

Apomorphine-induced sensitization in rats exposed to restraint stress: Relationship with adaptation to stress

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Abstract: Drug abuse and impaired adaptation to stress are inter-related. Drug abuse is more potentiated upon exposure to stress and an impairment to cope with stress may lead to depression. On the other hand, use of addictive compounds increase the vulnerability to depression by inhibiting the adaptation to stress. Present study investigates relationship between behavioral tolerance to repeated restraint stress and apomorphine-induced sensitization. Apomorphine was injected either before or after the restraint stress episode, to monitor drug-induced behavioral sensitization and place preference. Apomorphine-induced sensitization and place preference were enhanced if the drug is experiencing during restraint stress. Conversely, apomorphine-induced sensitization and place preference were attenuated if the drug is experiencing after restraint stress. It shows that apomorphine, if experienced during restraint stress, produces greater sensitization. Conversely, sensitization effects of apomorphine are blocked in animals receiving apomorphine after the termination of restraint stress. The results tend to show that drug of abuse may be effective for the treatment but not prevention of stress-induced depression.

Keywords: Apomorphine, behavioral sensitization, stress, open field, conditioned place preference.

INTRODUCTION

Stress is a pre-disposing and precipitating factor in the pathophysiology of addiction (Niemtzow, 2018) and depression (Struijs *et al.*, 2018). An inability of certain individual's capability to cope with stress, results in depression (Holubova *et al.*, 2018). An important role of 5-HT (5-Hydroxy Tryptamine; serotonin)_{1A} receptors is there in the pathophysiology of stress and depression (Albert, 2012). 5-HT_{1A} receptors are reported to be supersensitized in patients with major depressive disorders (Kaufman *et al.*, 2016). It has also been suggested that this supersensitivity of 5-HT_{1A} receptors may result in hypofunctioning of serotonergic neurons and is consistent with indoleamine hypothesis proposed for major depression (Clevenger *et al.*, 2018). Studies in animal models of depression have shown that modulation of serotonergic neurons may also play a very important role in adaptation to stress (Badawy, 2018) and a deficiency of 5-HT (mainly in the hippocampus) may impair adaptation to stress (Mineur *et al.*, 2015).

Apart from stress and depression, 5-HT also plays an important role in the pathophysiology of addiction (Kirby *et al.*, 2011) and modulate the addictive effects of drugs of abuse. 5-HT is involved in modulating different aspects of impulsivity. Abnormal levels of impulsivity have been shown to be a risk factor for compulsive drug use, as well as contributing to relapse following withdrawal. Therefore, effective pharmacological manipulation of the

serotonergic system may contribute to successful recovery from the repeating cycle of addiction by alleviating some of the neurochemical abnormalities associated with drugs of abuse, which may in turn affect the quantitative levels of impulsivity (Kuypers *et al.*, 2018). Therefore, we could suggest that a decrease in 5-HT levels may potentiate reinforcing effects of psychostimulants too. Therefore, drugs that may increase the release of serotonin may be the best medications for the pharmacotherapy of addiction (Rothman *et al.*, 2008).

Prevalence of depression is more in addicts. Whether drug addiction precipitates depression or depression predisposes an individual to addiction, is still a matter of current debate (DeVido and Weiss, 2012). Since a common feature of 5-HT_{1A} receptor supersensitivity is there in the pathophysiology of both addiction and depression, this might be the underlying common feature in these disorders. The present study was therefore designed to monitor the neurochemical and behavioral effects of apomorphine when experienced during and after restraint stress. Apomorphine, when experienced during or after stress, may affect the development of adaptation to restraint stress by altering the availability of 5-HT.

MATERIALS AND METHODS

Study was carried out on locally bred male Albino Wistar rats (150-250g) purchased from HEJ Research Institute of Chemistry. Rats were kept individually in specially designed cages in a quiet room with free access to water

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and cubes of standard rat food for at least 1 week before starting the experiment so that rats adopt the environment.

