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# BEHAVIORAL EVIDENCE OF NEUROPSYCHOPHARMACOLOGICAL EFFECT OF IMIPRAMINE IN ANIMAL MODEL OF UNPREDICTABLE STRESS INDUCED DEPRESSION

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## ABSTRACT

Stressful experiences embrace complex correlation concerning body's responses and brain function towards stress that leads to other psychological illnesses such as depression. Depression is a challenging provocative mental disorder with psychological and clinical consequence. Imipramine (IMI) is a tricycle antidepressant that constrains norepinephrine and serotonin reabsorption inside the synaptic cleft in the central nervous system and extensively prescribed to millions of depressive sufferers. Chronic stress models are considered as the most favorable and appropriate exemplar parallel to acute stress mockups for inspecting depression in experimental simulations. Reduced 5-Hydroxytryptamine and neuropeptide Y neurotransmission is significant in mediating depression provoked by chronic mild stress. The current study was intended to examine whether recurring administration of imipramine (1.0 mg/kg) may possibly attenuate the insufficiencies of behavior induced by chronic stress model in laboratory rats. Animals experiencing unpredictable chronic mild stress (UCMS) showed a significant hypophagia whereas, hyper-locomotive activity in activity box and open field and anxiolytic in light/dark transition box in imipramine administered groups of unstressed and stressed rats. Data indicates that Imipramine is efficacious in the attenuation of UCMS induced symptoms of depression in rats. Repeated administration of imipramine increased the activity in familiar and novel environment. Anxiolytic effect of Imipramine was higher in unstressed as compared to stressed animals. This study, therefore establish the stress and behavior scarcities relations and therapeutic approach of imipramine.

**Keywords**: Depression, Imipramine, Unpredictable Chronic Mild Stress (UCMS), 5-Hydroxytryptamine (5-HT), Hypophagia, Locomotor Activity.

## INTRODUCTION

Depression is a massive sociological and clinical relevance and understanding biology of its consequences is a modern challenging scientific problem. Plasticity of neuronal pathways is likely to involve in the pathogenesis and treatment of depression. Antidepressants innovation and investigation of their mechanisms of action has restructured our comprehension of function of neuron and the effective mechanisms relating to depression. Managements of mental illness with antidepressant may produce therapeutic consequences by stimulating applicable modifying variations in neuronal coordination (Vaidya and Duman 2001). After a long struggle, clinical effects mediation of ADs it is still ambiguous for scientist the field of depression research (Malberg and Blendy 2005).

Depression-like symptoms can be stimulated by exposure to chronic unpredictable mild stress (UCMS) such as lack of sucrose preference (Pothion *et al.,* 2004), augmented REM sleep (Gronli *et al.,* 2004), altered sympathetic cardiac regulations and decreased levels of cytokines (Grippo *et al.,* 2002, 2003a, b). For exploring depression in experimental models, chronic stress simulations are moderately more appropriate than acute stress (Katz *et al.,* 1981; Willner *et al.,* 1997). Studies have stated reduced serotonergic function in animal model of depression for instance chronic mild stress (Kang *et al.,* 2005) and learned helplessness (Sherman *et al.,* 1982). Scientists have reported significant role of dopamine, serotonin and adrenergic receptors in the pathophysiology of depression (Gamaro *et al.,* 2003; Harro *et al.,* 2001; Papp *et al.,* 2002). Luo and his colleagues (Luo *et al.,* 2008) determined reduced neuropeptide Y and 5-HT neurotransmission is vital to facilitate the depression induced by chronic unpredicted mild stress.

