TO STUDY THE RESPONSIVENESS OF 5HT-2C RECEPTORS FOLLOWING REPEATED APOMORPHINE ADMINISTRATION IN RATS MODEL

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Abstract: Present study was designed to elucidate serotonergic mechanisms in apomorphine-induced behavioral sensitization upon its repeated administration. Since dopaminergic mechanisms are reported to have well-established role in the pharmacotherapy of Parkinson's, Alzheimer's and related disorders as well as in the pathophysiology of addiction, this thesis has been conceived to improve therapeutic utility of apomorphine by attenuating the reinforcement and behavioral sensitization induced by apomorphine. In the present experiment, repeated apomorphine administration exhibited psychosis in rats upon repeated administration, as observed by monitoring locomotive activities in familiar environment of Skinner's box. Following the third apomorphine injection, locomotor sensitization was evaluated in the familiar Skinner's box environment. In the novel open field environment, we also recorded the locomotive effects of apomorphine. The hyperlocomotive effects of apomorphine are stated to be greater after the sixth injection than the first, implying the emergence of behavioural sensitization effects in a novel environment.

Keywords: Behavioral Sensitization, Locomotory *and Exploratory activity*, *5 HT-2C Receptor*, *Apomorphine*

1. INTRODUCTION

Among different receptors of serotonin system, 5-HT_{2C} receptor subtype is associated with neuropsychiatric disorders (Chagraoui, Thibaut, Skiba, Thuillez and Bourin, 2015) and is thought to be linked with control of executive functions i.e., mental processes and skills (Carli and Invernizzi, 2014). GABA and dopamine neurons located in VTA have receptors of 5-HT_{2C}

type, thus it was proposed that 5-HT_{2C} receptor may effect on the output of this midbrain nucleus through multiple action sites, however it is difficult to predict the function and role of these subpopulations of 5-HT_{2C} receptors since their stimulation may cause activation of multiple signaling cascade (Canal, Morgan and Felsing et al., 2014). Considering GABA neurons, 5-HT_{2C} receptor stimulation must intensify VTA GABA neuronal firing rate, and thus consequently reducing localized dopamine neuronal firing rate, which is one of the functions of GABA neurons originating from VTA, to inhibit localized firing of dopaminergic neurons (Tepper and Lee, 2007). In contrast, considering dopamine neurons, receptors of 5-HT_{2C} type found in dopamine releasing neurons at ventral tegmental area (Bubar, Stutz and Cunningham, 2011) are expected to have escalated rate of firing of dopamine neurons.

Further study is needed to be conducted to better understand the newly discovered dopaminergic neuronal subpopulation having 5-HT_{2C} receptors. It is therefore suggested in previous studies that stimulation of the both types of 5-HT_{2C} receptors of VTA, are believed posses opposite outcomes on output of dopaminergic neurons, and is considered to have a crucial mechanism in controlling VTA dopamine neuron output by keeping a balance in between the effects produced by two opposing subpopulations of 5-HT2C receptors (Fantegrossi, Simoneau and Cohen et al., 2010). Since dopaminergic release is under the inhibitory regulatory influence of serotonin via 5-HT_{2C} receptors, present experiment was therefore planned to track responsive ability of 5-HT_{2C} receptors after injecting apomorphine repeatedly.

2. METHODS AND MATERIALS

2.1. Animals

Locally bred male Albino-Wister rats weighing 180-220 g were purchased from The Aga Khan University, Karachi, Pakistan, and housed individually under 12hr light-dark cycle and controlled room temperature (25 ± 2) with free access to cubes of standard rodent diet and water, a week before experimentation to familiarize them with environment. They were also accustomed to various handling procedures to nullify the effects of stress.

2.2. Drugs

All chemicals used in the behavioral assessment study of rats were purchased from Sigma chemicals (USA), BDH chemicals pool (England), Merck firm, or Research Biochemical (RBI, USA) and was determined by specific action of drug and different parameters.

