

## Neurochemical profile of 1-(m-Chlorophenyl)piperazine (m-CPP) in the Striatum of a Rat Model of Tardive Dyskinesia

<sup>1</sup>N. SAMAD\*, <sup>2</sup>M. A. HALEEM AND <sup>1</sup>D. J. HALEEM

<sup>1</sup>*Department of Biochemistry,  
Neurochemistry and Biochemical Neuropharmacology Research Laboratory,  
University of Karachi, Karachi-75270, Pakistan.*

<sup>2</sup>*Department of Biomedical Engineering,  
Sir Syed University of Engineering and Technology, Karachi, Pakistan.*

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**Summary:** The traditional neuroleptic drugs are chronically used in the treatment of schizophrenia and other psychotic disorders. A serious side effect of long term use of traditional neuroleptics is the precipitation of tardive dyskinesia (TD), characterized by involuntary movements of the face and extremities. Vacuous chewing movements (VCMs) in animals following long term administration of haloperidol is often taken as a model of TD. Dopamine (DA) D2 receptors in the striatum are involved in the control of motor activity, while serotonin (5-hydroxy tryptamine; 5-HT) elicits an inhibitory influence on the activity of dopaminergic neurons. Previous studies from our laboratory show that the inhibitory influence of 5-HT on the activity of dopaminergic neurons is decreased following long term administration of haloperidol. This is because of the supersensitization of somatodendritic 5-HT-1A receptors. The inhibitory effects of 5-HT on the activity of dopaminergic neurons are mediated via 5-HT-2C receptors located on the cell body and nerve terminal ends of dopaminergic neurons. The present study therefore concerns neurochemical effects of 5-HT-2C receptor agonist, 1-(m-Chlorophenyl) piperazine (m-CPP) in the striatum of rats repeatedly treated with haloperidol and exhibiting orofacial dyskinesia. Results show that haloperidol injected at a dose of 1 mg/kg twice a day for two weeks elicited VCMs, which increased in a time dependent manner following the drug administration for 5 weeks. The neurochemical effects of m-CPP were monitored 48-h after the drug withdrawal. Administration of haloperidol increased 5HT and DA metabolism in the striatum. Administration of m-CPP increased 5-HT metabolism and decreased DA metabolism in repeated saline treated animals not exhibiting VCMs. Conversely, administration of m-CPP resulted in a decrease of 5-HT metabolism in repeated haloperidol treated animals exhibiting VCMs. m-CPP-induced decreases of DA metabolism were also greater in repeated haloperidol treated animals. The results suggest that a decrease in the responsiveness of 5-HT-2C receptors and an increase in the responsiveness of dopamine D2 receptors are involved in the precipitation of TD.

### Introduction

Schizophrenia is a life-long illness that affects approximately 1 % of the human population [1-3]. Although the traditional neuroleptic drugs are widely used for the treatment of schizophrenia but their beneficial effects are accompanied by movement disorders such as Parkinsonism and Tardive dyskinesia (TD) often described as extrapyramidal side effects (EPS). TD, manifested by involuntary movements particularly in the oral lingual masticatory movements area [4] of rats chronically treated with typical neuroleptics such as haloperidol [5]. It has been also seen that TD persists often haloperidol withdrawal and occasionally become irreversible, indicating that haloperidol has produced long lasting changes in brain function that are no longer related to the presence of the drug [6, 7]. Rats

treated with repeated high doses of haloperidol develop vacuous chewing movements (VCMs), a syndrome similar to human TD, for time course and sign [8, 9]. An increase in the responsiveness of postsynaptic as well as presynaptic 5-HT-1A receptors [10] and dopamine D2 receptor binding [11, 12] in rat brain has also been observed following prolonged neuroleptic treatment.

1-(m-Chlorophenyl)piperazine (m-CPP), acts as agonist at 5-HT-2C receptor [13, 14] increases the release of 5-HT [13] via the stimulation of postsynaptic 5-HT-2C receptors [14]. Studies have shown that systemic administration of m-CPP decreased dopamine in the striatum [14] and other regions [15] of rat brain. The present study is

\*To whom all correspondence should be addressed.

designed to monitor the responsiveness of 5-HT-2C receptors in rats exhibiting VCMs following long term administration of haloperidol.