Dibenzazepine-acquired tricycle, Imipramine (IMI) is an antidepressant that inhibits5-HT and nor-epinephrine reuptake inside the synaptic cleft of neurons. IMI is not specifically prescribed for depression treatment but also for enuresis children (Duda *et al.,* 2016). It is quite extensively prescribed to millions of suffers. Unpredictable chronic mild stress (UCMS) model is fundamentally considered as a depression simulation in laboratory animals and is commonly used for antidepressant's functional evolutions (Willner 2005). Stress-induced anhedonia is antagonized by repeated antidepressant treatments (Papp and Moryl, 1994; Kubera *et al.,* 2001) and behavior deficits in the

forced swim paradigm (Rogo´z *et al.,* 2009).Less precedence for palatable sucrose solution above water evidenced a noticeably declined interest in rewarding stimuli by exposure to CMS (Papp *et al.,* 1991, Kubera *et al.,* 1996). This behavior shows disturbance of the proficiency for experiencing pleasure advocated to mock-up human anhedonia, the foremost symptom of major depression incidents as stated by DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders Fourth Edition, American Psychiatric Association 1994; Willner 2005). Research suggests some animals were un- responsive to imipramine treatment when exposed to UCMS (Faron-Go´recka *et al.,* 2014). It is still ambiguous that why imipramine does not produce therapeutic effects in some rats exposed to CMS, this situation is similar to clinical condition, when some patients are unreactive to anti depressive medications (El-Hage *et al.,* 2013).

## MATERIALS AND METHODS

### Subjects

Animals selected were Albino-Wistar rats (weighing 180-220 grams) purchased from The Dow University of health and sciences Ojha campus, Karachi. Initially for three days, all rats were kept separately in cages made up of Perspex glass observing 12-hrs light/dark cycle and (25±2 $^{o}$C) controlled room temperature with access to standard rodent diet and water unmeasured for familiarization. All animal paradigms, permitted by the Institutional Ethics and Animal Care Committee and supervised in strict conformity to the National Institutes of Health Guide for Care and Use of Laboratory Animals.

### Drug

Imipramine was acquired from Sigma Aldrich (USA) and dissolved in distilled water by 1:1 v/v. Imipramine (1mg/kg /mL /body weight) was orally administered to all experimental animals for 14 days by feeding tubes (stainless steel).

### Experimental Protocol

Twenty four male Albino Wistar rats were arbitrarily distributed into four groups:
(i) Unstressed - Saline (ii) Unstressed - Imipramine, (iii) CMS – Saline and (iv) CMS - Imipramine. Imipramine (1.0 mg/kg/day) and saline (1.0 mg/mL) were monitored to all groups accordingly 1 hour prior to each stress. Unpredictable chronic stress scheduled for stressed rats as follows Cage agitation, Noise stress, Water deprivation stress, Cold stress (4 $^{o}$C), Cage tilted stress, Diurnal light off stress, Crowding stress, Repeated light/dark cycle for a period of 14 days, while unstressed animals stayed in their home cages. Locomotive activities in activity box, exploration in open field and food intake were observed on next day of 1$^{st}$, 7$^{th}$ and 14$^{th}$ day of drug administration. Light dark transition box was used to monitor anxiolytic produced by drug treatment.

### Behavior Evaluations

***a. Food intake:*** Quantified food pallets were provided in the Hooper of cage to each animal. Intake was observed by weighing the leftover food in the cage Hooper on 7$^{th}$ and 14$^{th}$ day of experimental protocol.

***b. Open Field Test:*** Open field activity is a paradigm used to assess locomotors activity to the unfamiliarity. The open field apparatus is considerable larger as compared to home cage and is unacquainted to animal. It comprised of plastic walls which are opaque to outer environment and 42 cm high and 76 x 76 cm$^2$ area dimensions. The floor comprises of 25 equal squares and rat is positioned in the middle square of the field. Observations are made on the numbers of square crossed by rat with all four paws within 5 minutes durations.

c. ***Activity Box:*** Activity box is used to determine locomotors activity in familiar environment. Apparatus consists of quadrangular Perspex cage having (26 x 26 x 26 cm) dimensions. The cage floor concealed by saw dust. The monitoring of stress or drug induced behavior is observed 10 minutes duration and documented simultaneously. Experiment is performed in balanced design.

