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Haloperidol-Induced Tardive Dyskinesia: Role of 5-HT_{2C} Receptors

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Abstract. Tardive dyskinesia (TD), an involuntary orofacial hyperkinetic disorder, is the major limitation of neuroleptic therapy. Role of 5-hydroxytryptamine (5-HT; serotonin) may be important in the treatment of schizophrenia and TD. Rats chronically treated with haloperidol exhibiting vacuous chewing movements (VCMs) with tongue protrusions and facial musculature are widely used as animal model of TD. Rats repeatedly injected with haloperidol at the dose of 1 mg/mL/kg twice a day for 2 weeks displayed VCMs that increased in a time dependent manner as the treatment was continued for 5 weeks. VCMs were produced two days after withdrawal; animals were given meta-chlorophenylpiperazine (m-CPP) challenge (3 mg/mL/kg) to monitor the responsiveness of 5-HT_{2C} receptors. The intensity of m-CPP induced hypophagia was more in repeated haloperidol + m-CPP injected rats after 4 h but not after 2 h post m-CPP challenge. m-CPP also attenuated haloperidol induced increased dopamine (DA) and 5-HT metabolism both in dorsal and ventral striatum. However, these effects were more pronounced in ventral striatum. Results are discussed in context with responsiveness of 5-HT_{2C} receptors. Findings may help in extending the therapeutics in schizophrenia.

Keywords. tardive dyskinesia, vacuous chewing, neuroleptics, 5-hydroxytryptamine receptors, haloperidol

Introduction

Schizophrenia results from the hyperactivity of nigrostriatal dopaminergic pathway (Mohammadi and Akhondzadeh, 2001). Neuroleptics/antipsychotics are the drugs which are effective in the treatment of schizophrenia due to their ability to seize the over activity of dopaminergic neurons (Armenteros and Davis, 2006). Long term administration of these drugs could lead to development of tardive dyskinesia (TD), which is characterized by difficulty in controlling involuntary movements of the small muscle groups producing tic-like orofacial reactions, muscle rigidity and difficulty in maintaining muscle tone (Jordan and Williams,1990).

Despite its high frequency, the exact mechanism underlying pathophysiology of TD is not known. Role of 5-hydroxytryptamine (5-HT; serotonin) and receptors may be important in the treatment of schizophrenia and pathophysiology of tardive dyskinesia. Enhancement of serotonin-1A receptor dependent responses following withdrawal of haloperidol in rats has been reported by Haleem and Khan (2003). 5-HT_{2C} receptors are important in this regard as dopaminergic transmission is regulated by the striatal 5-HT_{2C} receptors and they could play important role in the treatment of schizophrenia (Alex *et al.*, 2005). Clozapine and other atypical neuroleptics not only have the dopaminergic blocking effects but anti-serotonergic actions and they bind with the receptors only for short duration of time and, therefore, dissociate rapidly from dopaminergic receptors as compared to the typical ones. That is why they produce fewer side effects (Haleem *et al.*, 2004; Kapur and Seeman, 2001; Horacek, 2000).

Administration of haloperidol increases the concentration of 5-HT and 5-HIAA indicating increased rate of production of serotonin as well as metabolism. This increased metabolism of serotonin induced by the haloperidol correlates well with the behavioural response (Ali *et al.*, 2005; Haleem *et al.*, 2002). This suggests a possible alteration in the activity of 5-HT_{2C} receptors. The present study was designed to test the hypothesis that the 5-HT_{2C} receptors are supersensitized upon repeated administration of haloperidol.

Materials and Methods

Animals. Twenty-four female albino Wistar rats, weighing 180-200 g, were purchased from HEJ Research Institute of Chemistry, Karachi, Pakistan. The animals were housed individually in plastic cages under a 12 h light-dark cycle (lights on at 6:00 h) with free access to tap water and cubes of standard rodent diet for at least one week before the start of experiment, so that, they could become familiar with the environment. They were accustomed to various handling procedures to nullify stress effects. All experiments were performed according to the protocols approved by the local animal care committee.

Drugs. Haloperidol (Serenace, Searle, USA), purchased as injectable ampoules of 5 mg/mL, was injected intraperitoneally

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at a dose of 1 mg/kg body weight. m-CPP 2HCl, purchased from Sigma was dissolved in saline and injected intraperitoneally at the dose of 3 mg/mL/kg body weight. Control animals were injected with saline in volumes of 1 mL/kg body weight.

Experimental protocol. Twenty-four female rats were randomly divided into four groups as follows, each containing six animals: (i) repeated saline plus saline, (ii) repeated saline plus m-CPP, (iii) repeated haloperidol plus saline and (iv) repeated haloperidol plus m-CPP injected rats. These four groups of animals were then injected repeatedly (twice a day at 9:00-9:30 and 13:30-14:00 for 5 weeks) with saline (1 mL/kg of body weight) or haloperidol (1 mg/kg of body weight). Vacuous chewing movements and motor coordination were monitored on weekly basis. Two days after withdrawal, animals were injected with saline (1 mL/kg of body weight) or m-CPP (3 mg/mL/kg of body weight). Food intake 2 h and 4 h post-injection were monitored. The animals were then decapitated to collect dorsal and ventral striatum samples for the neurochemical analysis by HPLC-EC.