### Results and Discussion

The intensity of haloperidol-induced vacuous chewing movement (VCMs) is shown in Fig. 1. Data analyzed by two-way ANOVA showed significant effect of haloperidol ( $F = 879.72$   $df = 1,110$   $p < 0.01$ ), weeks ( $F = 39.10$   $df = 4,110$   $p < 0.01$ ) and a significant interaction between haloperidol\*weeks ( $F = 144.03$   $df = 4,110$   $p < 0.01$ ). Post-hoc analysis showed that administration of haloperidol elicited VCMs after 2 weeks of administration. The intensity of VCMs increased in a time dependent manner. Previous studies show that long term administration of haloperidol elicits orofacial dyskinesia where VCMs are one of the prominent features [16]. Withdrawal from repeated administration attenuated the intensity of VCMs. In the present study also haloperidol-induced VCMs persist after 2 days of drug withdrawal but its intensity was decreased (Fig. 2).

The effects of m-CPP on DA, DOPAC and HVA levels in the striatum of repeated haloperidol and repeated saline treated animals is shown in Fig. 3. Data on DA levels analyzed by two-way ANOVA ( $df = 1, 20$ ) showed significant effects of haloperidol ( $F = 3.18$   $p < 0.05$ ), m-CPP ( $F = 6.32$   $p < 0.01$ ) and significant interaction between haloperidol and m-CPP ( $F = 3.70$   $p < 0.05$ ). Post-hoc analysis showed that administration of m-CPP decreased DA levels in haloperidol but not in saline treated animals. The levels of DA were smaller in haloperidol plus m-CPP than saline plus m-CPP treated animals.

Data on DOPAC levels analyzed by two-way ANOVA ( $df = 1, 20$ ) showed that the effect of haloperidol ( $F = 1.21$   $p > 0.05$ ) was not significant. Effect of m-CPP ( $F = 14.61$   $p < 0.01$ ) was significant. Interaction between haloperidol\*m-CPP ( $F = 0.09$   $p > 0.05$ ) was not significant. Post-hoc analysis showed that administration of m-CPP decreased DOPAC levels in haloperidol and saline treated animals.

Data on HVA levels analyzed by two-way ANOVA ( $df = 1, 20$ ) showed that effect of haloperidol ( $F = 16.76$   $p < 0.01$ ) and m-CPP ( $F = 22.98$   $p < 0.01$ ) was significant. Interaction between haloperidol\*m-CPP ( $F = 5.55$   $p < 0.05$ ) was also significant. Post-hoc analysis showed that

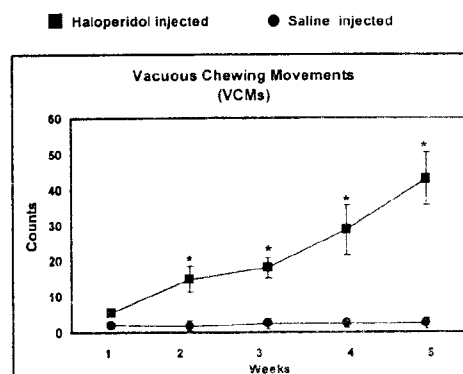


Fig. 1: The intensity of haloperidol-induced VCMs. Values are means  $\pm$  S.D. ( $n=12$ ). Significant differences by Newman-Keuls test:  $*p < 0.01$  from saline treated animals following two-way ANOVA.

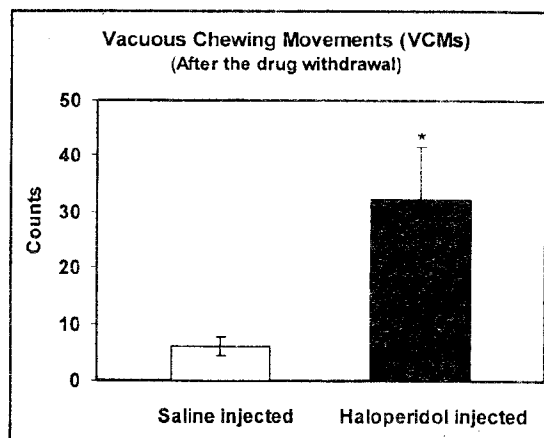


Fig. 2: The intensity of haloperidol-induced VCMs. Values are means  $\pm$  S.D. ( $n=12$ ). Significant differences  $*p < 0.01$  by t-test.

administration of m-CPP decreased HVA in haloperidol but not in saline treated animals. The levels of HVA were higher in haloperidol plus saline than saline plus saline treated animals.