***d. Light-Dark Transition Box:*** Rat has peculiarity to remain in shades and feel being protected over there. Escaping of lit area is considered as the anxiety provoking characteristic of rats. If a condition is given to stay in an illuminated uncovered area and a dim place, rodent usually favors the dark, laminated environment. Transition apparatus comprised of dual compartments having identical size of (26 x 26 x 26 cm) and an opening of (12 x 12 cm) among the boxes. One of the sections is transparent made up of Plexiglas and other is painted black and both are top closed. Animal is placed at the light box center. The light dark transition assessment evaluates the anxiogenic and anxiolytic reaction of a particular drug (Hascoët *et al.,* 2001). The observed parameter is time consumed in exploring the light slot in 5 minutes. All tests are performed in balanced design.

**Statistics**

Results are represented as mean ± S.D. All activities are evaluated by three-way ANOVA repeated measure designs (SPSS). SPSS (version 17) Software used was for the analysis. Individual assessments performed by Newman- Keuls test. Values of $p<0.05$ were considered as significant.

**RESULTS**

**Imipramine effects on food intake of CMS exposed rats**

Observed data (Fig. 1) evaluated by three-way ANOVA (repeated measured designing) presented the effects of stress ($F=108.241$; df=1, 21: $p<0.01$) and interaction between stress, drug and days of stress ($F=86.753$; df=1, 21: $p<0.01$) were found significant. Whereas, effects of repeated monitoring ($F=1.641$; df =1, 21) as well as effects of drug ($F=18.169$; df =1, 21) were found non-significant. Post-hoc analysis by Newman-Keuls test revealed decreased ($p<0.01$) food intake in stressed animals of saline and imipramine group when compared to their respective controls. However, on last ($2^{nd}$) administration food intake was more ($p<0.05$) in unstressed and ($p<0.01$) in stressed animals treated with imipramine than their saline controls.

**Imipramine effects on activity box cage crossings of UCMS exposed rats**

Data from Figure 2 as investigated by three-way ANOVA (repeated measured designing) revealed that the effects of repeated monitoring ($F=33.157$; df =1, 21: $p<0.01$), imipramine ($F=286.508$; df= 1, 21: $p<0.01$) and stress ($F=53.884$; df=1, 21: $p<0.01$) were significant. Whereas, the relations between drug, days and stress ($F=2.1788$; df =1, 21) was non-significant. Post-hoc analysis by Newman-Keuls test determined decrease ($p<0.01$) in cage counts on $2^{nd}$ week in stressed rats from their respective unstressed controls however, decrease ($p<0.05$) in saline administered stressed rats and increase ($p<0.01$) in imipramine administered unstressed rats was observed from $1^{st}$ week of drug administration. Furthermore, cage counts were more ($p<0.01$) in unstressed and stressed rats treated with imipramine on last ($2^{nd}$) administration and less ($p<0.05$) in stressed rats in $1^{st}$ week than their saline controls.

