Regional neurochemical profile following development of apomorphine-induced reinforcement

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Abstract: Dopamine is primary neurotransmitter which mediates the reinforcing effects of abused drugs, serotonin (5-Hydroxytryptamine; 5-HT) also has a crucial role in the pathophysiology of addiction. The binding sites of various drugs of abuse are different from each other, their final rewarding effects are mediated by an increase in the dopamine level in the Nucleus Accumbens. The present study used conditioned place preference (CPP) test to monitor apomorphine's reinforcing effects. Associated alterations in 5-HT and dopamine metabolism were also monitored in various brain regions by HPLC-EC. Withdrawal from apomorphine administration (at a dose of 1.0 mg/kg on six alternate days) induced reinforcement as monitored in the conditioned place preference (CPP) paradigm. Serotonin and dopamine metabolism was also changed particularly in the ventral and dorsal striatum. Results therefore suggest desensitization of dopamine receptors in the presynaptic site is involved in apomorphine-induced reinforcement. Desensitization of somatodendritic 5-HT_{1A} receptors resulting in increased availability of 5-HT at 5-HT_{2C} receptors could attenuate apomorphine-induced reinforcement. Therefore, further investigations in this area should focus on attempts to attenuate apomorphine-induced reinforcement by desensitizing somatodendritic 5-HT_{1A} receptors.

Keywords: Apomorphine, dopamine, serotonin, conditioned place preference, addiction, reinforcement.

INTRODUCTION

Current research in addiction is focused on a better understanding of underlying physiological, cellular and molecular mechanisms (Koob *et al*., 2004; Bailey and Connor, 2005; Ammon-Treiber and Hollt, 2005). Kreek *et al*. (2002) has also demonstrated vulnerability to environmental, drug induced as well as genetic factors in the development of addiction. Early life experiences greatly influence later neurobehavioral development (Bateson *et al*., 2004). Stress could also be one of the precipitating factors for the induction of relapse in (Sinha *et al*., 2002).

Drugs of abuse have different initial mechanisms and targets but final reinforcing effect involves few features in common (Kreek *et al*., 2002). It is also being suggested that apart from dopamine, serotonin also plays a very important role in the pathophysiology of addiction. Experimental studies in mice have shown self-administration of cocaine in mice with DA transporter knocked out. Sora *et al*., (2001) has suggested an important contribution of 5-HT in manifesting the reinforcing effects of abused drugs. Sensitivity to ethanol was increased in 5-HT knockout mice. The behavioral and neural effects of ethanol are altered upon loss of 5-HT gene function and an increased sensitivity to ethanol induced sedation/hypnosis was observed in 5-HT knockout mice (Boyce-Rustay *et al*., 2006).

Drug dependence arises as a result of habitual drug use and sudden discontinuation of drug could result in symptoms of physical withdrawal. These withdrawal symptoms make drug quitting more difficult. For example, alcohol withdrawal symptoms include: mild tremors, anxiety, hyperreflexia, seizures and severe convulsions (Chung *et al*., 2008). Similarly, nicotine withdrawal results in lack of concentration, hunger sleep disturbance, irritability, impatience, anxiety, and depression (Morrell *et al*., 2008). The present study involves the investigation of metabolism of biogenic amines in various brain regions of experimental animals after establishment of reinforcement as induced by repeated administration of apomorphine.

MATERIALS AND METHODS

Animals

Experimental protocol was started after taking approval from the Institutional Animal Ethics Committee (IAEC). Albino-Wistar rats (180±20 g) purchased from HEJ Research Institute of Chemistry, University of Karachi were housed in transparent perspex cages with dimensions 26×26×26 cm (one rat per cage). Animals were placed in environmentally controlled room under a 12:12 h light/dark cycle (lights on at 6:00 hr). An acquisition phase of 7 days was allowed to the animals to let them get familiarized with the environment.

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Drug and doses

Drug (Apomorphine-HCl; Sigma, St. Louis, USA) was dissolved in saline (0.9% NaCl solution) and injected intra-peritoneally at the dose of 1.0 mg/ml/kg to respective animals. Buspirone (Sigma, St. Louis, USA) was prepared in saline (0.9% NaCl) and injected intra-peritoneally. Drug was freshly prepared each day before starting the experiment. Saline was injected to control animals at the dose of 1.0 ml/kg.