2. 3. EXPERIMENTAL PROTOCOL

A total of 24 rats divided equally in a randomized fasion among 4 groups (i) saline and (ii) apomorphine injected rats. Saline injection was given to rats (0.9% NaCl) at the dosage 1ml/kg or apomorphine at doses (1.0 mg/kg) daily over a six-day cycle. Skinner's box response was observed on daily basis. On day 6, rats of each group were sub-divided into 2 groups, giving a total of four groups: (i) saline-saline, (ii) mCPP-saline, (iii) apomorphine-saline and (iv) apomorphine-mCPP injected rats. Rats were given a challenge of mCPP at the dose of 2.5mg/kg. Open field, Skinner's box and light dark box responses were observed post mCPP injection. 2hr and 4hr post injection food intakes were also monitored.

2.4 BEHAVIORAL ASSESSMENTS

2.4.1 HOME CAGE ACTIVITY:

Specifically designed Perspex home cages with a specific area (26x26x26 cm) with saw-dust covered floor were utilized for monitoring home cage activity. In a separate room, the experiment was carried out. For 30 minutes, all control and treated rats were housed in separate activity boxes. During this time, rats get familiar to their environments. The cage crossing of the rats was then recorded in 5 min.

2.4.2 OPEN FIELD ACTIVITY TEST:

The open field apparatus was a square area of 76x76 cm with 42 cm high opaque walls. The apparatus's floor was divided equally into 25 equal squares. The test consists of measuring the activity of rat in an open novel space, which is surrounded by a wall, rendering escape difficult

(Walsh and Cummins, 1976). The animal was introduced gently at the centre square of the open field to monitor the activity. For 5 minutes, the exploratory activity (number of squares traversed on all four paws) and latency to move (time it took a rat to leave the centre square) were measured.

2.4.3 BODY WEIGHTS (GROWTH RATE):

Body weight variations were tracked in order to visualize the results of respective treatment. The animals were weighed daily or weekly in the respective experiment. Changes in growth rates measured as a percent of starting day body mass upon daily or weekly basis.

$$\frac{\text{Body weight}}{\text{Preceding day or week body weight}} \times 100$$

2.5. STATISTICAL ANALYSIS:

Results are given as means ± SD. Analysis of the data was performed through 2-way ANOVA. Following ANOVA, Tukey's test was used for post hoc comparisons among groups. Value of $p<0.05$ was taken into consideration as significant.

3. RESULTS

Figure 3.1 expresses response of repeatedly injected apomorphine on activities in known situation of Skinner's box. Data analyzed by 2-way ANOVA expressed that apomorphine responses (df= 1, 60; F= 39.45; p= 0.0001), repeatedly observed (df= 5, 60; F= 94.15; p= 0.0001) & interaction of 2 (df= 5, 60; F= 86.42; p= 0.0001) were all significant. Post-hoc analysis through Tukey's test expressed that apomorphine raised ($p<0.01$) activities from 3rd to 6th dosage, in contrary with both corresponding saline administered- and corresponding Day 0 values.

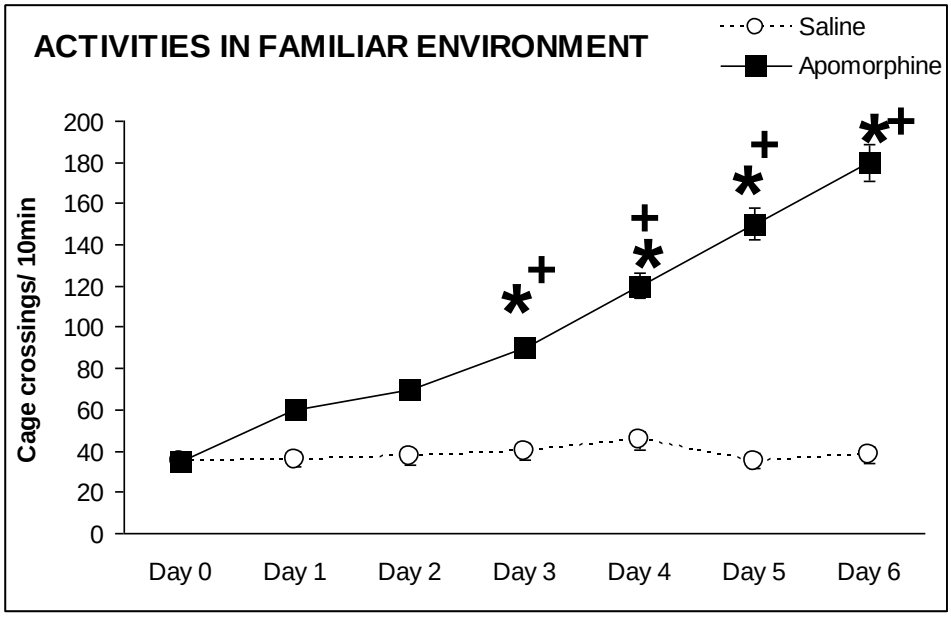

Figure 3.1: Responses of repeatedly injected apomorphine on activities of Skinner's box's known situation. Values are means+SD (n=6). Significant differences through Tukey's test: *$p<0.01$ from comparable saline injected rats; +$p<0.01$ from comparable Day 0 values, following2-way ANOVA.