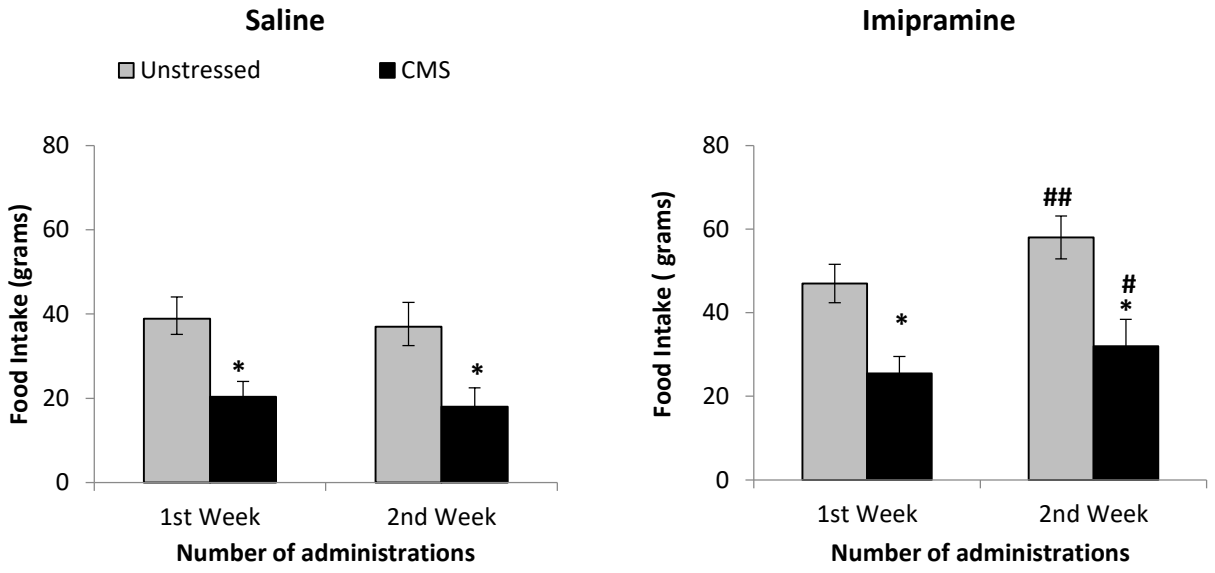


Fig.1. Effects of Imipramine on cumulative (weekly) food intake of rats exposed to UCMS.
Values are means ±SD (n=6) as monitored on next day of $7^{th}$ and $14^{th}$ day of stress. Significant differences by Newman-Keuls test: *$p<0.01$ from unstressed controls of same drug; #$p<0.05$, ##$p<0.01$ from similarly saline or imipramine administrated unstressed animals of same day following three-way ANOVA (repeated measures design).

**Imipramine effects on square crossed activity n open field of UCMS exposed rats**

Observed data (Fig. 3) studied by three-way ANOVA (repeated measured designing) displays that the effect of drug ($F = 510.663$; df = 1, 21; $p < 0.01$) and stress ($F = 171.059$; df = 1, 21: $p < 0.01$) were significant and the effect of drug ($F = 19.940$; df = 1, 21) and the interaction between drug, days and stress ($F = 6.933$; df = 1, 21) were found

non-significant. Post-hoc analysis by Newman-Keuls test indicated decrease ($p < 0.05$) square crossed in 1st week and ($p < 0.01$) in 2nd week in stressed saline rats moreover decreased ($p < 0.01$) in square crossed was observed in last week of imipramine administration in stressed rats however, square crossed was more ($p < 0.01$) in unstressed and ($p < 0.05$) in stressed rats treated with imipramine when compared to their 1st week of administration. Furthermore increase ($p < 0.01$) in 2nd week of administration in stressed and unstressed rats and decrease ($p < 0.05$) in 1st week of administration was observed in imipramine treated rats than their saline controls.