Experimental protocol

Twelve male Albino Wistar rats (180-220 g) were randomly assigned to two groups each containing six animals: (i) saline injected and (ii) apomorphine injected rats. The animals were injected with apomorphine on alternate days (i.e., day 2, 4, 6, 8, 10 and 12) and administration of apomorphine was paired with the sequestration in the light compartment of light dark activity box. Saline was injected on other set of alternate days (i.e., day 1, 3, 5, 7, 9 &11) and saline administration was paired with the sequestration in the dark compartment of light dark activity box. Entries and time spent in the light compartment of the light dark activity box, were monitored during a test session of 10 minutes on test day (i.e., day 13). Animals were decapitated on day 13 (12hr after last apomorphine injections).

Behavioral procedures

Phase I: Pre-conditioning place preference test

The test was conducted in locally made two compartment box. Both compartments were of equal size (26x26x26cm) with an access (12x12cm) between them. The two preference compartments differed in their sensory properties. Walls of one compartment were of transparent perspex (light compartment) and of other black (dark compartment).

Base line values of place preference (PP) for all animals were monitored a day before the start of the conditioning phase. Rats were placed in the shuttle compartment with doors of preference compartment kept open. Being nocturnal animals, they spent ~30% time in light compartment and ~70% in the dark one.

Phase II: Conditioning phase

In the conditioning phase access between the two compartments was closed. Less preferred (light) compartment (as observed in the preconditioning phase) was paired with the drug administration and more preferred (dark) compartment was paired with saline administration. Animals, after being exposed to 12 (once daily), place conditioning sessions of 30min each, were injected with saline on six alternating days (day 1, 3, 5, 7, 9 and 11). After injection each animal was sequestered in the dark compartment of CPP apparatus for a period of 30 minutes. Apomorphine was injected on days 2, 4, 6, 10 and 12. Immediately after the drug injections, animals were sequestered in the light compartment of CPP apparatus for 30 minutes. Animals were kept back in their home cages after conditioning sessions.

Phase III: Post-conditioning place preference test

On the test day (day 13) access between the compartments was opened. Animal to be tested was placed in the light compartment of the CPP apparatus and number of entries in the light compartment and time spent in the same were monitored during a test session of 10 minutes.

Decapitation of rat brain

Dissection procedure was essentially same as described elsewhere (Haleem *et al*., 2004). After decapitation, fresh brain was dipped in ice-cold saline and placed with its ventral side up in the molded cavity of brain slicer. Fine razor/blade was inserted between the slots to give brain slices containing particular brain regions (i.e., prefrontal cortex, dorsal striatum, ventral striatum, mid brain, hippocampus, hypothalamus and cortex) were isolated. The collected brain regions were stored at −70°C until analysis by HPLC-EC (High Performance Liquid Chromatography with Electrochemical detector).

HPLC-EC estimation of DA, 5-HT and metabolites

Biogenic amines and metabolites were extracted with perchloric acid ($HClO_4$; 70%) from brain tissue. 5 times volume of the extraction medium was added to the brain tissues. Samples were homogenized by using electrical homogenizer and subjected to ultracentrifugation at 6000 rpm for 20min at 4°C. The supernatant was transferred to eppendorf tubes and injected to HPLC-EC for neurochemical assay. HPLC-EC determination was carried out as described earlier (Ikram and Haleem, 2010; Ikram *et al*., 2007).

STATISTICAL ANALYSIS

Results are presented as means ± S.D. Statistical analysis was performed using Student's t-test and two-way ANOVA (analysis of variance). *Post hoc* comparisons among groups were done by the Newman-Keuls test. p values < 0.05 were considered significant.

