig.2A) in the chronic stress group rats of both sexes compared to control and recovery rat groups. A significant difference was also found among the mean FR-2 escape latencies (male: $F_{2,27}$=16.23; p<0.001 and female: $F_{2,27}$=33.71; p<0.001; Fig.2B) among them. Exposure to long term stress among the male rats and female rats for 42 days increased their escape latencies.

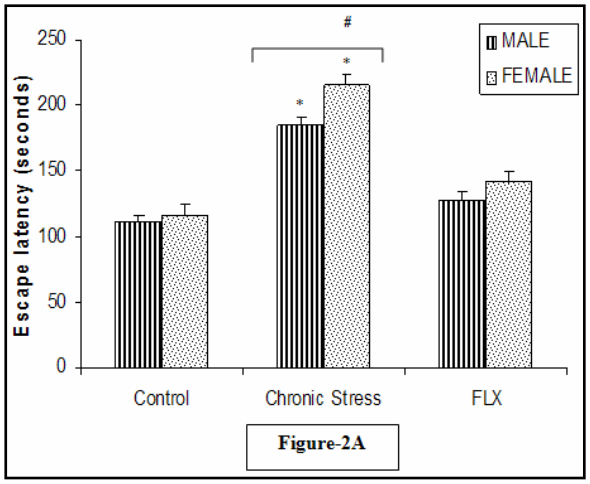


Figure-2A

Chronically stressed females exhibit higher escape latencies in relation to chronically stressed male rats compared with control group in both FR1(t=2.17; df=18; p<0.05) and FR2 (t=3.92; df=18; p<0.001) session. Rats of recovery group restored escape frequency throughout the chronic antidepressant drug treatment.

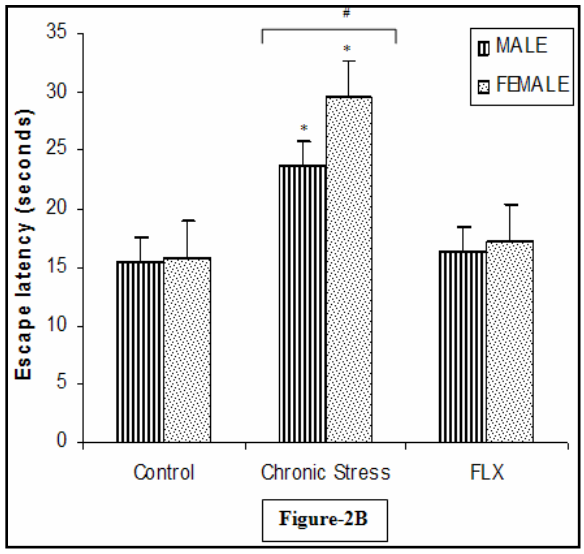


Figure-2B

Figure-2: FR-1 and FR-2 escape latencies (resp. Fig. 2A and Fig. 2B) of male and female rats of 3 experimental groups. The FR-1 and FR-2 escape latencies were significantly different in case of chronic stress group rats of both sexes (FR-1:$F_{male}(2,27)=10.19$; $p<0.001$; $F_{female}(2,27)=15.96$; $p<0.001$; Fig.2A and FR-2:$F_{male}(2,27)=16.23$; $p<0.001$ and $F_{female}(2,27)=33.71$; $p< 0.001$; Fig.2B). Further student t-test revealed a significance difference of escape latencies between chronically stressed male and female rats ($p<0.05$).

Alteration of Body Weight: All rats continued to grow throughout the experiment, and there was a significant effect of day on male and female body weight gain (resp. $F_{1,42}=2938.35$, $p<0.001$; $F_{1,42}=4554.74$, $p<0.001$, Fig. 3). Body weight gain was significantly affected by chronic stress in male rats ($F_{1,42}=16.46$, $p<0.001$, Fig. 3A) but not in female rats ($F_{1,42}=0.221$, $p=0.64$, Fig.3B). In recovery rat group after 21 days when stress was stopped and Fluoxetine was administered, the recovery male rats restored their significant body weight compared to stressed rats ($F_{1,42}=5.09$, $p=0.029$, Fig. 3A), whereas there was no significance between control male rats and recovery male rats ($p=0.072$, Fig. 3A) at the end of the procedure. Anti depressant drug treatment had no significant effect on the body weight of female rats (Fig. 3B).