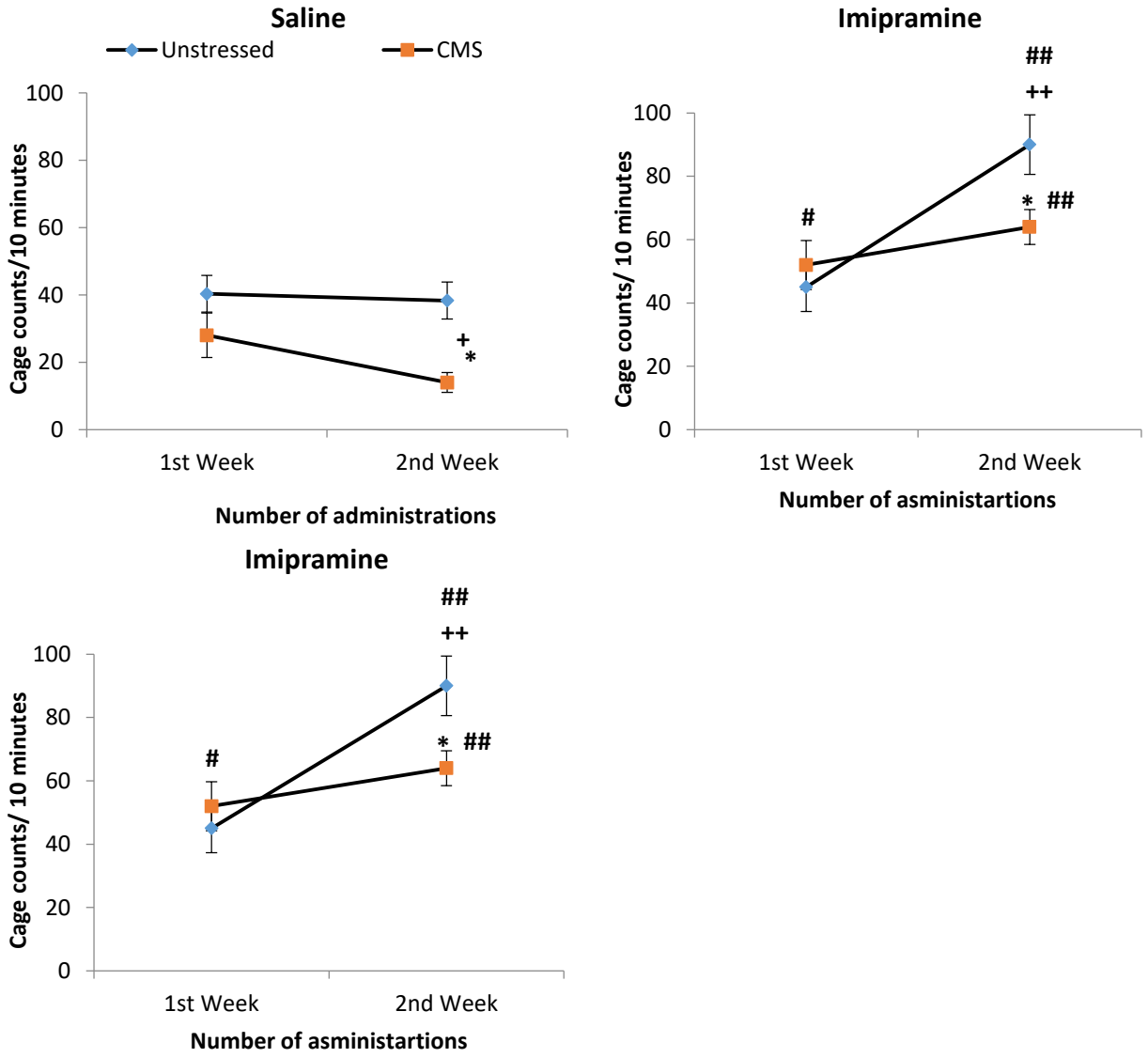


Fig. 2. Effects of Imipramine on activity in activity box of rats exposed to CMS.
Values are means ± SD (n=6) as monitored on next day of 7th and 14th day of stress. Significant differences by Newman-Keuls test: *p<0.01 from unstressed controls of same drug; +p<0.05, ++p<0.01 from similarly administrated unstressed or stressed animals of 1st week, #p<0.05, ##p<0.01 from similarly saline or imipramine administrated unstressed animals of same day following three-way ANOVA (repeated measures design).

**Imipramine effects on light dark box activity of UCMS exposed rats**

Data on time spent in light box (figure 4a) as analyzed by three-way ANOVA (repeated measured designing) shown that the effect of stress (F=26.901;df=1, 21: p<0.04), drug (F=31.898; df= 1,20: p<0.01) were significant but the effects of days (F=0.825; df=1,21) and the interaction between all factors (F=0.398; df=1, 21) were non-significant. Post-hoc analysis by Newman-Keuls test presented decrease (p<0.05) time spent in light box in 2nd week of administration in both saline and imipramine stressed rats. Whereas decrease (p<0.05) in time spent saline stressed rats and increase (p<0.05) in imipramine stressed and unstressed animals was observed as compared to first week of administration. However, time spent in light compartment was more (p<0.05) in unstressed and stressed rats in imipramine treated group than their saline controls.

The data (figure 4b) on number of entries in light box as investigated by three-way ANOVA (repeated measured designing) effect of drug (F=71.223; df=1,21: p<0.01) , the effect of stress (F=61.22; df=1, 21: p<0.01) were found significant Whereas, the effect of day (F=0.825; df=1,21) and the relation of drug, days and stress (F=0.398; df=1, 21) were non-significant. Post-hoc analysis by Newman-Keuls test showed decrease (p<0.05) in entries in light compartment in stressed saline rats in 2nd week of administration. However, entries were more (p<0.05) in stressed rats treated with imipramine as compared to their saline controls in last administration.