RESULTS

Effects of apomorphine administration on conditioned place preference

Fig. 1 shows effects of repeated apomorphine (1.0 mg/kg) administration on entries and time spent in the light compartment of the light dark activity box. Analysis of the data on entries in the light compartment of the light dark activity box as analyzed by the two-way ANOVA (df=1,20) showed significant effect of repeated monitoring ($F=110.34$; $p<0.01$), apomorphine ($F=115.93$; $p<0.01$) and interaction between the two ($F=152.34$;

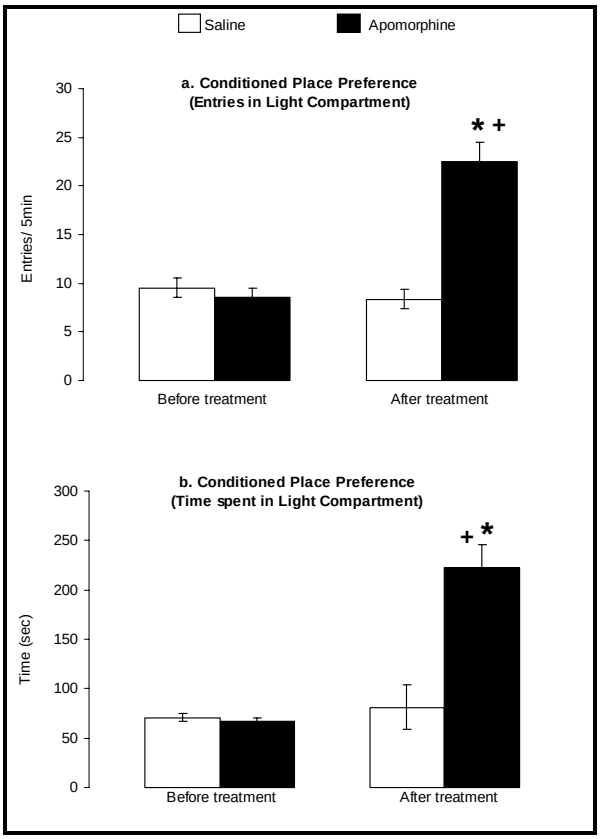

Fig. 1: Effects of repeated (12 days; alternate days) apomorphine (1.0 mg/kg) administration on (a) entries and (b) time spent in the light compartment of light dark activity box. Values are means ± SD before and after treatment (day 0 and day 13). Significant differences by Newman-Keuls test: *p<0.01 from respective saline injected rats; +p<0.01 from similarly injected rats before treatment following two-way ANOVA.

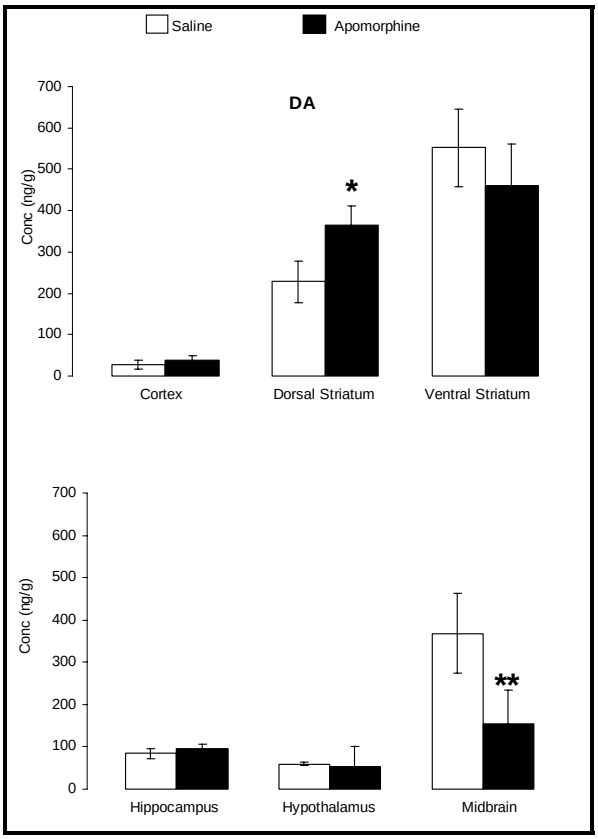

Fig. 2: Effects of repeated (12 days; alternate days) apomorphine (1.0 mg/kg) administration on DA levels in cortex, dorsal striatum, ventral striatum, hippocampus, hypothalamus and mid brain. Values are means ± SD 24hr after last injection (n=6). Significant differences: *p<0.05, **p<0.01 from respective saline injected rats following student's t-test.

p<0.01). Post hoc analysis by Newman-keuls test showed that repeated administration of apomorphine significantly increased (p<0.01) entries in the light compartment as compared to saline injected controls after treatment as well as same animals (before treatment).