Monitoring vacuous chewing movements, motor coordination and food intake. Vacuous chewing movements were recorded in the home cage apparatus as described by Ikram *et al.* (2007). Each burst of purposeless chewing movements, which remained continuous for at least 30 sec, was counted as one. Rotorod apparatus was used for the determination of motor coordination. It consists of drums moving on a pully. There is a timer on the base of apparatus. Experiment was performed in a quiet room. Animals were placed on the drums and as they started moving, the timer was started for recording the time. As soon as animal fell down, timer at the base was stopped and the time was recorded. Motor coordination was expressed as time in seconds for which animal maintained its grip on the moving drum.

Dissection of striatum. Dissection procedure was essentially same as described by Ikram *et al.* (2007). 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 of slicer to give slices of 2 mm thickness. The slices containing striatum were transferred to a slide kept on ice. Punches of 2.5 mm diameter were made bilaterally in the striatum to collect dorsal and ventral striatum. Samples were frozen at -70 ºC until analysis by HPLC-EC.

HPLC-EC determination of biogenic amine metabolites. HPLC-EC determination was carried out as described by Haleem *et al.* (2002). A 5 µ Shim-pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used. Separation was achieved by a mobile phase containing methanol (10%), octyl sodium sulphate (0.023%) and EDTA (0.001%) in 0.1 M phosphate buffer of pH 2.9 at an operating potential of 2000-3000 psi on Schimadzu HPLC pump. Electrochemical detection was achieved on Schimadzu LEC 6A detector at an operating potential of +0.8V. Levels of biogenic amines, i.e., dopamine (DA) and 5-hydroxytryptamine (5-HT) as well as their metabolites i.e., 3, 4-dihydroxyphenyl-acetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were estimated in the samples.

Statistical analysis. Results are represented as means ± S.D. Statistical analysis was performed by student's t-test and two-way analysis of variance (ANOVA), to see the effect of various factors involved. Post hoc comparison of groups was performed by Newman-Keul's test. Values of $P<0.05$ were considered as significant.

Results and Discussion

Development of vacuous chewing movements in repeated haloperidol injected rats. Figure 1(a) shows development of vacuous chewing movements in rats upon repeated administration of haloperidol. Repeated two-way ANOVA revealed significant effect on intensity of vacuous chewing movements, of both haloperidol ($F=928.8$, $df=1,132$, $P<0.01$) as well as weekly monitoring ($F=42.0$, $df=4,132$, $P<0.01$). Interaction between the two factors was also significant ($F=218.34$, $df=4,132$, $P<0.01$). Post hoc analysis by Newman-Keul's test showed significant ($P<0.01$) increase in the vacuous chewing movements by haloperidol administration after the 1st to the 5th week of repeated drug administration. The effect after the 1st injection, however, was not significant.

Figure 1(b) shows the effects of haloperidol withdrawal on vacuous chewing movements. Student's t-test revealed significant ($P<0.01$) increase in the vacuous chewing movements in repeated haloperidol injected group.

Effect of single dose of m-CPP in rats following withdrawal from repeated haloperidol administration on food intake. Figure 2(a) shows the effect of repeated haloperidol administration on weekly food intake. Repeated two-way ANOVA revealed significant effects of both haloperidol administration ($F=20.56$, $df = 1,110$, $P<0.01$) as well as weekly monitoring ($F=24.05$, $df = 4,110$, $P<0.01$) on the food intake. Interaction between the two factors was found to be significant as well ($F=17.09$, $df = 4,110$, $P<0.01$). Post hoc analysis by Newman-Keul's test showed that administration of haloperidol caused a significant ($P<0.01$) decrease in the food intake after the 1st injection but not afterwards.

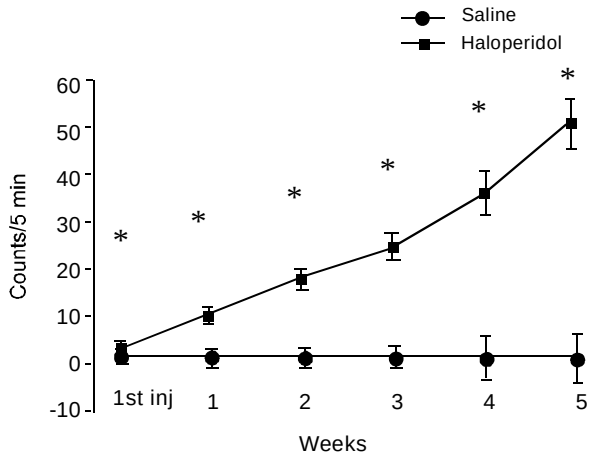

Fig. 1(a). Effects of repeated haloperidol (1 mg/mL/kg) administration (twice a day for 5 weeks) on vacuous chewing movements.