Figure 3.2 expresses responses of mCPP on growth rates, following repeated apomorphine administration. Data evaluated through 2-way ANOVA expressed apomorphine responses (df= 1, 20; F= 1.45; p= 0.25), mCPP (df= 1, 20; F= 1.56; p= 0.97) & interaction of 2 (df= 1, 20; F= 1.71; p= 0.08) all non-significantly. Post-hoc analysis through Tukey's test revealed no significant difference among groups.

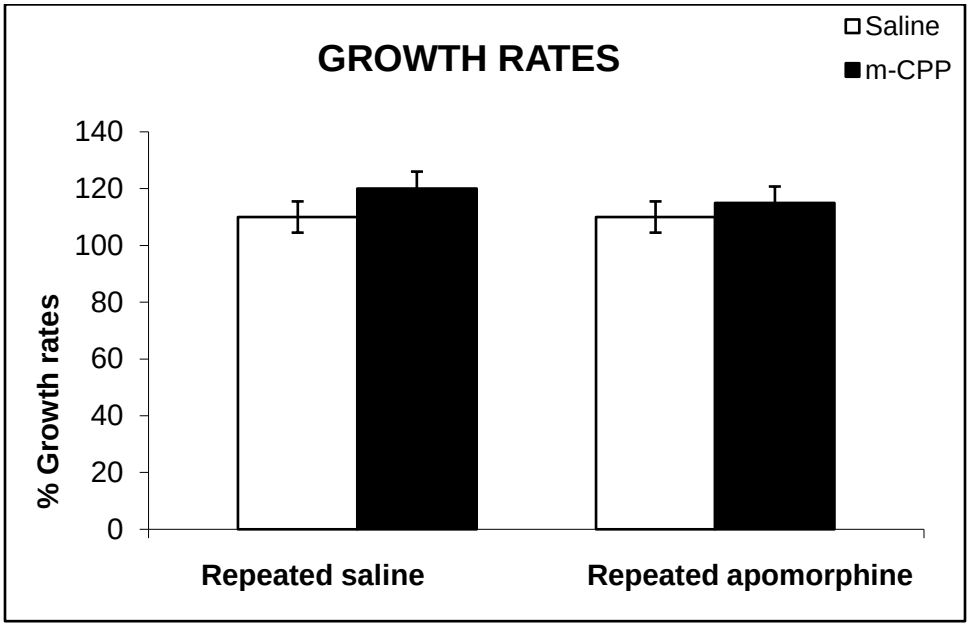

Figure 3.2: Effects of mCPP on growth rates, following apomorphine injected repeatedly. Values are means+SD (n=6). Post-hoc analysis through Tukey's test showed no significant difference among groups, following 2-way ANOVA.

Figure 3.3 expresses responses of mCPP over activities in familiar environment of Skinner's box, following repeated apomorphine administration. Data evaluated through 2-way ANOVA expressed that apomorphine response (df= 1, 20; F= 68.45; p= 0.0001), mCPP (df= 1, 20; F= 38.29; p= 0.0001) & interaction of 2(df= 1, 20; F= 45.33; p= 0.0001) all significantly. Post-hoc analysis through Tukey's test showed decreased ($p<0.01$) activities through mCPP in both repeated saline- as well as repeated apomorphine- administered rats, in contrast with their respective saline administered controls. Activities in repeated apomorphine-saline injected rats were increased ($p<0.01$) in contrast with corresponding repeated saline injected animals. While activities in repeated apomorphine-mCPP injected rats were increased ($p<0.01$) in contrast with corresponding repeated saline injected animals.