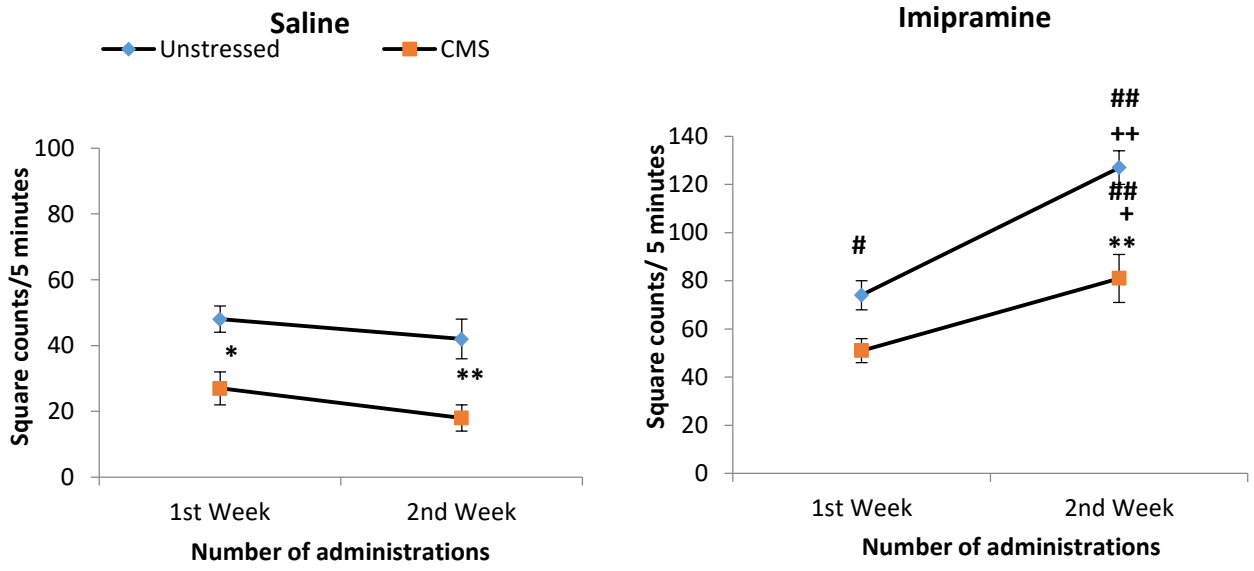


Fig. 3. Effects of Imipramine on number of square crossed in open field of rats exposed to UCMS.
Values are means ±D (n=6) as monitored on next day of 7th and 14th day of stress. Significant differences by Newman-Keuls test: *p<0.01 from unstressed controls of same drug; +p<0.05, ++p<0.01 from similarly administrated unstressed or stressed animals of 1st week, #p<0.05, ##p<0.01 from similarly saline or imipramine administrated unstressed animals of same day following three-way ANOVA (repeated measures design).

## DISCUSSION

The current study was intended to examine whether recurring administration of imipramine (1.0 mg/kg) may possibly attenuate the insufficiencies of behavior induced by chronic stress model in laboratory rats. Chronic unpredictable mild stress causes a distinctive anhedonia consequence in the sucrose intake test (Willner 1997, 2005), in addition to a cognitive impairment in object recognition test and anxiogenic effect in elevated plus maze. These effects were upturned by the classical antidepressant imipramine. This study reveals that chronic stress can be attenuated and behavior deficits due to stressful situations can be attenuated by the management of antidepressants predominantly imipramine.