Drugs and doses

Apomorphine-HCl purchased from Sigma (St. Louis, USA) was dissolved in saline (0.9% NaCl) and injected intraperitoneally at a dose of 1.0 mg/kg to the rats. Drug was freshly prepared before starting the experiment. Controls were injected with saline (0.9% NaCl) in volumes of 1ml/kg.

Experimental protocol

Experiment No. 1: Apomorphine experienced during restraint stress

Twenty four male rats were randomly divided into four groups, each containing 6 rats each: (i) saline-unrestraint (ii) saline-restraint (iii) apomorphine- unrestraint and (iv) apomorphine-restraint rats. rats were injected with saline (1ml/kg) or apomorphine (1mg/kg) respectively. 1hr post injection, rats of restraint groups were restrained (in restraining tubes) for 1hr, while rats of unrestraint groups were kept in their home cages. Rats were exposed to daily restraint stress sessions of 1hr each from day 1-12. Apomorphine was injected on alternate days (day 2, 4, 6, 8, 10, and 12) for a period of 12 days and rats were sequestered in 'apomorphine-paired compartment' of CPP apparatus for 20min. On days 1, 3, 5, 7, 9 and 11, rats were sequestered in 'saline-paired compartment' of CPP apparatus for 20min. Place preference was monitored before (basal values) and after (conditioned place preference) repeated apomorphine administration. Motor activities in familiar environment were monitored daily. While activities in novel environment of an open field were monitored post first and sixth apomorphine injection.

Experiment No. 2: Apomorphine experienced after restraint stress

Twenty four male rats were randomly divided into four groups, each containing 6 rats each: (i) unrestraint-saline, (ii) unrestraint-apomorphine, (iii) restraint-saline, and (iv) restraint-apomorphine injected rats. Rats of restraint groups were restrained (in restraining tubes) for 1hr, while rats of unrestraint groups were kept in their home cages. 1hr post restraint stress, rats were injected with saline (1ml/kg) or apomorphine (1mg/kg) respectively. Rats were exposed to daily restraint stress sessions of 1hr each from day 1-12. Apomorphine was injected on alternate days (day 2, 4, 6, 8, 10, and 12) for a period of 12 days and rats were sequestered in 'apomorphine-paired compartment' of CPP apparatus for 20min. On days 1, 3, 5, 7, 9 and 11, rats were sequestered in 'saline-paired compartment' of CPP apparatus for 20min. Place preference was monitored before (basal values) and after (conditioned place preference) repeated apomorphine administration. Motor activities in familiar environment were monitored daily. While activities in novel

environment of an open field were monitored post first and sixth apomorphine injection.

Restraining procedure

Restraint stress was produced by placing rats in adjustable (8" long and 2" diameter) plexiglas tubes with air holes in the front, top and back. Restraining was achieved by allowing the rats to enter into tubes and pressing their bodies gently to let them fit into the tube. Tail was passed through the lid hole at the back and that adjustable lid was fixed to prevent any movement of rat. Method was essentially same as described elsewhere (Weinberg *et al.*, 2007).

Behavioral assessment

Monitoring motor activities in familiar environment

Video recording was used to determine motor activities in familiar environment during drug conditioning phase. Rats confined to a compartment were moving across the compartment. Activity scores were counted as number of compartment crossings for 10 min starting 5 min post-injection. The small area (26×26×26 cm³) of the compartment enabled giving a score of 1 for one crossing. Method was same as described earlier (Ikram and Haleem, 2017).

Monitoring motor activities in novel environment

Open field consists of a square area (76×76 cm) with walls 42 cm high. Floor of the apparatus is divided by lines into 25 squares of equal size. The method is essentially similar as described elsewhere (Ikram and Haleem, 2017). Experiment was conducted in a quiet room under white light. An animal was taken out from home cage and placed in the centre of open field apparatus. Numbers of squares crossed with all four paws and latency to move from the central square were scored for a period of 5 minutes.

