

Apomorphine induced conditioned place preference and sensitization is greater in rats exposed to unpredictable chronic mild stress

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Abstract: CNS stimulants are the class of the drugs that may be used to get relief from depression. Apomorphine is a D1 and D2 receptor agonist with a CNS stimulatory effect used for the treatment of Parkinson's disease is also abused. Although many drugs of abuse produce tolerance and dependence. Long term use of psychostimulants produce reverse tolerance described as sensitization. These drugs also have a number of other beneficial effects but their therapeutic use is limited because of abuse potential. Conditioned place preference (CPP) test is used to monitor the reinforcing effect of drugs of abuse. Stress is an important factor that precipitates and potentiates addictive effects of different drugs of abuse. The present study was designed to investigate the addictive effect of apomorphine (1mg/kg) in rats previously exposed to repeated unpredictable chronic mild stress for 10 days (animal model of depression). Results from present study illustrate that unpredictable chronic mild stress potentiates the reinforcing effects of apomorphine as the number of entries and the time spent in the CPP compartment associated with drug administration is increased. Motor activity was taken as a parameter for behavioral sensitization which is induced by repeated administration of apomorphine, monitored as the number of cage crossings in light compartment of the CPP apparatus, also increased.

Keywords: Unpredictable chronic mild stress, Depression, CPP, Sensitization, Drug addiction.

INTRODUCTION

CNS stimulants are class of the drugs that produce different kinds of effects by improving the activity of the central and peripheral nervous systems. They increase locomotor activity, alertness, awareness, wakefulness, increased arousal, improve mood and relieving anxiety, induce feelings of euphoria. Stimulants produce their effects by a variety of different pharmacological mechanisms, the most prominent is facilitation of norepinephrine (noradrenalin) and/or dopamine activity (Riddle *et al.*, 2005). It has also been suggested that addiction is mostly associated with depressive symptoms (Yen *et al.*, 2007). Although the environmental and the neurobiological factors that are involved in depression and drug addiction are separately but it has been observed that depression is followed by drug abuse (Volkow, 2004).

Apomorphine is a dopaminergic D1/D2 receptors agonist (Carlos *et al.*, 2006), which is used to provide symptomatic relief in Parkinson's disease but it has been observed that the drug is reinforcing (Ranaldi *et al.*, 2001; Ikram and Haleem, 2011).

Drug addiction is a chronic relapsing brain disorder that produces neurobiological changes, which lead to a compulsion to take a drug with loss of control over drug intake (Koob, 2000). Drugs of abuse are both rewarding and reinforcing. It has been proposed that addiction may

develop in response to some neuroadaptations that underlie behavioral sensitization (Maidment *et al.*, 2008). Repeated exposure of the neural system to psychostimulants would result in alteration of various behaviors. Behavioral sensitization primarily reflects neuroadaptive changes, which are induced by repeated administration of a psychostimulant. Animals given a stimulant repeatedly in a test cage but not in other environments may show enhanced drug-induced behavior in the test cage (Ohmori *et al.*, 2000).

Stress is an important factor that play vital role in precipitation of depression and the changes in different systems of body that occur in depression are similar to those observed in response to stress (Leanard, 2001). It has been shown in different studies that exposure to inescapable stress produces some behavioral changes (Amat *et al.*, 2001). Chronic stress precipitates drug seeking behavior and alters the effects of drugs of abuse (Doyle *et al.*, 2010). Stress can enhance the effects of different drugs and their consumption by acting directly on neural circuit, brain reward pathway and it can also enhance conditioned place preference, self-administration and seeking of addictive drugs by the activation of the mesocorticolimbic dopamine system (Yap & Miczek, 2008). Preclinical literature suggests that stress increases reward associated with psychomotor stimulants, possibly through a process similar to sensitization and a clinical literature indicates that there is a link between substance abuse and stress.

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MATERIALS AND METHODS

24 Male Albino Wistar rats (150-200 gm) were purchased from Agha Khan University Hospital, Karachi and housed individually under a 12-hour light and dark cycle (lights on at 06:00 hours) with free access to cubes of standard rodent diet and tap water 3 days before experimentation. All experiments were performed according to a protocol approved by a local Animal Care Committee.