Figure-3: Effects of stress, recovery after long-term stress and chronic anti depressant treatment on body weight gain of male rats (3A) and female rats (3B). Data were expressed as mean ±SEM, n=9, #p<0.05, recovery group versus chronic stress group; *p<0.001, control group versus chronic stress group.

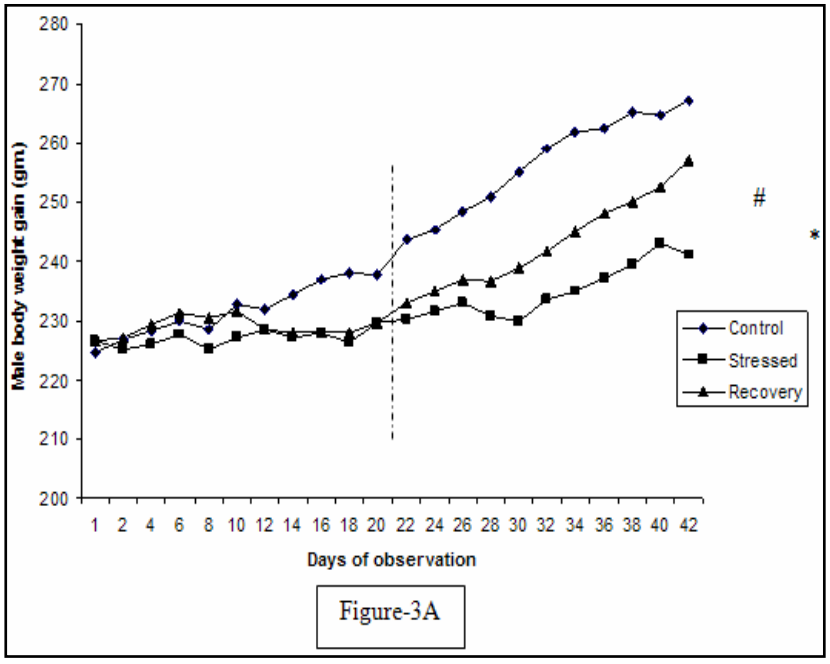


Figure-3A

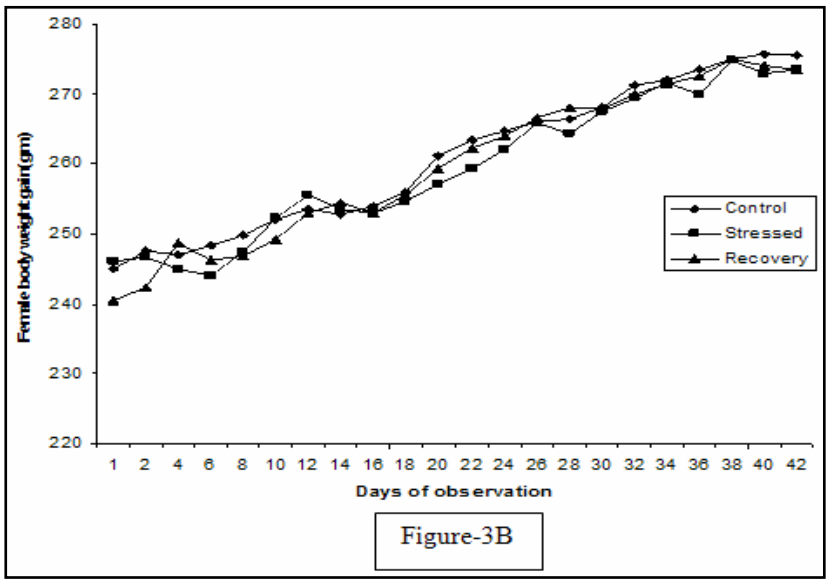
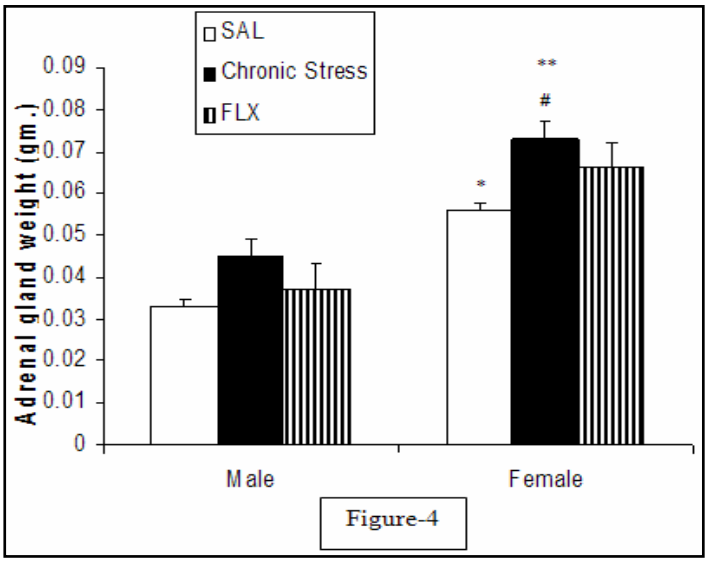