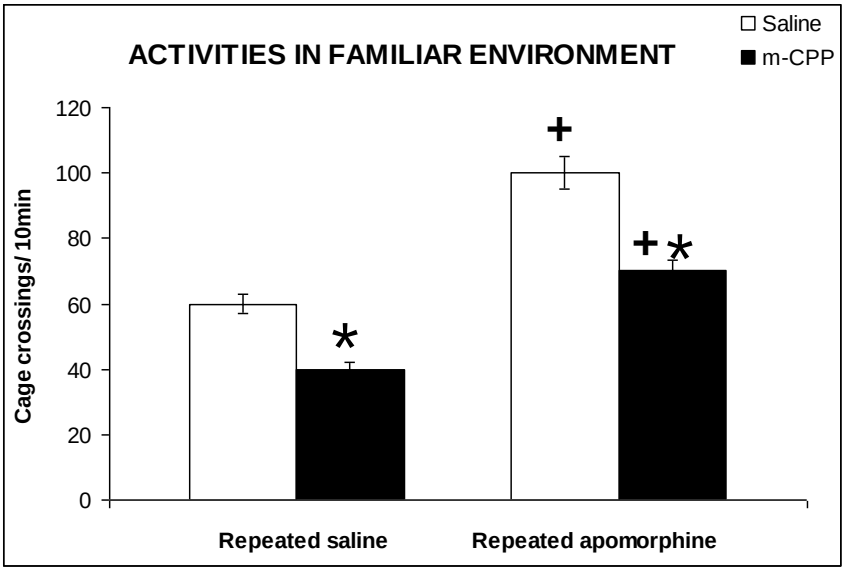

Figure 3.3: Effects of mCPP on activities in familiar environment of Skinner's box, following repeated apomorphine administration. Values are means+SD (n=6). Significant differences through Tukey's test: *$p<0.01$ from comparable saline injected rats; +$p<0.01$ from comparable repeated saline administered rats, following 2-way ANOVA.

Figure 3.4 expresses responses of mCPP on activities in new situation of open-field apparatus, following repeated apomorphine administration. Data evaluated through 2-way ANOVA expressed that apomorphine response (df= 1, 20; F= 92.15; p= 0.0001), mCPP (df= 1, 20; F= 29.83; p= 0.0001) & interaction of 2 (df= 1, 20; F= 37.49; p= 0.0001) all significantly. Post-hoc analysis through Tukey's test showed decreased ($p<0.01$) activities through mCPP in rats both injected repeatedly with saline and also injected repeatedly with apomorphine, in contrast with corresponding saline injected controls. Activities in apomorphine injected animals were increased ($p<0.01$) in contrast with corresponding repeated saline injected animals. While activities in repeated apomorphine-mCPP injected rats were increased ($p<0.01$) in contrast with corresponding repeated saline injected animals.

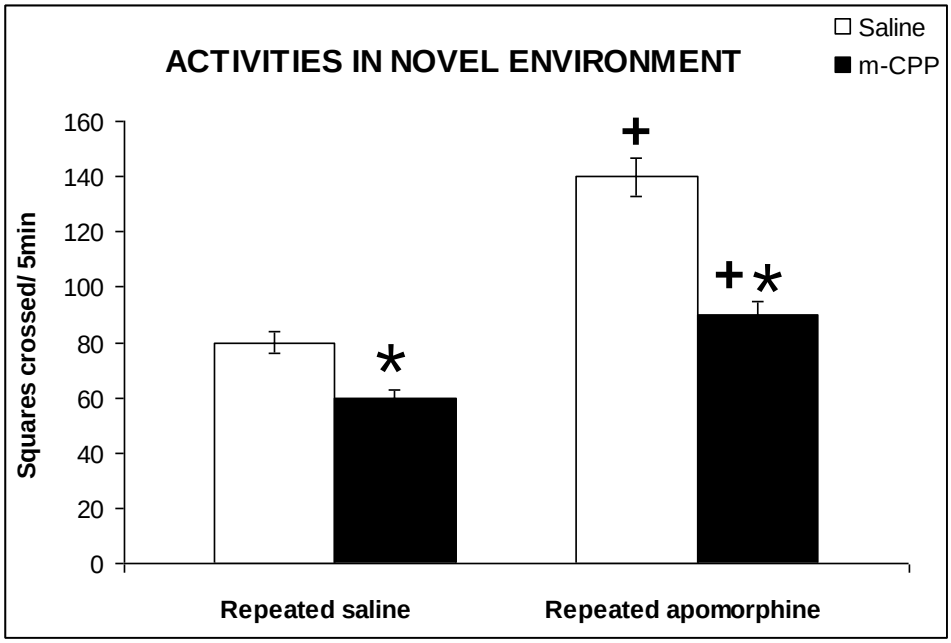

Figure 3.4: Effects of mCPP on activities in novel environment of open field, following repeated apomorphine administration. Values are means+SD (n=6). Significant differences through Tukey's test: *$p<0.01$ from corresponding saline injected rats; +$p<0.01$ from corresponding repeated saline administered rats, following 2-way ANOVA.