Experimental protocol

12 rats were exposed to unpredictable chronic mild stress for 10 days and remaining rats were unstressed. After this period of stress animals were assigned as test and control in such a manner that unstressed control, unstressed test, stressed control and stressed test. All test animals were injected intraperitoneally and repeatedly with saline on day 1,3,5,7,9 and 11 and with apomorphine (1mg/Kg/ml) on day 2,4,6,8,10 and 12. Whereas control animals were injected repeatedly with saline. Body weight, food and water intake of all animals were monitored daily. Reinforcing effects of apomorphine were monitored in a CPP paradigm on test days and after 6 injections of the drug. For behavioral sensitization, motor activity of all rats was monitored as the number of cage crossings in light compartment of CPP apparatus, on test days.

Chronic unpredictable stress

Rats were exposed to 10 days of chronic unpredictable mild stress: Day 1 10:00h 50 min cold room (4°C); Day 2 13:00 60min cage agitation; Day 3 10:00h 60 min restraint stress; Day 4 18:00 food and water deprivation overnight (12 h); Day 5 10:00h 3 h lights off. All these stresses were repeated in the same order on day 6-10 respectively.

Reinforcing effects of apomorphine

The test was conducted in a locally made two-compartment box of equal size (26x26x26cm each) and had an access (12x12cm), but differed in their sensory properties. Walls of one compartment were light (transparent) and other dark (black). The experiment was performed in three phases as follows:

Phase 1: Pre-Conditioning-PP-test

Base line values of place preference (PP) of all rats in the light dark box were monitored a day before the start of the conditioning phase. Access from one to the other compartment was open. An animal to be tested was placed in the light compartment of the box. Time spent and number of entries in the light compartment was monitored for a cut off time of 10 min.

Phase 2: Conditioning phase

In the conditioning phase access from one to the other compartment in the light box was closed. During pre-conditioning PP-test animals stayed more than 70% of the

total time in the dark compartment with only more than 30% time in the light compartment. Therefore in the conditioning phase, less preferred (light) compartment was paired with the drug administration and more preferred (dark) compartment was paired with the saline administration.

Animals were exposed to twelve, one daily, place conditioning sessions of 30 min duration each. On six alternating days (1,3,5,7,9 and 11) animals of test as well as control were treated with saline. After injection each animal was sequestered for 30 min in the dark compartment of the box before transferring in its home cage.

On the other six days (day 2,4,6,8,10 and 12) each animal of the control group or treated with drug was sequestered for 30 min in the light compartment of the CPP box. Motor activity of each rat was monitored for 10 min as the number of compartment crossings. After 30 min of conditioning session the animals were transferred in their home cages.

Phase 3: Post-Conditioning-PP-test

Reinforcing effects of apomorphine were monitored in a test session on day 13 of treatment. On the test day animals were given free access to move between the two compartments. An animal to be tested was placed in the light compartment of a CPP box. Time spent and number of entries in the light compartment was monitored during a test session of 10 minutes.

Behavioral sensitization induced by apomorphine

Behavioral Sensitization induced by repeated administration of apomorphine was monitored as the locomotor activity in light compartment of CPP apparatus on test day 10 min post-injection of drug for a cut-off time of 10 minutes (Ikram *et al.*, 2012; Mirza *et al.*, 2013).

RESULTS

Fig. 1 (a) shows effects of unpredictable chronic mild stress on cumulative food intake of rats. Analysis of data on cumulative food intake by Students t-test exhibit significant effect of UCMS on cumulative food intake ($t=6.89$; $p<0.01$). UCMS for a period of 10 days caused reduction in cumulative food intake of stressed rats as compared to unstressed rats.

Fig. 1 (b) shows effects of unpredictable chronic mild stress on % growth rate of rats. Analysis of data on % growth rate by Students t-test exhibit significant effect of UCMS on % growth rate ($t=4.88$; $p<0.01$). UCMS for a period of 10 days caused reduction in % growth rate of stressed rats as compared to unstressed rats.