Figure-3B

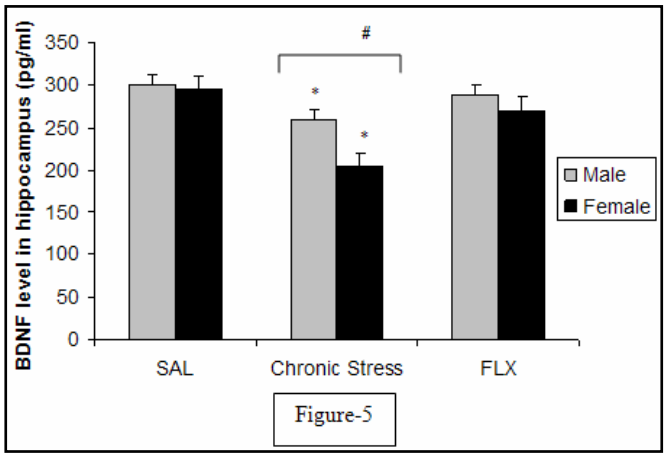
Alteration of Adrenal Gland Weight: Gender differences was found in relative adrenal weight, the female adrenal was significantly larger than the male adrenal ($F_{1,18}$= 10.83, p= 0.004, Fig. 4). Anti depressant drug treatment also had no significant effect on female adrenal weight compared to chronic stress group rats. However there was a significant increase of relative adrenal weight in stress group rats of both sexes compared to control group rats of the same gender (t_{male}=6.56; df=18; p<0.001; t_{female}= 6.89; df=18; p<0.001). In comparison between the chronic stress group rats of both sexes, female stressed rats exhibited significant enlargement of adrenal weight (t=5.48; df= 18; p<0.001).

Figure-4: Effects of chronic stress, recovery after long term stress by anti depressant treatment on adrenal gland weight measured on day 42 in male and female rats. Data were expressed as mean ±SEM, n= 10. *p<0.001 versus control male group; #p<0.05 versus control female group; **p<0.001 versus stressed male group.