Analysis of the data on time spent in the light compartment of the light dark activity box as analyzed by the two-way ANOVA (df= 1,20) showed significant effect of repeated monitoring (F= 91.97; p<0.01), apomorphine (F=87.70; p<0.01) and interaction between the two (F=96.55; p<0.01). Post hoc analysis by Newman-keuls test showed that repeated administration of apomorphine increased (p<0.01) time spent in the light compartment as compared to saline injected controls after treatment as well as same animals (before treatment).

Effects of apomorphine administration on biogenic amines and metabolites

Fig. 2 shows effects of repeated apomorphine (1.0 mg/kg) administration on levels of dopamine in cortex, dorsal striatum, ventral striatum, hippocampus, hypothalamus and mid brain. One-way ANOVA (df=1,60) showed significant (F=63.25; p<0.01) effects of treatment. Post hoc analysis by Newman-keuls test showed that repeated administration of apomorphine significantly increased (p<0.05) dopamine levels in the dorsal striatum. Decrease in the DA levels in the ventral striatum was not significant. In the mid brain samples levels of DA were found to be decreased (p<0.01) in repeated apomorphine injected animals as compared to saline injected controls.

Fig. 3 shows effects of repeated apomorphine (1.0 mg/kg) administration on levels of DOPAC in cortex, dorsal striatum, ventral striatum, hippocampus, hypothalamus and mid brain. One-way ANOVA (df=1,60) showed that the effects of treatment were significant (F=30.42; p<0.01). Post hoc analysis by Newman-keuls test showed that repeated administration of apomorphine significantly decreased (p<0.01) DOPAC levels in the ventral striatum. Whereas, DOPAC levels were decreased (p<0.01) in the hippocampus samples.

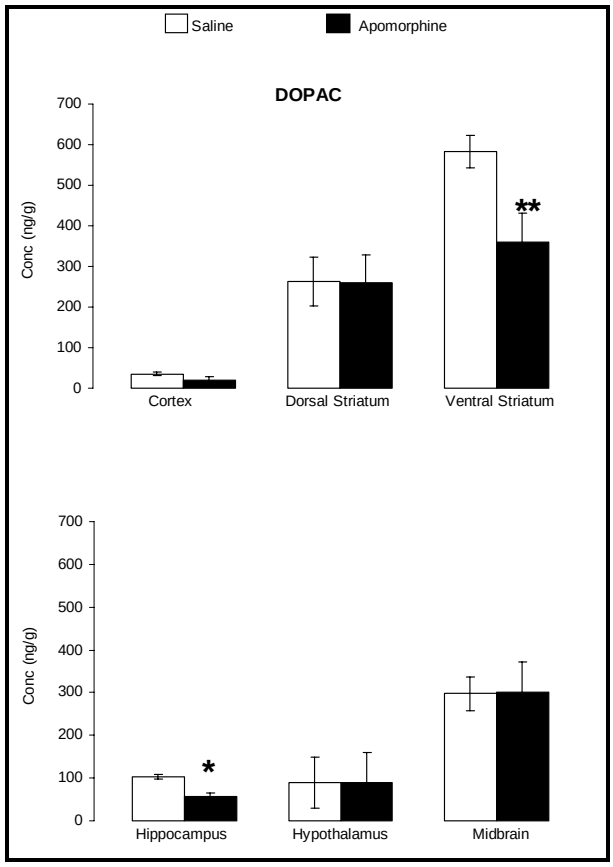

Fig. 3: Effects of repeated (12 days; alternate days) apomorphine (1.0 mg/kg) administration on DOPAC levels in cortex, dorsal striatum, ventral striatum, hippocampus, hypothalamus and mid brain. Values are means ± SD 24hr after last injection (n=6). Significant differences: *p<0.05, **p<0.01 from respective saline injected rats following student's t-test.