Conditioned place preference

Conditioned place preference (CPP) was conducted in a three-compartment apparatus with an unbiased design. The compartments were separated by sliding guillotine doors. The middle (shuttle) compartment (10×26×26 cm³) had a smooth floor. The end (preference) compartments (26×26×26 cm³ each) provided distinct contexts, with one compartment having black horizontal stripes on side walls and grid rod floor. The other compartment had vertical stripes and stainless steel mesh floor.

Phase I: Pre-conditioning place preference

Rats were tested for CPP using a 14-day procedure. On day 1 all rats were tested, before any treatment, to establish pre-conditioning responses and any possible bias for either compartment. Pre-conditioning place preference testing involved placing individual rats in the central shuttle compartment; after 10 s the guillotine doors were

removed and animal allowed exploring all three compartments for a time period of 10 min. The time spent in end (preference) compartments were recorded. The rats exhibited no preference for either compartment.

Phase II: Drug conditioning

In 12 days rats went through conditioning (one session per day) in which they were confined to either the horizontal or vertical strip compartment by raising the respective guillotine door. On days 2, 4, 6, 8, 10 and 12 rats were injected with saline (1 ml/kg at 9:00-11:00 h) and placed immediately in the assigned 'Non-Drug' compartment for 30 min. On every other day, i.e. days 3, 5, 7, 9, 11 and 13 rats of each group were injected (as assigned for group) with drug or saline and placed immediately in the 'Drug' compartment for 30 min.

Phase III: Post-conditioning place preference

The post-conditioning testing was carried out on day 14. As in the pre-conditioning phase, both guillotine doors were raised and the rats were allowed free access to all compartments for 10 min and time spent in 'Non-Drug' and 'Drug' assigned compartment was monitored to determine place preference.

Ethical approval

All experimental protocol and experimental procedures were approved by Institutional Ethical Approval Committee (IEAC).

STATISTICAL ANALYSIS

Results are represented as means \pm S.D. Statistical analysis was performed by three-way ANOVA (SPSS ver 17.0). Post hoc comparison of groups was performed by Tukey's test. Values of $p < 0.05$ were considered significant.

RESULTS

Fig. 1 shows motor activities in a familiar environment for apomorphine experienced during restraint stress (fig. 1a) and apomorphine experienced after restraint stress (fig. 1b). Data on apomorphine experienced during stress (fig. 1a) as analyzed by three-way ANOVA (repeated measure design) showed significant effects of apomorphine ($df = 1,120$; $F = 32.45$; $p = 0.0001$), restraint stress ($df = 1,120$; $F = 64.90$; $p = 0.0001$) and repeated monitoring ($df = 5,120$; $F = 18.56$; $p = 0.0001$). Interactions of apomorphine*restraint stress ($df = 1,120$; $F = 72.15$; $p = 0.0001$), apomorphine*repeated monitoring ($df = 5,120$; $F = 68.13$; $p = 0.0001$), restraint stress*repeated monitoring ($df = 5,120$; $F = 85.23$; $p = 0.0001$) and apomorphine*restraint stress*repeated monitoring ($df = 5,120$; $F = 64.23$; $p = 0.0001$) were all significant. Post hoc analysis by Tukey's test showed increased ($p < 0.01$) activities of apomorphine-unrestraint- as well as

apomorphine-restraint rats as compared to their respective saline injected controls, post 4th injection till 6th injection. Activities of apomorphine-restraint rats were significantly increased ($p < 0.01$) as compared to their respective unrestraint controls as well.