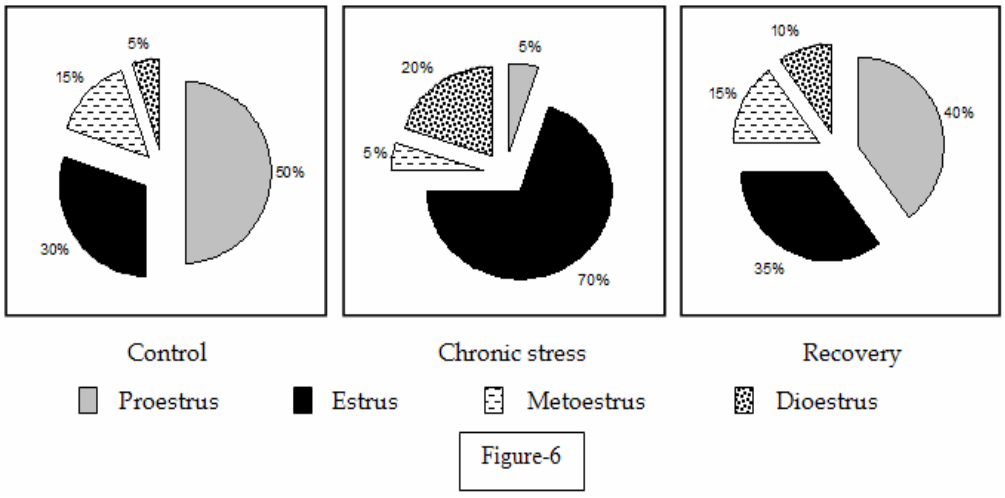
BDNF level in Hippocampus: Chronic stress procedure significantly modify the availability of BDNF protein levels in the hippocampus of both male and female rats compared to control (t_{male}=5.43; df=18; p<0.001; t_{female}=5.03; df=; p<0.001; Fig. 5) and chronically Fluoxetine (t_{male}=3.96; df=18; p<0.001; t_{female}=3.55; df=18; p=0.001; Fig. 5) treated groups. After long term stress female rat group exhibited significantly higher level of BDNF reduction compared to chronically stressed males (t=3.41; df=18; p<0.01; Fig. 5).

Figure-5: Effects of chronic stress, recovery after long term stress by anti depressant treatment on hippocampus BDNF level measured on day 42 in male and female rats. Data were expressed as mean ±SEM, n= 10. *p<0.001 versus control (SAL) and recovery (FLX) groups of both sexes; #p<0.001 versus stressed male group.



Estrous stages Analysis: We directly evaluated whether the vulnerability in females to the induction of the immobility behavior fluctuated across the estrous cycle phases. Chronic stress procedure induced the persistence of estrus phase among the female LH rats of chronic stress group. Significant increase ($F_{2,27}$=14.68, P<0.001; Fig. 6) of prolonged persistency of estrus stage among the chronic stress group rats clearly highlighted the effects of repeated stress and depression over the estrus stage biasness among them.

Figure-6: Effects of chronic stress and recovery after long term stress by anti depressant treatment on estrous stage frequencies (no. of rats/stage) in comparison to control group.



Discussion

The comparative study on escape latency magnitudes between male and female LH rats of chronic stress groups indicates a significant increase of depression over the female individuals due to the effect of repeated stress (Fig. 2). From the statistical analysis on the said data it is predicted that a greater depression induction is associated with the female LH rats than males. As discussed many of the instances of depression in females are associated with the levels of reproductive hormones and their fluctuation across time [29-30]. Thus there is a difference in the base line response that is dependent on sex and moreover, dependent on the stages of estrous cycle [30]. Body weight gain was significantly affected by chronic stress in male rats whereas there was no significance between control male and recovery male rats. There were no effects of stress in the body weight gain of female rats. Such observation indicates that body weight alteration can be considered as a parameter to asses depressogenic induction in male only where as it has no significant role in female sex (Fig. 3).

Shock exposed females in the estrus and dioestrus were significantly different in their susceptibility to develop learned helplessness. The response is stress dependent since there were not significant differences between the control groups. The result presented a clear distinction in development of learned helplessness between two stages of estrous cycle. Therefore, the learned helplessness paradigm may be useful as an animal model to investigate menstrual related mood disorders. In the present study the linear relationship between the percentage of rats in estrus stage and the relative escape latency, suggests that reduced levels of estrogen might have influence in estrus phase associated with high escape latency (Fig.6). Specifically females acquire the conditioned response faster during proestrus when estrogen levels appear in higher percentage relative to the females in estrus and dioestrus phases [31-32].