Haloperidol blocks inhibitory DA autoreceptors [17] and increased cell firing, is responsible for the increase in DA turnover [18]. It is now generally agreed that the clinical responses to neuroleptics are associated with an increase in HVA concentration [19]. The proposed mechanism of HVA changes involves a strong blockade of pre- and post-synaptic D2 receptors. The pre-synaptic

blockade increases the amount of DA released on subsequent action potential but because D2 post-synaptic receptors are also blockade, acute DA transmission is attenuated. Other researchers have reported that chronic and sub-chronic treatment of rat with haloperidol results in upregulation of D2 receptors [10, 18], even after the drug withdrawal [20]. The effect of haloperidol 48 hrs after the drug withdrawal on HVA increases in the striatum is consistent with previous reports of an increase in DA turnover and release [21] following withdrawal from repeated administration of the drug.

The serotonin agonist is 1-(m-Chlorophenyl) piperazine (m-CPP) a metabolite of the antidepressant drug, trazodone binds to various 5-HT receptors in rat brain and of these its binding affinity is more potent at 5-HT-2C receptor. The m-CPP increase the release of 5-HT [13] via the stimulation of post-synaptic 5-HT-2C receptor [13, 14]. Studies have shown that systemic administration of m-CPP decreased DA in the striatum [14] and other regions [15] of rat brain.

Alex *et al.*, 2005 [14] have reported that m-CPP decreased striatal DA via inhibition of nigrostriatal dopaminergic transmission. Systemic administration of m-CPP decreased striatal DA and attenuated the 5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrodo [2, 3-f] indole hydrochloride (SB-206553; selective 5-HT-2B/2C inverse agonist)-induced increases the striatal DA [14]. Other studies have also reported that in the hypothalamus chronic m-CPP treatment decreased the level of DA [22]. In the present study a decrease in DA metabolism following the administration of m-CPP (Fig. 3) in repeated saline treated animals provides an evidence of its inhibitory influence on dopaminergic neuron. In addition, we report that the inhibitory influence of m-CPP on the activity of dopaminergic neurons is enhanced in rats repeatedly injected with haloperidol (Fig. 3).

The effects of m-CPP on 5-HT and 5-HIAA levels in the dorsal and ventral striatum of repeated saline and repeated haloperidol treated animals is shown in fig. 4. Data on 5-HT levels analyzed by two-way ANOVA ( $df = 1, 20$ ) revealed that effect of haloperidol ( $F = 0.058$   $p > 0.05$ ) was not significant. Effect of m-CPP ( $F = 14.39$   $p < 0.01$ ) was significant. Interaction between haloperidol\*m-CPP ( $F = 3.6$   $p < 0.05$ ) was also significant. Post-hoc analysis showed

that administration of m-CPP increased 5-HT concentration in saline but not haloperidol injected animals.

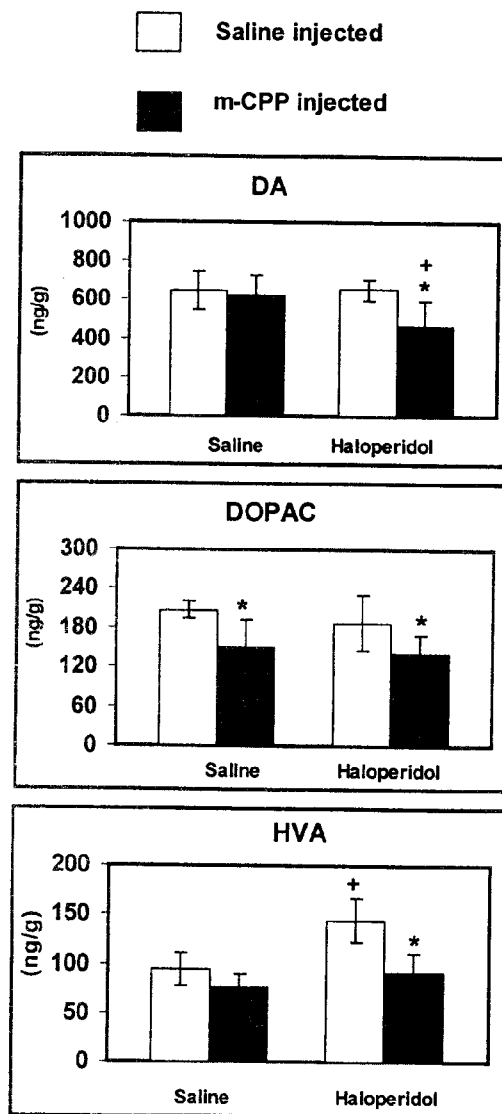


Fig. 3: Effect of m-CPP on DA, DOPAC and HVA levels in the striatum of repeated saline and repeated haloperidol treated animals. Values are means  $\pm$  S.D. ( $n=6$ ). Significant differences by Newman-Keuls test: \* $p < 0.01$  from saline plus saline and saline plus haloperidol treated animals, + $p < 0.01$  from saline plus saline treated animals following two-way ANOVA.