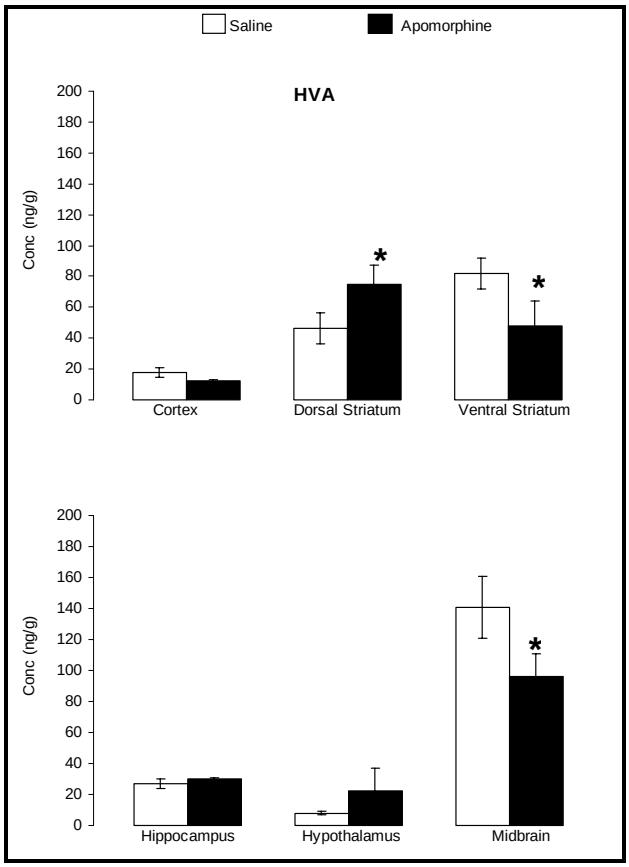

Fig. 4: Effects of repeated (12 days; alternate days) apomorphine (1.0 mg/kg) administration on HVA levels in cortex, dorsal striatum, ventral striatum, hippocampus, hypothalamus and mid brain. Values are means ± SD 24hr after last injection (n=6). Significant differences: *p<0.05 from respective saline injected rats following student's t-test.

Fig. 4 shows effects of repeated apomorphine (1.0 mg/kg) administration on levels of HVA in cortex, dorsal striatum, ventral striatum, hippocampus, hypothalamus and mid brain. One-way ANOVA (df=1,60) showed that the effects of treatment were significant (F=23.69; p<0.01). Post hoc analysis by Newman-keuls test showed that repeated administration of apomorphine significantly increased (p<0.05) HVA levels in the dorsal striatum samples. Whereas, HVA levels were found to be decreased (p<0.05) in the mid brain samples.

Fig. 5 shows effects of repeated apomorphine (1.0 mg/kg) administration on levels of 5-HT in cortex, dorsal striatum, ventral striatum, hippocampus, hypothalamus and mid brain. One-way ANOVA (df=1,60) showed that the effects of treatment were significant (F=52.39; p<0.01). Post hoc analysis by Newman-keuls test showed that repeated administration of apomorphine significantly increased (p<0.01) 5-HT levels in dorsal as well as ventral striatum.

Fig. 6 shows effects of repeated apomorphine (1.0 mg/kg) administration on levels of 5-HIAA in cortex, dorsal striatum, ventral striatum, hippocampus, hypothalamus and mid brain. One-way ANOVA (df=1,60) showed that the effects of treatment were significant (F=51.30; p<0.01). Post hoc analysis by Newman-keuls test showed that repeated administration of apomorphine decreased (p<0.05) 5-HIAA levels in the mid brain samples.

DISCUSSION

Conditioned place preference (CPP) test is widely used model to assess the reinforcing potential of the drugs in animal research. In this experiment, administration of drug is paired with a particular environment (Risinger and Oakes, 1996). Two-compartment light dark activity box with an entrance between two compartments was used in present study. Rats being nocturnal animals, try to spent ~70% time in the dark compartment and ~30% time in the dark one (). Drug injection was therefore, paired with sequestration in the light compartment.