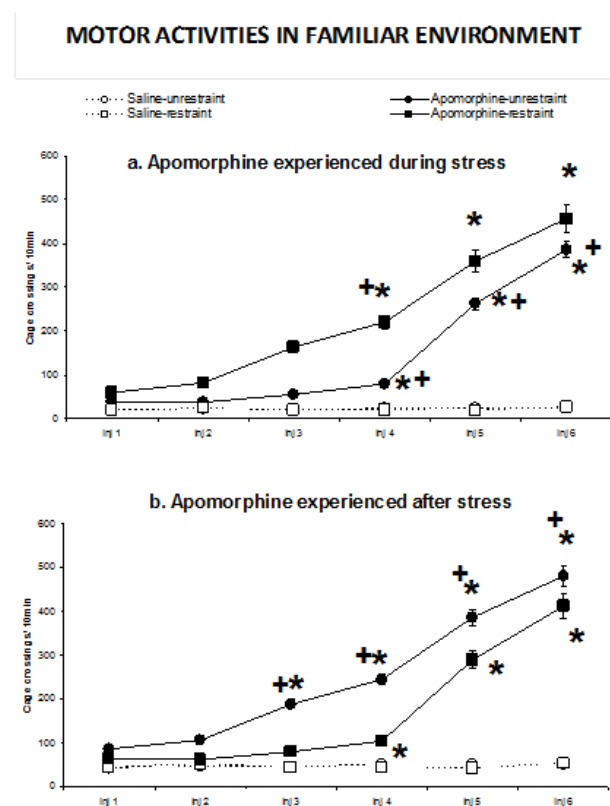


Fig. 1: Effects of restraint-stress, apomorphine and their interaction on motor activities in familiar environment. Values are means \pm SD ($n = 6$). Significant differences by Tukey's test: * $p < 0.01$ as compared to respective saline injected controls; + $p < 0.01$ as compared to respective unrestraint rats, following three-way ANOVA (repeated measure design).

Data on apomorphine experienced after restraint stress (fig. 1b) as analyzed by three-way ANOVA (repeated measure design) showed significant effects of apomorphine ($df = 1,120$; $F = 61.02$; $p = 0.0001$), restraint stress ($df = 1,120$; $F = 57.26$; $p = 0.0001$) and repeated monitoring ($df = 5,120$; $F = 64.14$; $p = 0.0001$). Interactions of apomorphine*restraint stress ($df = 1,120$; $F = 68.12$; $p = 0.0001$), apomorphine*repeated monitoring ($df = 5,120$; $F = 54.23$; $p = 0.0001$), restraint stress*repeated monitoring ($df = 5,120$; $F = 16.13$; $p = 0.0001$) and apomorphine*restraint stress*repeated monitoring ($df = 5,120$; $F = 23.16$; $p = 0.0001$) were all significant. Post hoc analysis by Tukey's test showed increased ($p < 0.01$) activities of unrestraint-apomorphine- as well as restraint-apomorphine injected rats as compared to their respective saline injected controls, post 3rd injection till 6th injection. Activities of restraint-apomorphine injected rats

were significantly decreased ($p < 0.01$) as compared to their respective unrestraint controls as well.

Fig. 2 shows motor activities in a novel environment for apomorphine experienced during restraint stress (fig. 2a) and apomorphine experienced after restraint stress (fig. 2b). Data on apomorphine experienced during stress (fig. 2a) as analyzed by three-way ANOVA showed significant effects of apomorphine ($df = 1,24$; $F = 23.56$; $p = 0.0001$), restraint stress ($df = 1,24$; $F = 39.18$; $p = 0.0001$) and repeated monitoring ($df = 1,24$; $F = 49.25$; $p = 0.0001$). Interactions of apomorphine*restraint stress ($df = 1,24$; $F = 56.25$; $p = 0.0001$), apomorphine*repeated monitoring ($df = 1,24$; $F = 41.09$; $p = 0.0001$), restraint stress*repeated monitoring ($df = 1,24$; $F = 36.46$; $p = 0.0001$) and apomorphine*restraint stress*repeated monitoring ($df = 1,24$; $F = 32.46$; $p = 0.0001$) were all significant. Post hoc analysis by Tukey's test showed decreased ($p < 0.01$) activities of saline-restraint (post 1st injection) and apomorphine-restraint (post 1st as well as post 6th injection) rats as compared to their respective unrestraint controls. Activities of saline-restraint- and apomorphine-unrestraint rats were significantly increased ($p < 0.01$) post 6th injection as compared to their respective post 1st injection values.