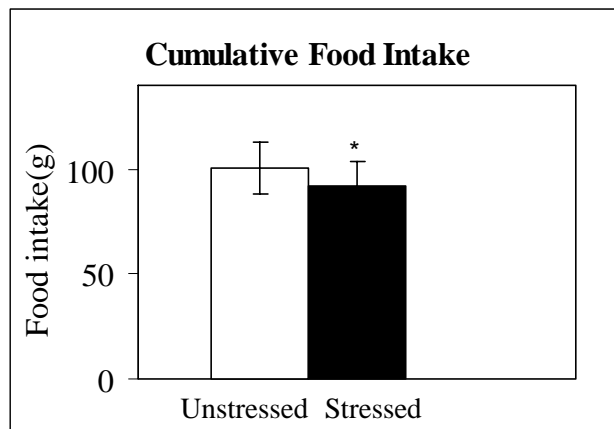


Fig. 1(a): Effects of unpredictable chronic mild stress on cumulative food intake in rats. Values are means \pm SD (n=12). Significant differences by students t-test * $p < 0.01$

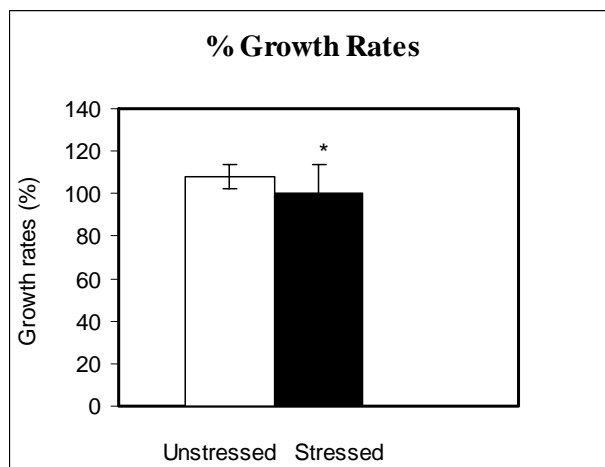


Fig. 1 (b): Effects of unpredictable chronic mild stress on % growth rates of rats. Values are means \pm SD (n=12). Significant differences by Students t-test * $p < 0.01$.

Fig. 2 shows effects of apomorphine administration on cage crossings (in a familiar environment) of rats previously exposed to unpredictable chronic mild stress. Analysis of data on cage crossings (in a familiar environment) by two-way ANOVA showed that effects of stress ($F=22.09$; $df=1,120$, $p < 0.01$), apomorphine ($F=22.09$; $df=1,120$, $p < 0.01$) and Interaction between apomorphine and stress ($F=22.09$; $df=1,120$, $p < 0.01$) were significant. Post hoc analysis by Newman-Keuls test showed that unstressed saline injected animals showed increased ($p < 0.01$) activity after 2nd, 3rd and 5th injection as compared to activity after 1st injection. Similarly saline injected stressed animals showed decreased ($p < 0.01$) activity after 4th and 5th injection as compared to activity after 1st injection. Saline injected stressed animals showed increased activity ($p < 0.01$) after 3rd and 5th injection as compared to 3rd and 5th injection of unstressed saline injected animals. However apomorphine injected

unstressed animals showed increased activity after 3rd injection as compare to 1st injection.

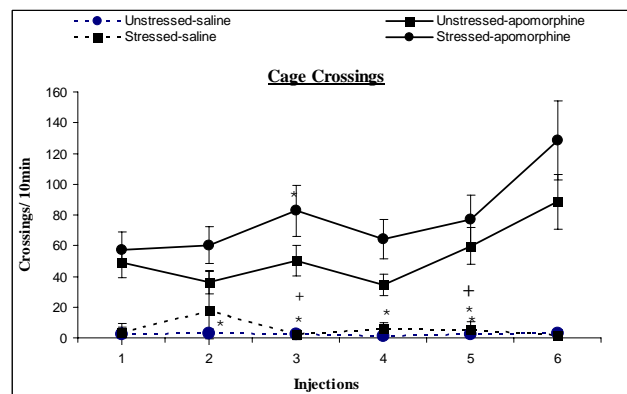


Fig. 2: Effects of apomorphine administration on cage crossings (familiar environment) of rats previously exposed to unpredictable chronic mild stress. Values are means \pm SD (n=6). Significant differences by Newman-Keuls test: * $p < 0.01$ from similarly treated rats and + $p < 0.01$ from similarly injected animals following two-way ANOVA.