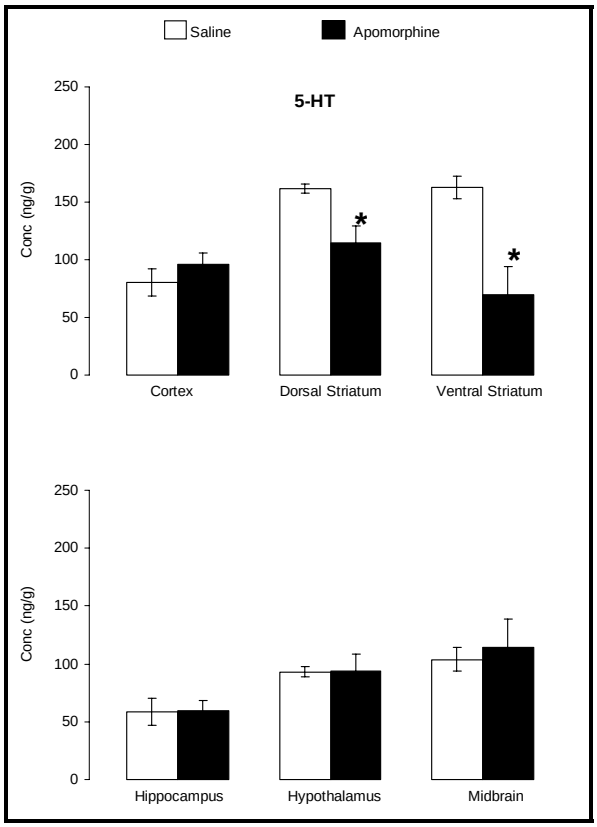

Fig. 5: Effects of repeated (12 days; alternate days) apomorphine (1.0 mg/kg) administration on 5-HT levels in cortex, dorsal striatum, ventral striatum, hippocampus, hypothalamus and mid brain. Values are means ± SD 24hr after last injection (n=6). Significant differences: *p<0.01 from respective saline injected rats following student's t-test.

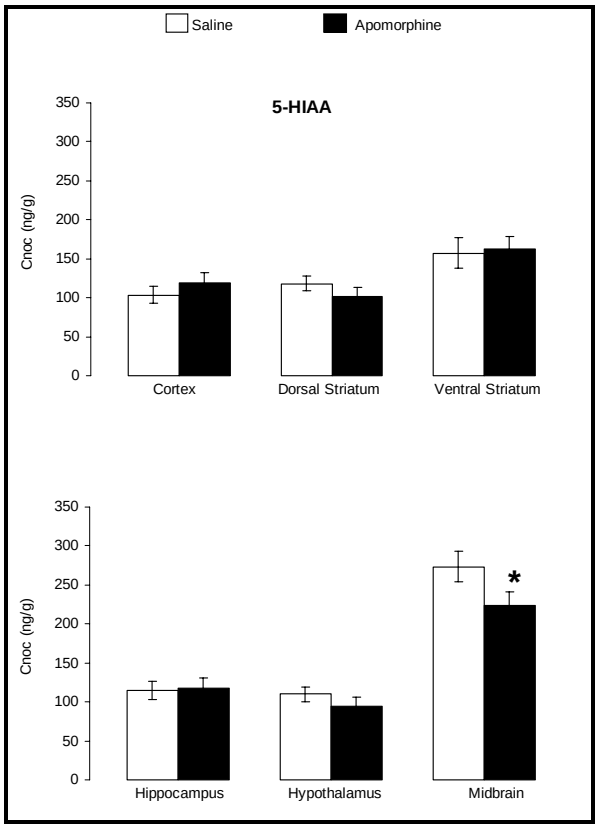

Fig. 6: Effects of repeated (12 days; alternate days) apomorphine (1.0 mg/kg) administration on 5-HIAA levels in cortex, dorsal striatum, ventral striatum, hippocampus, hypothalamus and mid brain. Values are means ± SD 24hr after last injection (n=6). Significant differences: *p<0.05 from respective saline injected rats following student's t-test.

During the conditioning/training phase, animals learn to associate apomorphine injection with placement in light compartment and saline injection with placement in either light or dark compartment. In the post-conditioning phase, animals were introduced to the light dark activity box with the entrance between the two compartments opened. Apomorphine injected animals enter more in the light compartment and spent more than 50% time in the light compartment (fig. 1). Present results show that the reinforcing effects of apomorphine are established after repeated administration of apomorphine (6 injections on alternate days) and these reinforcing effects of apomorphine, could be observed in a CPP paradigm.