Our findings observed an increase in adrenal gland weight among the chronically stressed rats of both sexes. Distinct authors have already suggested an increase of the rat adrenal weight after 14 [33] or 28 [34] or 42 [5] days of stress paradigm. These changes in adrenal gland could be due to the increase of adrenocorticotrophin circulating hormone which is released in high concentration during stressful situations by anterior pituitary gland [35]. We also found that Adrenal weight of female stressed rats showed significant increase in comparison to stressed male rats. Significant reduction of BDNF levels in hippocampus of both sexes clearly indicates the effects of depression on neurotrophin levels (Fig.5). After chronic FLX treatment significant recovery of BDNF levels of both male and female rats were investigated. Our findings also revealed that BDNF protein levels were significantly decreased in female LH rats over the male LH rats. In conclusion it could be said that among the experimental rats the depressogenic effect favors females than males with i) the persistence of estrus phase imparting the low level of estrogen secretion during stressed condition ii) significant enlargement of adrenal glands iii) significant reduction of BDNF levels in hippocampus. This scenario clearly denotes the Differential gender-specific vulnerability to depression induction in rats is beyond doubt.

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References

1. Kesslar RC. McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, Wittchen H, Kendler KS. Lifetime and 12 month prevalence of DSM-III-R psychiatric disorders in the United States: results from the national comordity survey. *Arch Gen Psychiatry* 1994; 51: 8-19.
2. Briley M. Understanding antidepressants; Londona: *Martin Dunitz* 2000.
3. Kesslar RC, McGonagle KA, Swartz M, Blazer DG, Nelson CB. Sex and depression in the National Comordity Survey. I Lifetime prevalence, chronicity and recurrence. *J Affect disord* 1993; 29: 85-96.
4. Sun MK and Alkon DL. Differential gender related vulnerability to depression induction and converging antidepressant responses in rats. *J Pharmacol Exp Ther,* 2006; 316: 926-932
5. Lin Y, Westenbroek C, Bakker P, Termeer J, Liu A, Li X and Ter Horst G J. Effects of long-term stress and recovery on the prefrontal cortex and dentate gyrus in male and female rats. *Cerebral cortex.* 2008; 18: 2762-2774.
6. Matheson K, Anisman H. Systems of coping associated with dysphoria, anxiety, and depressive illness: A multivariate profile perspective. *Stress* 2003; 6: 223-224.
7. Howell MP, Muglia LJ. Effects of genetically altered brain glucocorticoid receptor action on behavior and adrenal axis regulation in mice.*Front Neuroendocrinol* 2006;27:275-284
8. Luders E, Narr KL, Thompson PM, Rex DE, Jancke L, Steinmetz H, Toga AW. Gender differences in cortical complexity. *Nat Neurosci* 2004; 7: 799-800.
9. Rasmusson AM, Shi L and Duman R. Down regulation of BDNF mRNA in the hippocampal dentate gyrus after re-exposure to cues previously associated with footshock. *Neuropsychopharmacology* 2002; 271: 133-142.
10. Saarelainen T, hendolin P, Lucas G, Koponen E, Sairanen M, McDonald E, Agerman K, Haapasalo A, Nawa H, Aloyz R, Ernfors P and Castren E. Activation of the Trk B neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioural effects. *J Neurosci.* 2003; 23: 349-357.
11. Banerjee, R., Ghosh, A.K., Mondal, A.C., Ghosh, B. (2011). Stress: The Negative Modulator of NGF. *Research & Reviews: A J Life Sci.* 1 (2), 1-7.
12. Shirayama Y, Andrew C, Chen H, Nakagawa S, Russell DS and Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci.* 2002; 22(8): 3251-3261.
13. Sjoberg RL, Nilsson KW, Nordquist N, Ohrvik J, Leppert J, Lindstrom L . Development of depression: sex and the interaction between environment and promoter polymorphism of the serotonine transporter gene. *Int J Neuropshycopharmacol* 2006; 9: 443-449.
14. Palanza P. Animal model of anxiety and depression how are females different? *Neurosci Biobehav Rev.* 2001; 25: 219-233.
15. Jenkins JA, Williams P, Kramer GL, Davis LL, Petty F. The influence of gender and the estrous cycle on learned helplessness in the rat. *Biological Psychology* 2001; 58:147-158.
16. Shors TJ. Stress and sex effects on associative learning: for better or for worse. *Neuroscientist* 1998; 4: 353-364.
17. Nakagawa S, Kim J, Lee R, Malberg JE, Chen J, Steffen C, Zhang Y, Nestler EJ and duman RS. Regulation of neurogenesis in adult mouse hippocampus by cAMP and the cAMP response element-binding protein. *J. Neurosci.* 2002; 22: 3673-3682.