Values are means ± SD (n=12). Significant differences by Newman-Keul's test: * =P<0.01 from their respective repeatedly saline injected controls following repeated two-way ANOVA.

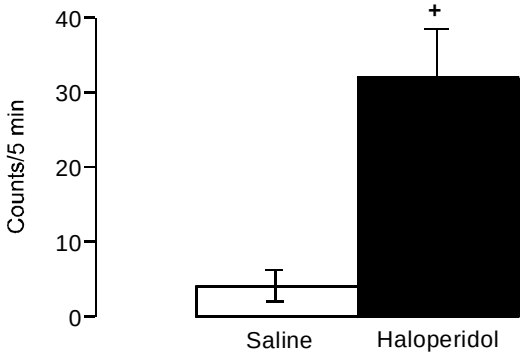

Fig. 1(b). Effects of withdrawal after repeated haloperidol (1 mg/mL/kg) administration (twice a day for 5 weeks) on vacuous chewing movements.

Values are means ± SD (n=12). Significant differences by student's t-test: **+** = P<0.01 from respective repeatedly saline injected controls.

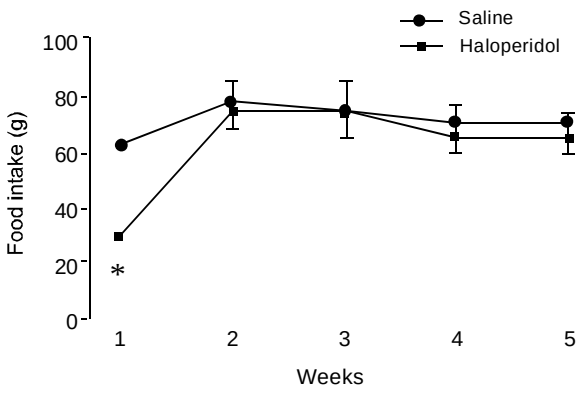

Fig. 2(a). Effects of repeated haloperidol (1 mg/mL/kg) administration (twice a day for 5 weeks) on food intake.

Values are means ± SD (n=12). Significant differences by Newman-Keul's test: * = P<0.01 from their respective repeatedly saline injected controls following repeated two-way ANOVA.

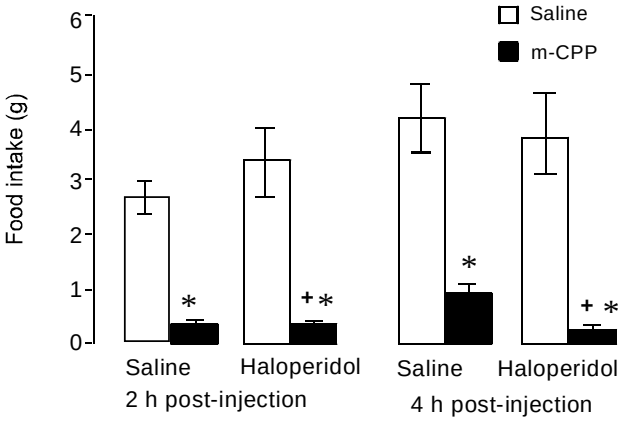

Fig. 2(b). Effects of single dose of m-CPP (3 mg/mL/kg) in rats repeatedly injected with haloperidol (1 mg/mL/kg; twice a day for 5 weeks) on food intake (2 h and 4 h post-injection).

Values are means ± SD (n=6). Significant differences by Newman-Keul's test: * = P<0.01; **+** = P<0.01 from their respective saline and repeatedly saline injected controls following two-way ANOVA.

Figure 2(b) shows the effects of single dose of m-CPP (as monitored 2 h and 4 h post m-CPP injection) on food intake. Data on 2 h post injection food intake analyzed by two-way ANOVA demonstrated significant effect on food intake, of both haloperidol (F=8.79, df=1,20, P<0.01) as well as m-CPP (F=344.0, df = 1,20, P<0.01) administration. The interaction between both of them was also significant (F=9.49, df=1,20, P<0.01). Post-hoc analysis by Newman-Keul's test indicated significant decrease in food intake by m-CPP in both the repeated saline-(P<0.01) as well as the repeated haloperidol-injected rats (P<0.01) from their respective repeated saline plus saline and repeated haloperidol plus saline injected rats. Repeated haloperidol plus saline injected rats exhibited significant (P<0.01) increase in food intake than their respective repeated saline plus saline injected rats.