Apomorphine is a derivative of morphine but it does not bind to opioid receptors. It is a non-selective agonist of dopamine receptors, but its affinity for D_2-like dopamine receptors is relatively higher than that for D_2-like receptors (Wang *et al.*, 2007). Sensitization to apomorphine (1.0 mg/kg) is reported following its repeated administration and is suggested to be mediated by stimulation of dopamine autoreceptors (Mattingly *et al.*, 2001; Ikram and Haleem, 2011; Ikram *et al.,* 2011). Reinforcing effects of apomorphine (1.0 mg/kg) could be monitored in a CPP paradigm, following withdrawal from its repeated administration. Decreased activity of dopaminergic neurons following its withdrawal, could be a possible reason for the compulsive use of apomorphine (Haleem *et al.*, 2005).

In the present study apomorphine altered metabolism of dopamine and serotonin particularly in dorsal striatum, ventral striatum and mid brain. Dopamine and HVA levels were elevated ($p<0.01$) in dorsal striatum (figs. 2 and 4). This suggests that apomorphine could be effectively used for the pharmacotherapy of Parkinson's and related disorders. Given subcutaneously, apomorphine has a rapid onset of anti-parkinsonian action qualitatively comparable to that of levodopa (Hagell and Odin, 2001).

In the present study, levels of DOPAC and HVA were found to be significantly decreased in the ventral striatum (figs. 3 and 4) suggesting that decreased release of

dopamine in the ventral striatum might be involved in the reinforcing effects of drugs of abuse after withdrawal. Several studies have reported that dopamine plays a pivotal role in the establishment of reinforcement. Drugs of abuse have different initial targets and actions but the resultant reinforcing effect shares several key features (Kreek *et al*., 2002) including increase in extracellular Dopamine (DA) levels, particularly in Nucleus Accumbens (NAcc) (Corominas-Roso *et al*., 2007). Self-administration of cocaine is reported to be related with dopamine transporters (Ritz *et al*., 1998; Kiyatkin, Kiyatkin and Rebec, 2000). Large and extracellular dopamine release is reported to be associated with reinforcing effects of drugs of abuse (Volkow *et al*., 2003).

Despite the fact that final rewarding effect is connected with elevation of DA release in the NAcc, different parts of Limbic system seem to be the targets of addictive drugs such as ventral tegmental area, frontal cortex, hippocampus, pedunculopontine nucleus (Vetulani, 2001). In the present study, levels of dopamine and HVA (figs. 2 and 4) were found to be decreased ($p<0.01$ and $p<0.05$ respectively) in the mid brain suggesting an important role of this brain region in mediating the reinforcing effects of apomorphine. Any significant alteration in the dopamine metabolism was not observed in other regions like cortex, hippocampus and hypothalamus. This could be due to the reason that apomorphine was administered sub-chronically in present study and this treatment (6 injections on alternate days) might not be enough to produce significant changes in the dopamine metabolism in these brain regions.

Results from present study show that 5-HT levels were decreased ($p<0.01$) in the dorsal and ventral striatum of apomorphine injected rats as compared to saline injected controls (fig. 5). Whereas, levels of 5-HIAA were also decreased in the mid brain (fig. 6). This suggests that withdrawal from repeated apomorphine administration induces a decrease in the serotonergic tone which could be a possible predisposing and precipitating agent in drug craving and dependency (Hamon, 2002). Studies have suggested that 5-HT_{2C} receptor antagonist *SB242084* enhances Cocaine primed reinstantement of Cocaine-seeking behavior after a 10mg/Kg i.p. Cocaine prime (Fletcher *et al*., 2002). Whereas, *WAY100635*, a 5-HT_{1A} antagonist, attenuates Cocaine primed reinstantement (Schenk, 2000). It attenuates Cocaine induced locomotor activity and increases extracellular 5-HT, but not DA in NAcc and Hippocampus (Muller *et al*., 2002). But this is unclear that whether effects of *WAY100635* are due to the blockade of post synaptic 5-HT_{1A} receptors or due to an increase in extracellular 5-HT levels via blockade of somatodendritic 5-HT-$_{1A}$ receptors (Burmeister *et al*., 2004).

This decrease in serotonin metabolism could also be due to the supersensitization of the somatodendritic 5-HT_{1A} receptors and strategies involving desensitization of these receptors could help in attenuating the reinforcing effects of apomorphine and CNS stimulants. Therefore, in future it would be interesting to monitor the responsiveness of somatodendritic 5-HT_{1A} receptors following repeated apomorphine administration.

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