Fig. 3 shows effects of apomorphine administration on conditioned place preference (entries in light compartment) of rats previously exposed to unpredictable chronic mild stress. Analysis of data on conditioned place preference (entries in light compartment) by two-way ANOVA showed that effects of stress ($F=33.98$; $df=1,40$, $p < 0.01$), apomorphine ($F=34.04$; $df=1,40$, $p < 0.01$) and Interaction between apomorphine and stress ($F=35.25$; $df=1,40$, $p < 0.01$) were significant. Post hoc analysis by Newman-Keuls test showed that apomorphine increases entries in the light compartment of light dark activity box in stressed animals as compared to unstressed-apomorphine injected animals. Group of animals, which are previously exposed to chronic mild stress showed significant differences among the groups. Saline injected stressed animals showed decreased entries in light compartment of light dark activity box as compare to unstressed saline injected animals before the treatment ($p < 0.01$) and similarly these animals also showed decreased activity after the treatment ($p < 0.01$). Apomorphine injected stressed animals showed increased entries in light compartment of light dark activity box after the treatment of apomorphine ($p < 0.01$), as compare to unstressed apomorphine injected animals ($p < 0.01$) and as compare to saline injected stressed animals ($p < 0.01$). Apomorphine injected stressed animals before the treatment exhibit decreased activity as compare to apomorphine injected unstressed animals ($p < 0.01$) and as compare to saline injected stressed animals before treatment ($p < 0.01$).

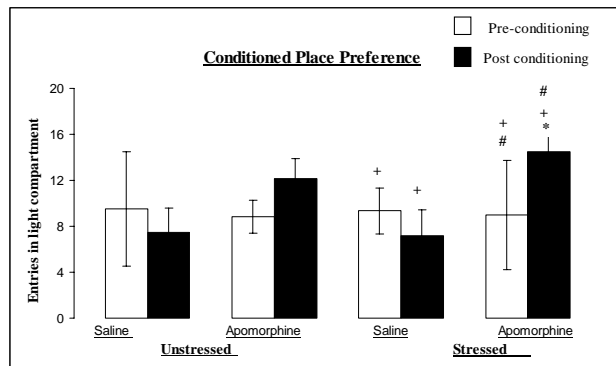


Fig. 3: Effects of apomorphine administration on conditioned place preference (entries in light compartment) of rats previously exposed to chronic mild stress. Values are means \pm SD (n=6). Significant differences by Newman-Keuls test: +p<0.01 from untreated (before treatment); *p<0.01 from similarly injected animals following two-way ANOVA.

Fig. 4 shows effects of apomorphine administration on conditioned place preference (time spent in light compartment) of rats previously exposed to unpredictable chronic mild stress. Analysis of data on conditioned place preference (time spent in light compartment) by two-way ANOVA showed that effects of stress ($F=31.74$; $df=1,40$, $p<0.01$), apomorphine ($F=32.12$; $df=1,40$, $p<0.01$) and Interaction between apomorphine and stress ($F=34.11$; $df=1,40$, $p<0.01$) were significant. Post hoc analysis by Newman-Keuls test showed that apomorphine increases time spent in the light compartment of light dark activity box in stressed animals as compared to unstressed-apomorphine injected animals. Apomorphine injected unstressed animals spent more time in light compartment of light dark activity box after the treatment ($p<0.05$) and as compare to saline injected unstressed animals after treatment ($p<0.01$). Similarly, animals of the same group before treatment spent less time in light compartment as compare to unstressed saline injected animals before treatment ($p<0.05$). Saline injected stressed animals showed decreased activity after treatment as compare to unstressed saline injected animals after treatment ($p<0.01$). Whereas apomorphine injected stressed animals before treatment showed decreased activity as compare to saline injected stressed animals before treatment ($p<0.01$). Similarly, apomorphine injected stressed animals spent more time in light compartment of light dark activity box after the treatment ($p<0.01$) and as compare to unstressed apomorphine injected animals after treatment ($p<0.05$) and saline injected unstressed animals ($p<0.01$).