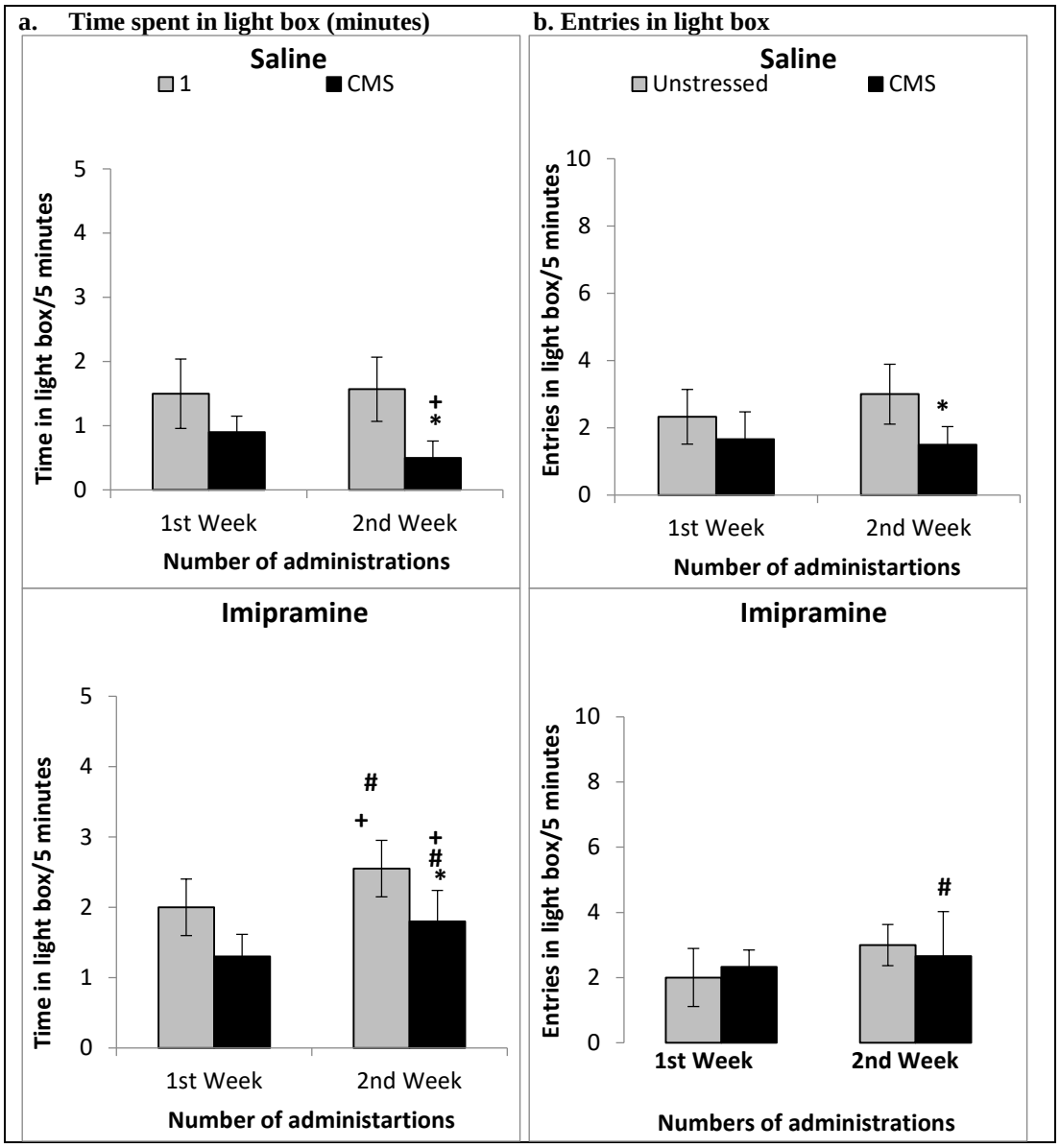


Fig. 4. Effectsof Imipramine on activity in light/dark box of rats exposed to UCMS.

Values are means ± D (n=6) as monitored on next day of 7th and 14th day of stress. Significant differences by Newman-Keuls test:*p<0.01 from unstressed controls of same drug; +p<0.05, ++p<0.01 from similarly administrated unstressed or stressed animals of 1st week, #p<0.05, ##p<0.01 from similarly saline or imipramine administrated unstressed animals of same day following three-way ANOVA (repeated measures design).