Data on 4 h post-injection food intake as analyzed by two-way ANOVA demonstrated significant effect on food intake, upon both haloperidol (F=12.05, df=1,20, P<0.01) as well as m-CPP (F=245.27, df=1,20, P<0.01) administration. The interaction between the two was not significant (F=0.14,

df=1,20, P>0.01). Post-hoc analysis by Newman-Keul's test indicated significant decrease in food intake by m-CPP in both the repeated saline-(P<0.05) as well as the repeated haloperidol-injected rats (P<0.05)from their respective repeated saline plus saline and repeated haloperidol plus saline injected rats. Repeated haloperidol plus m-CPP injected rats exhibited significant (P<0.01) decrease in food intake than their respective repeated saline plus m-CPP injected rats. No significant decrease was observed in repeated haloperidol plus saline injected rats.

Effect of single dose of m-CPP in rats following withdrawal from repeated haloperidol administration on motor coordination: Figure 3(a) shows the effect of repeated haloperidol administration on motor coordination. Repeated two-way ANOVA revealed significant effect on motor coordination of both haloperidol administration (F=1075.3, df=1,132, P<0.01) as well as weekly monitoring (F=100.28, df = 4,132, P<0.01). Interaction between the two factors was also significant (F=246.58, df=4,132, P<0.01). Post hoc analysis by Newman-Keul's test showed significant (P<0.01) decrease in the motor coordination after 1st injection as well as after the 1st to the 3rd week of haloperidol administration. The decrease in the motor coordination was not observed after the 4th and the 5th week of drug administration.

Figure 3(b) demonstrates the effect of single dose of m-CPP in rats following withdrawal from repeated administration of haloperidol on motor coordination. Two-way ANOVA revealed significant effect on motor coordination, of haloperidol (F=152.37, df=1,20, P<0.01), m-CPP (F=807.76, df=1, 20, P<0.01) and a significant interaction between the two

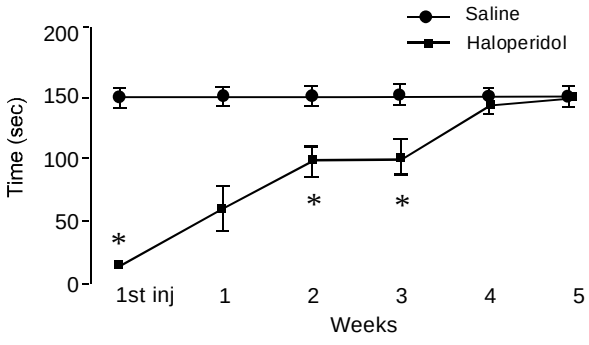

Fig. 3(a). Effects of repeated haloperidol (1 mg/mL/kg) administration (twice a day for 5 weeks) on motor coordination.

Values are means ± SD (n=12). Significant differences by Newman-Keul's test: * = P<0.01 from their respective repeatedly saline injected controls following repeated two-way ANOVA.

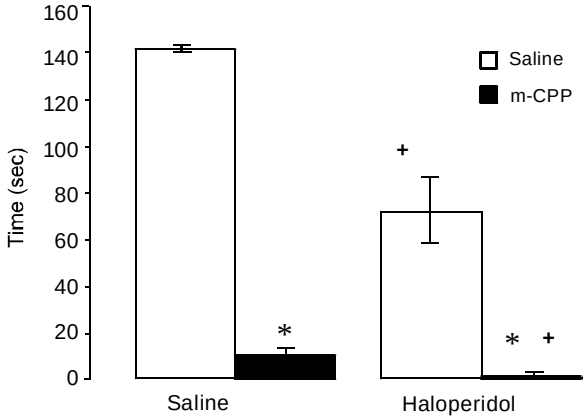

Fig. 3(b). Effects of single dose of m-CPP (3 mg/mL/kg) in rats repeatedly injected with haloperidol (1 mg/mL/kg; twice a day for 5 weeks) on motor coordination.

Values are means ± SD, (n=6). Significant differences by Newman-Keul's test: * = P<0.01; **++** = P< 0.01, **+**P<0.05 from their respective saline and repeatedly saline injected controls following two-way ANOVA.

(F=90.33, df=1,20, P<0.01). Post hoc analysis by Newman-Keul's test showed significant decrease in the motor coordination by m-CPP in both the repeated saline- (P<0.01) as well as repeated haloperidol-injected rats (P<0.01) from their respective repeated saline plus saline and repeated haloperidol plus saline injected control rats. Repeated haloperidol plus saline- (P<0.01) as well as repeated haloperidol plus m-CPP injected rats (P<0.01) exhibited a significant decrease in the motor coordination than their respective repeated saline plus saline and repeated saline plus haloperidol injected rats.