DISCUSSION

The present findings demonstrate that apomorphine induces CPP and sensitization in rats whereas the combination of unpredictable chronic mild stress and apomorphine potentiates the same effect. This potentiated

effect indicates that apomorphine produces more rewarding and reinforcing effects when rats are previously exposed to unpredictable chronic mild stress.

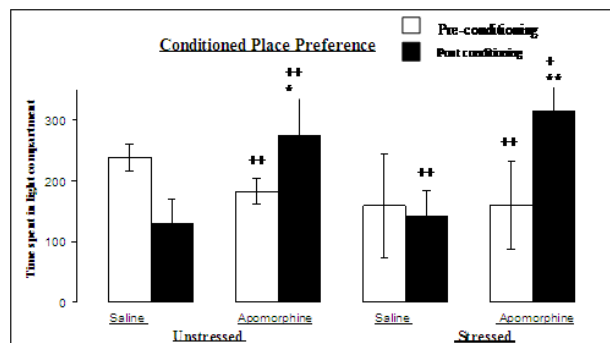


Fig. 4: Effects of apomorphine administration on conditioned place preference (time spent in light compartment) of rats previously exposed to chronic mild stress. Values are means + SD (n=6). Significant differences by Newman-Keuls test: +p<0.05 from similarly treated saline injected controls; *p<0.05, **p<0.01 from similarly treated and +p<0.05, ++p<0.01 from similarly injected animals following two-way ANOVA.

The reinforcing effects of apomorphine monitored in a CPP paradigm in rats has been reported previously (Haleem *et al.*, 2005; Ikram *et al.*, 2012). Animal studies have found that all drugs mediate their rewarding effects through a direct or indirect increase in dopamine neurotransmission (Grace, 2000; Koob., 1992). Results of the present study revealed that apomorphine (1 mg/kg) has rewarding and reinforcing effects because it produces place preference in test animals as the number of entries in light compartment as well as time spent in the light compartment of CPP apparatus is increased (fig 3 & 4).

It has been reported that all stimulatory drugs induces hyperactivity (Aizenstein *et al.*, 1995). Apomorphine (1 mg/kg) increases motor activity in rats (fig 2), which is associated with an increase in the dopaminergic neuronal firing in different regions of brain as Apomorphine is a D2 receptor agonist (Ikram *et al.*, 2011). This increased motor activity of apomorphine injected rats showed behavioral sensitization which is induced by the repeated administration of apomorphine and the effect is more in animals which are previously exposed to unpredictable chronic mild stress.

In previous studies it has been concluded that chronic exposure to stress of a certain severity induces anorexia and reduces overall body weight of the rats. More intense the stressor and the longer the duration, the greater reduction in food intake and body weight in the rats (Marti *et al.*, 1994). Results of the present study also revealed that food intake and growth rate of rats were reduced, when they were exposed to unpredictable chronic mild stress (fig 1 a & b).

It has been reported in previous studies that exposure to chronic mild stress has a significant impact on drug addiction. The preclinical literature which were reviewed by Goeders in 2003, suggests that reward associated with psychomotor stimulants is increased by stress, possibly through a process which is similar to sensitization and a clinical literature indicates a link between substance abuse and stress (Goeders, 2003). From results of the present study it has been suggested that the I.P. administration of apomorphine (1mg/kg) produces rewarding and reinforcing effects (which were observed by a CPP test paradigm) in rats, and this effect was observed more in those rats, which were previously exposed to unpredictable chronic mild stress.

CONCLUSION

Apomorphine (1mg/kg) produces a significant place preference and sensitization in rats and the same effect is greater in rats, which were previously exposed to unpredictable chronic mild stress for 10 days (animal model of depression). In conclusion, present study provides evidence that stress precipitates and potentiates the reinforcing and rewarding effects of apomorphine.

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