4. DISCUSION

In the present experiment, repeated apomorphine administration exhibited psychosis in rats upon repeated administration, as observed by monitoring locomotive activities in familiar environment of Skinner's box. Following the third apomorphine injection, locomotor sensitization was evaluated in the familiar Skinner's box environment (Ikram and Haleem, 2011). In the novel open field environment, we also recorded the locomotive effects of apomorphine. The hyperlocomotive effects of apomorphine are stated to be greater after the sixth injection than the first, implying the emergence of behavioural sensitization effects in a novel environment (Ikram ad Haleem, 2011).

We monitored anxiogenic effects of mCPP as monitored in light dark activity box. Apparatus of light dark activity box is generally used to monitor anxiogenic effects of drugs. The apparatus comprises of a dark chamber and a brightly lit chamber, and the test is vulnerable to anxiolytic drug therapy. The two chambers are linked by a narrow passageway. The rats are able to move freely between the two chambers. Mice's bright-space anxiety is measured by the amount of times they enter the bright chamber and the length of time they spend there (Takao and Miyakawa, 2006). mCPP being $5HT_{2C}$ receptor agonist exerts anxiogenic effects as exhibited by greater entries and also duration consumed in light compartment of light & dark activity box. On the other hand, antagonists of $5HT_{2C}$ receptors, exerts anxiolytic effects by blocking these receptors (Martin, Martin and Trigo et al., 2015). In the present study, mCPP-induced anxiogenic effects as recorded in light dark activity box were decreased in apomorphine-injected rats, suggesting a desensitization of $5HT_{2C}$ receptors upon repeated apomorphine administration. In present study, mCPP-induced hypophagia as monitored 2hr- and 4hr post mCPP challenge was observed. This was in accordance with the findings reported by others. This mCPP-induced hypophagia is mediated by $5HT_{2C}$ receptor and is independent of dietary composition (Rice and Corwin, 2002). In apomorphine-injected rats, mCPP-induced hypophagic effects were not pronounced, suggesting a desensitization of $5HT_{2C}$ receptors upon repeated apomorphine administration.

In the present study, 5HT metabolism was not altered by mCPP- as well as apomorphine treatment. Being $5HT_{2C}$ receptor agonist, mCPP could decrease dopamine metabolism resulting

in increased serotonin levels as nigrostriatal dopaminergic system is regulated by 5-HT_{2C} receptor localized in dorsal striatum (Alex, Yavanian, McFarlane, Pluto and Pehek, 2005). However, no such effect of mCPP on serotonin metabolism was observed in the present experiment.

Present results show that dopaminergic metabolism was increased upon repeated apomorphine administration. Abusive drugs increase dopamine's release and its neurotransmission (Yoshimoto, Watanabe, Tanaka and Kimura, 2012); they could do this by desensitization of 5-HT_{2C} receptors, resulting from the repeated injection of abusive compounds like apomorphine or other CNS stimulants (Martin, Hamon, Lanfumey and Mongeau, 2010). In repeated apomorphine-mCPP injected rats, apomorphine-induced increase in dopamine metabolism was not observed, suggesting a desensitization of 5-HT_{2C} receptors.

CONCLUSION

According to the outcomes of this investigation, it may be concluded that repeatedly injected apomorphine results in desensitization of 5-HT_{2C} receptors resulting increased dopaminergic neurotransmission & potentiation of reinforcing/ psychosis-like effects of apomorphine. However, besides 5-HT_{2C}, 5-HT_{1A} receptors could also take part in indirect control of dopaminergic release since they are localized on cell body region of the serotonergic neuron. Therefore, when 5-HT binds with the 5-HT_{1A} receptors, dopaminergic synthesis would be indirectly inhibited

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