Effect of single dose of m-CPP following withdrawal from repeated haloperidol administration, on 5-HIAA levels in the dorsal and ventral striatum of rats. Figure 4 shows the effect of single dose of m-CPP in rats following withdrawal from repeated administration of haloperidol on 5-HIAA levels in the dorsal and ventral striatum of rats. Data on 5-HIAA levels in the dorsal striatum levels were analyzed by two-way ANOVA and revealed significant effect of m-CPP administration (F=0.308, df=1,20, P<0.01) on 5-HIAA levels in the dorsal striatum. Both the effect of haloperidol on 5HIAA levels (F=0.308, df=1,20, P>0.01) as well as interaction between haloperidol and m-CPP (F=30.48, df=1,20, P>0.01) were not significant. Post hoc analysis by Newman-Keul's test revealed significant (P<0.01) decrease by m-CPP in 5-HIAA levels in the dorsal striatum of repeated haloperidol injected rats from their respective repeated haloperidol plus m-CPP injected rats.

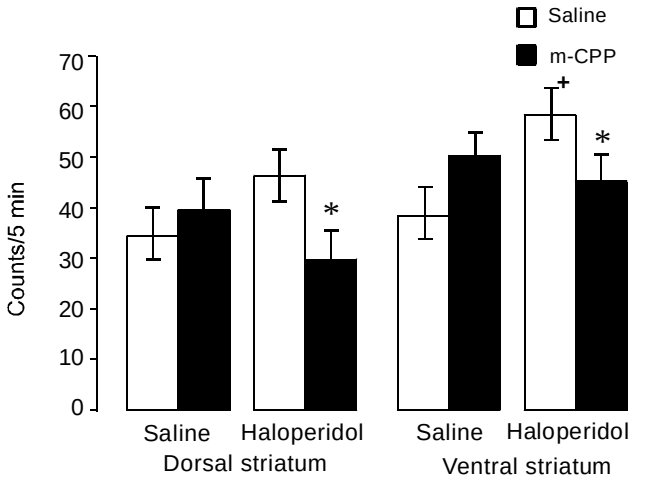

Fig. 4. Effects of single dose of m-CPP (3 mg/mL/kg) on 5-HIAA levels in the dorsal and ventral striatum in rats repeatedly injected with haloperidol (1mg/mL/kg; twice a day for 5 weeks).

Values are means ± SD (n=6). Significant differences by Newman-Keul's test: * = P<0.01; **+** = P<0.01 from their respective saline and repeatedly saline injected controls following two-way ANOVA.

Data on 5-HIAA levels as in the ventral striatum analyzed by two-way ANOVA revealed significant effect of haloperidol administration (F=7.069, df=1,20, P<0.05) on 5-HIAA levels in the ventral striatum of rats. While, the effect of m-CPP on 5-HIAA levels was not significant (F=3.10, df=1,20, P>0.01), interaction between the two was found to be significant (F=12.85, df = 1,20, P<0.01). Post hoc analysis by Newman-Keul's test revealed significant (P<0.05) decrease in the 5-HIAA levels in the ventral striatum of repeated haloperidol injected rats from their respective repeated haloperidol plus saline injected controls. Increase in 5-HT levels in repeated saline injected rats by m-CPP was not significant. Repeated haloperidol plus saline injected rats showed significantly (P<0.01) high levels of 5-HIAA levels than their respective repeated saline plus saline injected rats.

Effect of single dose of m-CPP following withdrawal from repeated haloperidol administration, on DOPAC and HVA levels in the dorsal and ventral striatum of rats. Figure 5(a) shows the effect of single dose of m-CPP in rats following withdrawal from repeated administration of haloperidol on HVA levels in the dorsal and ventral striatum of rats. Data on HVA levels in the dorsal striatum as analyzed by two-way ANOVA revealed significant effects of both haloperidol (F=16.76, df=1,20, P<0.01) as well as m-CPP (F=22.98, df=1,20,0 P<0.01) administration on HVA levels in the dorsal striatum of rats.