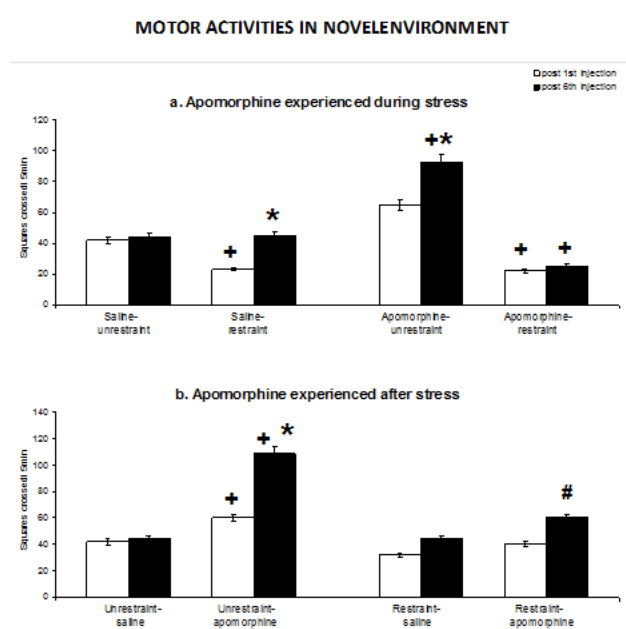


Fig. 2: Effects of restraint-stress, apomorphine and their interaction on motor activities in novel environment. Values are means \pm SD ($n = 6$). Significant differences by Tukey's test: * $p < 0.01$ as compared to post 1st injection values; + $p < 0.01$ as compared to respective unrestraint controls, following three-way ANOVA.

Data on apomorphine experienced after stress (fig. 2b) as analyzed by three-way ANOVA showed significant effects of apomorphine ($df = 1,24$; $F = 61.58$; $p = 0.0001$), restraint stress ($df = 1,24$; $F = 29.46$; $p = 0.0001$) and

repeated monitoring ($df = 1,24$; $F = 32.51$; $p = 0.0001$). Interactions of apomorphine*restraint stress ($df = 1,24$; $F = 48.32$; $p = 0.0001$), apomorphine*repeated monitoring ($df = 1,24$; $F = 34.03$; $p = 0.0001$), restraint stress*repeated monitoring ($df = 1,24$; $F = 25.12$; $p = 0.0001$) and apomorphine*restraint stress*repeated monitoring ($df = 1,24$; $F = 64.76$; $p = 0.0001$) were all significant. Post hoc analysis by Tukey's test showed increased ($p < 0.01$) activities of unrestraint-apomorphine (post 1st injection) injected rats as compared to their respective saline injected controls. Activities of unrestraint-apomorphine (post 6th injection) were increased ($p < 0.01$) as compared to respective saline injected as well as post 1st injection values. Restraint-apomorphine injected rats showed significantly decreased ($p < 0.01$) activities post 6th injection as compared to their respective unrestraint controls.