18. Nair A, Vaidya VA. Cyclic AMP response element binding protein and brain derived neurotrophic factors: Molecules that modulate our mood?; *J.Biosci.* 2006; 31: 423-434.
19. Ueyama T, Kawai Y, Nemoto K, Sekimoto M, Tone S, and Senba E. Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. *Neuroscience Research* 1997; 28: 103-110.
20. Hashimoto K, Shimizu E, Iyo M. Critical role of brain-derived neurotrophic factor in mood disorders. *Brain Res Rev* 2004; 45: 104-114.
21. Lucca G, Comim CM, Valvassori SS, Pereira JG, Stertz L, Gavioli EC, Kapczinski F, Quevedo J. Chronic Mild Stress Paradigm Reduces Sweet Food Intake in Rats without Affecting Brain Derived Neurotrophic Factor Protein Levels. *Current neurovascular Research* 2008; 5: 207-213.
22. Banerjee, R., Das, M., Mondal, A.C., Ghosh, A.K., Ghosh, B. (2011). Influence of chronic stress and antidepressant treatment on the level of BDNF in rat hippocampus: A study in animal model of depression . *Asian J Microbio Biotech EnvSci.* 13 (4), 625-632.
23. Vaidya VA, Siuciak JA, Du F and Duman RS. Hippocampal mossy fiber srouting induced by chronic electroconvulsive seizures. *Neuroscience.* 1999; 89: 157-166.
24. Vaidya VA and Duman RS. Depression- emerging insights from neurobiology. *Br. Med. Bull.* 2001; 57: 61-79.
25. Valentine G, Dow A, Banasr M, Pittman B, Duman R. Differential effects of chronic antidepressant treatment on shuttle box escape deficits induced by uncontrollable stress. *Psychopharmacology* 2008; 200: 585-596.
26. Chen H, Pandey GN, Dwivedi Y. Hippocampal cell proliferation regulation by repeated stress and antidepressants. *Neuroreport* 2006; 17: 863-867.
27. Gambarana C, Scheggi S, Tagliamonte A, Tolu P, De Montis MG. Animal models for the study of antidepressant activity. *Brain Res Protoc.* 2001; 7: 11-20.
28. Mandl AM. Cyclical changes in the vaginal smear of adult ovariectomized rats. *J Exp Biol* 1951; 28: 585-592.
29. Caldarone BJ, George TP, Zachariou V, Picciotto MR. Gender differences in learned helplessness behavior are influenced by genetic background. *Pharmacol. Biochem. Behav.* 2000; 66: 811-817.
30. Buckwalter JG, Buckwalter DK, Bluestein BW and Stanczyk FZ. Pregnancy and postpartum changes in cognition and mood. *Prog. Brain Res.* 2001; 133: 303-319.
31. Shors TJ and Leuner B. Estrogen-mediated effect on depression and memory formation in females. *J affect disord.* 2003; 74: 85-96.
32. Chang YJ, Yang CH, Liang YC, Yeh CM, Huang CC, Hsu KS. Estrogen modulates sexually dimorphic contextual fear extinction in rats through estrogen receptor beta. *Hippocampus.* 2009; 19: 1142-1150.
33. Harro J, Tonnissar M, Eller M, Kask A, Oreland L. Chronic variable stress and partial 5H-T denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry. *Brain Res* 2001; 899: 227-239.
34. Konarska M, Stewart RE, McCarty R, Predictability of chronic intermittent stress: effects on sympathetic-adrenal medullary response of laboratory rats. *Behav Neural Biol* 1990; 53: 231-243.
35. O'Connor TM, O'Halloran DJ, Shanahan F. The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. *QJM* 2000; 93: 323-333.

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