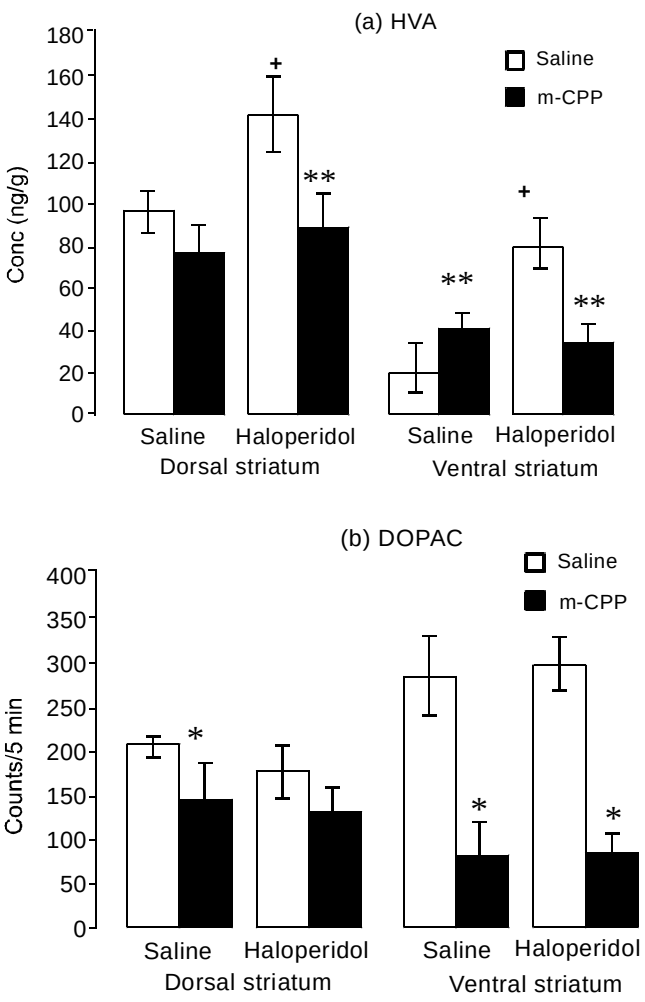

Fig. 5. Effects of single dose of m-CPP (3 mg/mL/kg) on (a) HVA and (b) DOPAC levels in the dorsal and ventral striatum in rats repeatedly injected with haloperidol (1 mg/mL/kg; twice a day for 5 weeks).

Values are means ± SD (n=6). Significant differences by Newman-Keul's test: * = P<0.05, ** = P<0.01; **+** = P<0.01 from their respective saline and repeatedly saline injected controls following two-way ANOVA.

Interaction between haloperidol and m-CPP was found to be significant (F=9.57, df = 1,20, P<0.01). However, the effect of haloperidol on DOPAC levels in the DS was found to be significant as well (F=5.55, P>0.05).

Post hoc analysis by Newman-Keul's test revealed significant (P<0.01) decrease in the HVA levels by m-CPP in the dorsal striatum of repeated haloperidol injected rats from their respective repeated haloperidol plus saline injected rats. In repeated saline plus m-CPP injected rats, this decrease was not significant. HVA levels were found to be significantly

(P<0.01) high in the repeated haloperidol plus saline injected rats than their respective repeated saline plus saline injected rats.

Data on HVA levels as analyzed by two-way ANOVA revealed a significant effect of both the haloperidol (F=5.71, df=1,20, P<0.05) as well as the m-CPP administration (F=45.11, df= 1,20, P<0.01) on HVA levels in the ventral striatum of rats. Interaction between m-CPP and haloperidol was also significant (F=5.148, df=1,20, P<0.05). Post hoc analysis by Newman-Keul's test demonstrated significant (P<0.01) increase by m-CPP in HVA level in the ventral striatum of rats following repeated saline administration from their respective repeated saline plus saline injected rats. Levels of HVA in the ventral striatum of repeated haloperidol plus m-CPP injected rats decreased significantly (P<0.05) than their respective repeated saline plus m-CPP injected rats.

Figure 5(b) shows the effect of single dose of m-CPP in rats following withdrawal from repeated administration of haloperidol on DOPAC levels in the dorsal and ventral striatum. Two-way ANOVA revealed significant effects of m-CPP administration (F=14.59, df=1,20, P<0.01) on DOPAC levels in the dorsal striatum as well as interaction between haloperidol and m-CPP (F=9.57, df=1,20, P<0.01).

However, the effect of haloperidol on DOPAC levels in the DS was not significant (F=10.2, df=1,20, P>0.01). Post hoc analysis by Newman-Keul's test showed significant (P<0.05) decrease of DOPAC levels in the dorsal striatum of repeated saline plus m-CPP injected rats than their respective repeated saline plus saline injected rats.

Data on DOPAC levels in the ventral striatum as analyzed by two-way ANOVA revealed a significant effect of m-CPP administration (F=26.07, df=1,20, P<0.01) but not that of haloperidol (F=0.177, df=1,20, P>0.01) on DOPAC levels in the ventral striatum of rats. However, the interaction between m-CPP and haloperidol (F=5.148, df=1,20, P<0.05) was significant. Post hoc analysis by Newman-Keul's test showed significant decrease in the DOPAC levels by m-CPP in the ventral striatum of both repeated saline (P<0.01) as well as repeated haloperidol injected (P<0.01) rats from their respective repeated saline plus saline and repeated haloperidol plus saline injected rats.

Haloperidol induced tardive dyskinesia. Results of the present study showed that repeated haloperidol administration in rats produced 'vacuous chewing movements' which developed after the 1st week of repeated haloperidol administration and continued to persist over a period of 5 weeks (upon repeated haloperidol administration) and even after the withdrawal. Vacuous chewing movements (VCMs), induced in rats, upon repeated/chronic haloperidol administration, are often used as an animal model of tardive dyskinesia which is widely used for the experimental purposes (Ikeda *et al.*, 1999).