Fig. 3 shows conditioned place preference for apomorphine experienced during restraint stress (fig 3a) and apomorphine experienced after restraint stress (fig 3b). Data on basal values for apomorphine experienced during stress (fig. 2a) as analyzed by three-way ANOVA showed non-significant effects of apomorphine ($df = 1,24$; $F = 0.64$; $p = 1.35$), restraint stress ($df = 1,24$; $F = 6.26$; $p = 0.92$) and repeated monitoring ($df = 1,24$; $F = 1.46$; $p = 0.92$). Interactions of apomorphine*restraint stress ($df = 1,24$; $F = 9.25$; $p = 0.28$), apomorphine*repeated monitoring ($df = 1,24$; $F = 5.48$; $p = 0.56$), restraint stress*repeated monitoring ($df = 1,24$; $F = 1.67$; $p = 0.09$) and apomorphine*restraint stress*repeated monitoring ($df = 1,24$; $F = 2.33$; $p = 0.08$) were all non-significant. Post hoc analysis by Tukey's test showed no significant difference among groups. While data on conditioned place preference for apomorphine experienced during stress (fig 2a) as analyzed by three-way ANOVA showed significant effects of apomorphine ($df = 1,24$; $F = 46.12$; $p = 0.0001$), restraint stress ($df = 1,24$; $F = 16.98$; $p = 0.0001$) and repeated monitoring ($df = 1,24$; $F = 29.41$; $p = 0.0001$). Interactions of apomorphine*restraint stress ($df = 1,24$; $F = 25.46$; $p = 0.0001$), apomorphine*repeated monitoring ($df = 1,24$; $F = 65.12$; $p = 0.0001$), restraint stress*repeated monitoring ($df = 1,24$; $F = 25.19$; $p = 0.0001$) and apomorphine*restraint stress*repeated monitoring ($df = 1,24$; $F = 15.81$; $p = 0.0001$) were all significant. Post hoc analysis by Tukey's test showed increased ($p < 0.01$) time spent in apomorphine-paired compartment by apomorphine-unrestraint rats as compared to their respective time spent in saline-paired compartment. Time spent in saline-paired compartment by apomorphine-restraint rats (post 1st injection) were decreased ($p < 0.01$) as compared to the same in respective apomorphine-unrestraint rats. While in apomorphine-restraint rats, entries in apomorphine-paired compartment were increased ($p < 0.01$) as compared to their respective unrestraint- rats , saline- injected rats as well as time spent in saline-paired compartment values.