This present study exhibits that the food intake decreased in CMS treated rats in 1st and last 2nd week administration in both groups of saline and imipramine when compared with unstressed animals whereas rapid increase in food intake is observed in imipramine treated stressed rats in contrast to saline administered rats (Fig. 1). The food intake was monitored weekly for 14 days under unstressed and UCMS conditions and a distinctive modification is observed which displays hyperphagic effects in imipramine treated rats in both unstressed and stressed environments. Loss in body weight and decrease in food consumption are common consequences of depression but treatment with antidepressants such as imipramine facilitates increased appetite although the circumstances are stressful.

Activity box test is used to determine the locomotion in rats in acquainted conditions in stressful or stress-free paradigm (Ganea *et al.*, 2007). Results show increase in locomotion in imipramine (1.0 mg/kg) treated group of stressed and UCMS rats in contrast to saline animals (Fig. 2). Fatigue, desperation and hopelessness are common in

depression. Even though activity box was recognized environment for animals but rats are less mobile in stressed condition in both saline and imipramine treatment. As previous findings showed that a low dose 3-week management of imipramine via drinking water to C57BL6J mice decreased such depressive symptoms as stress provoked diminution in sucrose intake and preference, hyper-locomotion, and elevated aggressive behavior (Cline *et al.,* 2014). Similar behaviors were observed in the chronic stress model of depression with CD1 mice (Cline *et al.,* 2012) and elderly depression model of in 18-month-old C57Bl6N mice (Malatynska *et al.,* 2012). Imipramine low dose effects were accompanied by conservancy of brain per-oxidation enzymes normal activity which were inhibited by chronic stress (Cline *et al.,* 2014). These observations are characteristic for antidepressant demonstration induced by tricyclics in rats (Cryan *et al.,* 2005; Frijtag *et al.,* 2002).

The present study illustrates the antidepressant effect of imipramine in an open field by square crossed parameters. Figure 3 shows escalate square counts in imipramine monitored stressed and unstressed rats. However, a reduced amount of square counts in rats treated by saline of stressed and unstressed groups are observed. The increase in square crossed is mainly due to antidepressant effect of imipramine in novel environment, it improves locomotion in rats in both stressed and unstressed condition. Novelty and unfamiliarity to the environment caused less activity in rodents. The considerable increase locomotors activity of UCMS rats compared to control rats in Open Field Test indicate stress induced hyperactivity in the Chronic stress model (Gao *et al.,* 2011). Interestingly, the interpretations determine that psychomotor behavior was distressed by UCMS however, fluoxetine and imipramine attenuate the effect of UCMS at various grades (Jing Zhao *et al.,* 2015). Anxiogenic effects have repeatedly been defined in rodents exposed to chronic mild stress such as anhedonia and are retreated with prolonged antidepressant treatment (Surget *et al.,* 2008; Farley *et al.,* 2010; Wang *et al.,* 2014). The light/dark box test is established on the instinctive abhorrence of rodents to lit area. In the course of a 5-min period, rats are permitted to explore a unique situation that comprises of dual sections: shady (secured) and lit (insecure). In rodents, this paradigm produces a distinctive conflict concerning their exploration and their avoidance of two environments (Crawley and Goodwin, 1980; Crawley *et al.,* 1981). Treatment with anxiolytic drugs such as benzodiazepines increases the time spent in the lit compartment as well as the number of transitions between the two areas (Crawley and Goodwin, 1980; Crawley *et al.,* 1981).

The present study has revealed that time period consumed in bright compartment was greater in imipramine treated rats of unstressed and stressed group relatively to saline administrated animals (Fig. 4a) whereas, the accesses in light area in saline unstressed and imipramine monitored animals are increased than stressed rats moreover, imipramine administered rats demonstrated more admittances in bright box relatively to rats treated by saline (Fig 4b). Imipramine shows antidepressants effect by accumulating the time period spent of rodents in unfamiliar illuminated region even though the entrances of rats treated with imipramine were in a lesser amount as compared to saline rats in both stressed and unstressed groups.

## CONCLUSION

Chronic exposure to traumatic conditions consequence numerous behavior deficiencies that can be decreased or reversed by imipramine recurrent management. Imipramine causes hyperphagia in unstressed and stressed rats. Administration of imipramine produced anxiolytic and antidepressant effects but these behaviors were observed less in animals exposed to stress.

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