Rats repeatedly administered with haloperidol, display a significant increase in these VCM which is both time and dose dependant. Significant correlation has also been found between doses of haloperidol and VCMs as well as both D2 receptor occupancy and VCM scores. Along these VCMs, animals also show tongue protrusions, facial twitching and bursts of jaw tremors at high doses of haloperidol (about 5 mg/kg), administered repeatedly (over 21 days). Individual and strain differences are also there in the development of VCMs (Marchese *et al.*, 2004; Turrone *et al.*, 2003). The repeated jaw movement responses remain high during washout from drug and haloperidol was found to contain 70% D2 receptor occupancy (Rosenqarten and Quartermain, 2002). Hypersensitivity of D2 receptors after long term treatment with haloperidol, is responsible for tardive dyskinesia (Kulkarni and Naidu, 2000).

Effect of repeated haloperidol administration and m-CPP challenge on feeding behaviour. In the present study, haloperidol administration decreased food intake after the 1st week but not after further administration (i.e., from the 2nd to the 5th week). One of the side effects of neuroleptic therapy is obesity and about 50% of the patients maintained under chronic programme of neuroleptic administration suffer this problem (Hartfield *et al.*, 2003). In accordance with our results, several studies have shown significant decrease in weight upon haloperidol administration (Pouzet *et al.*, 2003) and this hypophagic response to haloperidol is dose dependant (Wolgin and Dalzell, 1992; Wolgin and Moore, 1992). The degree of suppression of feeding behaviour is inversely related to the doses of drugs (Wolgin and Thompson, 1989). This hypophagic effect of haloperidol is mediated as a result of its antagonistic effects towards D2 receptors and is also shown by other selective D2 receptor antagonists (N0437) when injected intraperitoneally (Clifton *et al.*, 1989).

The present study revealed hypophagic effects of m-CPP in rats after repeated administration of haloperidol. Schuhler *et al.* (2005) have also reported that m-CPP upon acute administration (at a dose of 3 mg/kg) markedly reduced food intake in the experimental animals. Several studies have revealed the hypophagic response upon administration of m-CPP. High doses of m-CPP (i.e., 10 mg/kg) produced marked decrease in the body weight and this effect was reversible upon withdrawal (Vickers *et al.*, 2003; Schreiber andVry, 2002; Fone *et al.*, 1998). Same hypophagic responses were also observed upon administration of moderate doses of m-CPP i.e., 3.3 mg/kg (Yamauchi *et al.*, 2004).Local perfusions of m-CPP into ventricular hypothalamic nucleus (which is involved in the

control of feeding behaviour) showed to inhibit food intake in rats (Hikiji *et al.,* 2004). m-CPP produces this hypophagic response by stimulating 5-HT_{2C} receptors and thus reducing feeding behaviour (Simansky *et al.,* 2004; Yamada *et al.,* 1996) as the stimulation of these receptors leads to diminished feeding behaviour (Zittel *et al.,* 2002). m-CPP has also shown to decrease food intake in hyperphagia-induced rats (by 2-deoxy-D-glucose) as reported by Sugimoto *et al.* (2001).

Administration of m-CPP decreases food intake in a dose dependant manner. The response appears very high on acute administration and further reduces upon chronic administration (Antonatos and Galanopoulou, 2006). Agonists of 5-HT_{2C} receptors have shown to decrease food intake. Whereas administration of the antagonists of these receptors have shown to increase it. This further confirms that hypophagic effects of m-CPP are mediated by its binding to 5-HT_{2C} receptors but not with 5-HT_{1B} receptors (Hewitt *et al.,* 2002).

Attenuation of parkinsonian-like effects of haloperidol by m-CPP. In the present study, catalepsy (as monitored in terms of decreased motor coordination on a rotorod) was observed in repeated haloperidol administered rats from the 1st to the 3rd week. However, these cataleptogenic effects were not observed from the 3rd to the 5th week of repeated haloperidol administration. Using rotorod, marked deficit in motor coordination in haloperidol injected rats (after the 1st injection) has also been reported by Johnson *et al.* (1992). This catalepsy induced in experimental animals is an equivalent of parkinsonism in humans. It is induced by haloperidol and other D_2 receptor antagonists which bind to D_2 receptors present in the extra pyramidal regions like striatum (Coppens *et al.,* 1995; (Merchant *et al.,* 1994). Haloperidol induced cataleptogenic effects are mediated *via* D_2 receptor occupancies of >57% (Crocker and Hamsley, 2001) and this, also increases the expression of c-Fos protein in rat brain (Hattori *et al.,* 2006; Binder *et al.,* 2004).