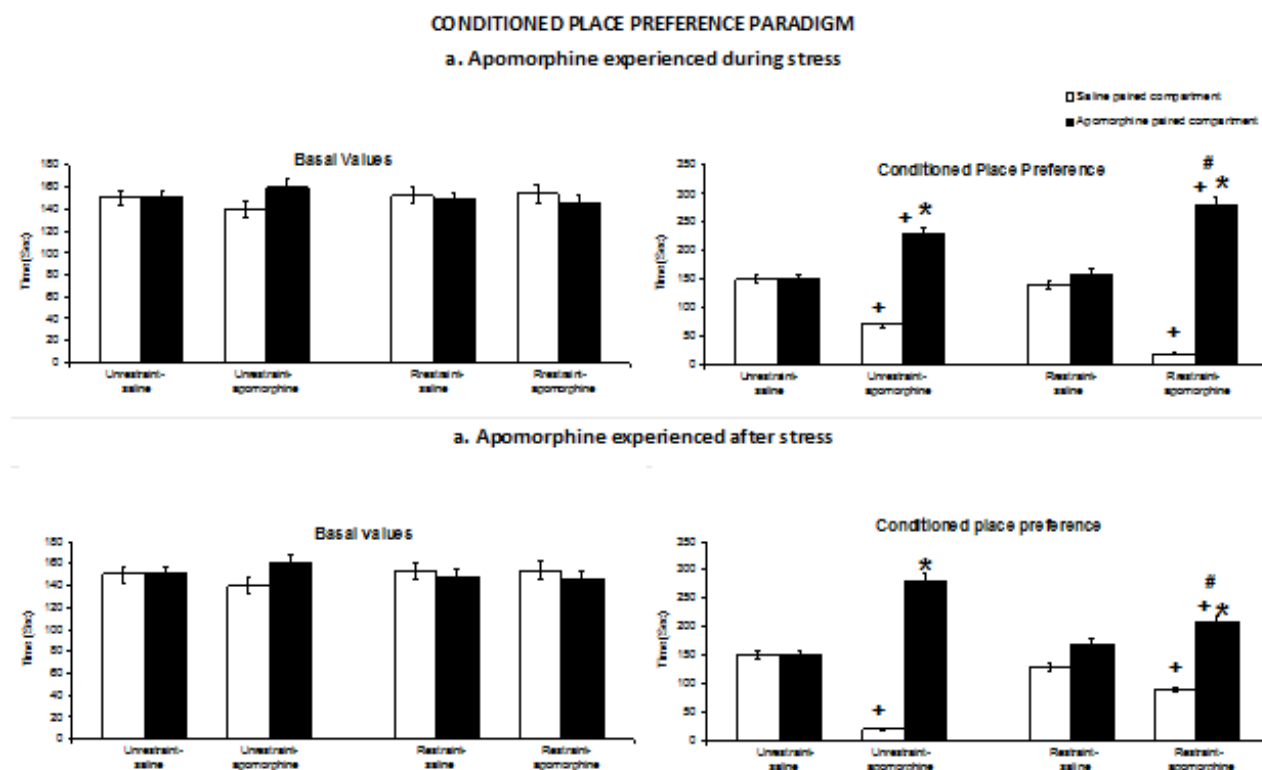


Fig. 3: Effects of restraint-stress, apomorphine and their interaction on motor activities on conditioned place preference. Values are means \pm SD (n=6). Significant differences by Tukey's test: * $p < 0.01$ as compared to respective values of saline paired compartment; + $p < 0.01$ as compared to respective saline injected controls; # $p < 0.01$ as compared to respective unrestraint controls, following three-way ANOVA.

Data on basal values for apomorphine experienced after stress (fig 2a) as analyzed by three-way ANOVA showed non-significant effects of apomorphine (df= 1,24; F= 1.98; $p = 0.64$), restraint stress (df= 1,24; F=21.64; $p = 0.09$) and repeated monitoring (df= 1,24; F= 4.56; $p = 1.48$). Interactions of apomorphine*restraint stress (df= 1,24; F= 9.25; $p = 0.28$), apomorphine*repeated monitoring (df= 1,24; F= 0.48; $p = 0.62$), restraint stress*repeated monitoring (df= 1,24; F= 0.67; $p = 0.28$) and apomorphine*restraint stress*repeated monitoring (df= 1,24; F= 1.53; $p = 0.54$) were all non-significant. Post hoc analysis by Tukey's test showed no significant difference among groups. While data on conditioned place preference for apomorphine experienced after stress (fig. 2a) as analyzed by three-way ANOVA showed significant effects of apomorphine (df= 1,24; F= 54.19; $p = 0.0001$), restraint stress (df= 1,24; F= 11.56; $p = 0.0001$) and repeated monitoring (df= 1,24; F= 14.28; $p = 0.0001$). Interactions of apomorphine*restraint stress (df= 1,24; F= 41.25; $p = 0.0001$), apomorphine*repeated monitoring (df= 1,24; F= 12.54; $p = 0.0001$), restraint stress*repeated monitoring (df= 1,24; F= 19.58; $p = 0.0001$) and apomorphine*restraint stress*repeated monitoring (df= 1,24; F= 18.45; $p = 0.0001$) were all significant. Post hoc analysis by Tukey's test showed increased ($p < 0.01$) time spent in apomorphine-paired

compartment by unrestraint-apomorphine injected rats as compared to respective time spent in saline-paired compartment. Time spent in saline-paired compartment by unrestraint-apomorphine- as well as restraint-apomorphine injected rats (post 1st injection) were decreased ($p < 0.01$) as compared to respective time spent by saline injected rats in saline paired compartment. While in restraint-apomorphine injected rats, time spent in apomorphine-paired compartment was increased ($p < 0.01$) as compared to their respective saline injected rats but was decreased ($p < 0.01$) as compared to respective unrestraint rats.

DISCUSSION

Results from the present study show that if experienced during restraint stress, apomorphine-induced sensitization was greater in rats exposed to restraint stress. Also, the behavioral deficits produced by the first episode of restraint stress, were not adapted upon repeated exposure to restraint stress, in apomorphine injected rats. Since apomorphine was experienced during restraint stress, we suggest that a decrease in serotonergic function as induced by apomorphine injection (Ikram *et al.*, 2018) would have been potential enough to hinder the desensitization of somatodendritic 5-HT_{1A} receptors or

increase in serotonergic function which is an important contributing factor for the development of adaptation to repeated restraint stress (Assié *et al.*, 2006).

Conversely, when apomorphine was administered after the termination of restraint stress period, i.e., experienced during stress, behavioral sensitization to apomorphine did not develop. Moreover, adaptation to repeated restraint stress was more “potentiated” in apomorphine injected rats. The common mechanism underlying adaptation and attenuation of addiction seems to be desensitization of somatodendritic 5-HT_{1A} receptors. Since desensitization of somatodendritic 5-HT_{1A} receptors may help in attenuation of apomorphine-induced sensitization (Ikram and Haleem, 2018), they very same could also be attenuated by adaptation to stress.

Stress alone, decreased activity of saline injected rats (after first episode of restraint stress) in novel environment. Lipatova *et al* (2018) have also reported reduced ambulation and rearing in open field in restrained juvenile males and female rats. This increase in activities was normalized after fifth restraint stress suggesting adaptation to it (Seibenhener and Wooten, 2015). Apomorphine increased activities in unrestrained rats, when monitored on day 6 suggesting expression of sensitization. When experienced during restraint stress, apomorphine-induced behavioral sensitization ‘established’ in a familiar environment, was also expressed novel environment. While when experienced after restraint stress, activities of restrained-apomorphine injected rats were greater than restrained-saline injected rats after first restraint stress. However, repeated exposure to restraint stress failed to attenuate apomorphine-induced hyperactivity in a novel environment.

Imperato *et al* (1992) have reported that single restraint stress (for 60 min) increases the release of dopamine in nucleus accumbens as determined by micro dialysis. While exposure to repeat restraint stress (daily 60 min, for 6 days) produced no significant changes. Kim *et al* (2008) have reported a decrease in the caudate volume in women with major depressive disorders may well be related to the pathophysiology. A down-regulation of vesicular monoamine transporter 2 is also reported by Zucker *et al* (2005). They also have suggested that down-regulation of this transporter in the dopaminergic regions is important for mediating the effects of chronic stress. Exposure to chronic stress induces variations in brain morphology including a decrease in volumes of hippocampal grey matter (Gianaros *et al.*, 2007). This decreased dopamine metabolism may well be co-related with adaptation to repeated restraint stress.

CONCLUSION

In conclusion, the present results suggest that apomorphine and also possibly the other CNS stimulants

may help to cope stress by attenuating stress-induced behavioral deficits. The finding that apomorphine-induced sensitization is smaller in rats exposed to repeated restraint stress supports previous findings from our laboratory that addictive drugs increase the sensitivity of somatodendritic 5-HT_{1A} receptors (Haleem *et al*, 2002) to decrease the availability of 5-HT in the terminal region, while adaptation to stress involves a decrease in the sensitivity of somatodendritic 5-HT_{1A} receptors (Haleem *et al.*, 2007) resulting in an increase in availability of 5-HT in the terminal region. It may be relevant that apomorphine-induced sensitization (chapter 4) is due to an increase in the sensitivity of somatodendritic 5-HT_{1A} receptors that tend to release dopaminergic neurons from the inhibitory influence of 5-HT. While adaptation to stress opposes this effect of apomorphine to inhibit drug-induced sensitization. The finding that apomorphine if experienced during stress potentiates behavioral sensitization and if experienced after stress could attenuate behavioral sensitization, tend to show that drug of abuse may be effective for the treatment but not prevention of stress-induced depression.

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