A decrease in motor coordination (assessed on a rotorod) and enhancement of catalepsy has also been reported by Karl *et al.* (2006), which is a major side effect of haloperidol therapy; haloperidol upon acute administration produces marked catalepsy (Bardin *et al.,* 2006). Several studies had reported that the administration of haloperidol induced increased catalepsy in a dose dependent manner (as monitored either on an inclined plane or placing the animal in an odd body posture in home cage). Upon repeated administration of haloperidol, tolerance to these cataleptogenic effects develops as these cataleptogenic effects are acute side effects (Nakai *et al.,* 2003; Bazyan *et al.,* 2002; Fischer *et al.,* 2002; Haleem *et al.,* 2002).

Administration of m-CPP in the haloperidol pre-treated rats has shown to increase catalepsy in the present study. This increased catalepsy could be due to the reason that m-CPP decreases firing rate of dopaminergic neurons in specific regions of brain (Di Giovanni *et al.,* 2000; Lucas and Spampinatos 2000; Lucas *et al.,* 2000). This decreased release of dopamine then leads to decreased motor coordination or catalepsy (Tada *et al.,* 2004) as dopamine is required for motor regulation acting via D_2 receptors (Wadenberg *et al.,* 2000).

Effect of m-CPP on dopamine metabolism in the dorsal and ventral striatum of repeated haloperidol injected rats. In the present study, m-CPP decreased DOPAC levels in the dorsal as well as ventral striatum, whereas, it increased HVA levels in the ventral striatum of repeatedly saline injected animals. This suggests that dopamine is not degraded within the neuron as DOPAC is intra-neuronal product of dopamine metabolism (Synder, 2006). m-CPP has been reported to decrease striatal dopamine by 5-HT_{2C} receptors (Pozzi *et al.,* 2002) and reverses the effects of serotonin $5HT_{2C}$ receptor antagonists which increase dopaminergic transmission in mesolimbic system (Di Giovanni *et al.,* 2000; Di Matteo *et al.,* 1999) and other regions of brain (De Deurwaerdere *et al.,* 2004). As DOPAC is an intra neuronal product of dopamine degradation, decreased DOPAC levels show that release of dopamine is increased by m-CPP under the normal physiological conditions. This could be due to the dopamine auto-receptors.

On the other hand, in the repeatedly haloperidol injected animals, m-CPP decreased HVA and dopamine levels in the dorsal striatum and both HVA and DOPAC levels in the ventral striatum. This shows that m-CPP decreased the release of dopamine in both the dorsal as well as the ventral striatum as a result of decreased dopamine synthesis. This decreased release of dopamine has also been reported by Lucas and Spampinato (2000) which could be explained on the basis of $5HT_{2C}$ receptor activation; $5HT_{2C}$ receptor antagonists; increase the release of dopamine (Porras *et al.,* 2002; Broderick and Piercey, 1998). It has also been reported that $5HT_{2C}$ receptor agonists could modulate the dopamine release induced by other drugs as well (Di Matteo, *et al.,* 2004).

Effect of m-CPP on 5-HT metabolism in the dorsal and ventral striatum of repeated haloperidol injected rats. Results of the present study that m-CPP decreased 5-HIAA levels in both the dorsal and the ventral striatum, suggested m-CPP-induced increased synthesis of 5-HT in the ventral striatum, which could be due to its affinity for 5-HT_{1B} receptors, while it increased both synthesis and release of 5-HT in the dorsal striatum. Various studies have reported increased release of 5-HT by m-CPP, not only via its interaction with 5-HT_{2C} receptors (Baumann *et al.,* 1995; 1993) but also by interaction with 5-HT transporters (Baumann *et al.,* 2001). These

5-HT levels increase in a dose dependant manner, upon m-CPP administration (Erikkson *et al.,* 1999). The increased release of 5-HT in the dorsal striatum (which is involved in locomotion control) might be the cause of increased dopamine, acting via 5-HT_{2C} receptors, as suggested in other studies (Di Giovanni *et al.,* 2000), while in the ventral striatum, m-CPP also increased synthesis of 5-HT. Since VS is involved in the emotional control, this could explain anxiogenic effect upon m-CPP administration.

Conclusion

From the present results, it can be concluded that 5-HT_{2C} receptor agonists may further exacerbate the EPS induced by haloperidol and that there is an important role of 5HT in the haloperidol induced extra pyramidal symptoms. An increase in the responsiveness of $5HT_{2C}$ receptors as observed in the present study could be explained in terms of increased effectiveness of $5HT_{2C}$ receptors present presynaptically on dopaminergic neurons. In conclusion, the present study supports the notion that $5HT_{2C}$ receptors become hypersensitive upon repeated haloperidol administration. Since 5-HT_{2C} receptors inhibit release of dopamine, m-CPP further exacerbated the extrapyramidal symptoms in the present study. As 5-HT_{2C} antagonists could reverse the inhibitory influence of 5-HT over dopaminergic neurons, they could be used as adjuvant therapies to prevent onset of extrapyramidal side effects, especially in those patients which have to be maintained over typical anti-psychotics for some reasons.

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