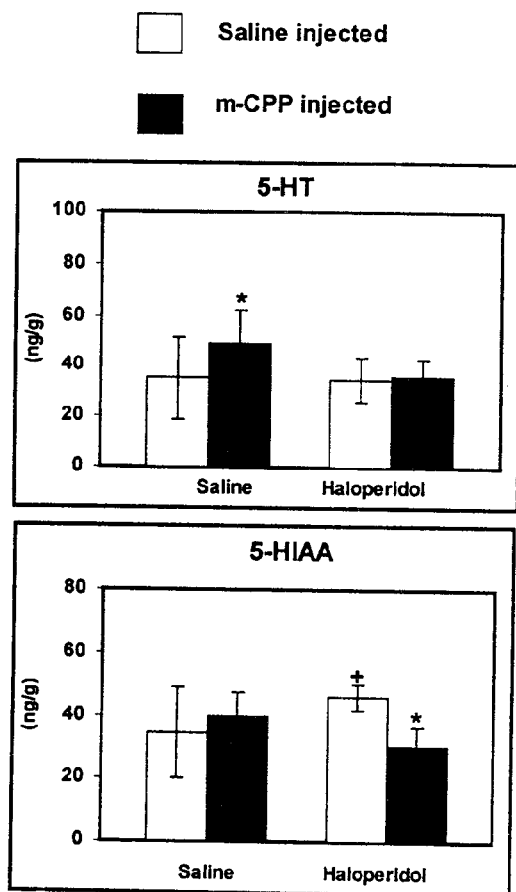


Fig. 4: Effect of m-CPP on 5-HT and 5-HIAA levels in the striatum of repeated saline and repeated haloperidol treated animals. Values are means  $\pm$  S.D. (n=6). Significant differences by Newman-Keuls test: \* $p < 0.01$  from saline plus saline and saline plus haloperidol treated animals, + $p < 0.01$  from saline plus saline treated animals following two-way ANOVA.

Data on 5-HIAA levels analyzed by two-way ANOVA (df = 1, 20) revealed that effect of haloperidol ( $F = 5.45$   $p > 0.05$ ) was significant. Effect of m-CPP ( $F = 0.30$   $p > 0.05$ ) was not significant. Interaction between haloperidol and m-CPP ( $F = 3.48$   $p > 0.05$ ) was also not significant. Post-hoc analysis showed that administration of m-CPP decreased 5-HIAA levels in haloperidol injected animals but not in saline injected animals. The levels of 5-HIAA were higher in haloperidol plus saline than saline plus saline treated animals.

The serotonergic system is known to inhibit DA neurotransmission at the level of the origin of DA system in the midbrain as well as in the terminal regions. It is reported that 5-HT-2C agonist and antagonist inhibit and enhance respectively, basal DA cell firing and DA release at terminals [23, 24].

The serotonergic system is an other neurochemical system that prominently regulates the oral activity. Striatum is a brain region rich in DA nerve terminals [25]. The administration of haloperidol increased 5-HT and 5-HIAA concentration in many brain regions including striatum [26], even after the drug withdrawal [10, 27]. Results of the present study also show an increase in 5-HIAA concentration (fig. 4) 48 hrs after withdrawal from repeated haloperidol treatment. Pre-clinical and clinical studies suggest that an increase in serotonin transmission may be important contributing factor in the onset of dyskinesia [28, 29]. The present study represents an increase in 5-HT metabolism in animal model of TD.

Bauman et al, 2001 [30] have reported that intravenous injection of d-fenfluramine and m-CPP exhibited similar potency in their ability to stimulate 5-HT efflux in vivo and interact with 5-HT transporters in vitro. This effect may well be involved in the increases of 5-HT levels that occur following the administration of m-CPP. Conversely, administration of m-CPP in saline injected animals lack of this effect in rats treated with haloperidol (Fig. 4) suggests a decrease in the responsiveness of 5-HT-2C receptors.

## Experimental

### Animals

Locally bred female albino Wistar rats weighing 180-220 g purchased from HEJ Research Institute of Chemistry, University of Karachi, Pakistan, were housed individually with free access to cubes of standard rodent diet and tap water 3 days before starting the experiment.

### Drugs

Haloperidol (Serenace, Searle, USA) purchased as injectable ampoules of 5 mg/ ml, was injected intraperitoneally at a dose of 1 mg/ kg body weight twice daily. The m-CPP purchased from Sigma was dissolved at a dose of 3 mg/ kg body weight. Control animals were injected with saline in volume of 1 ml/ kg body weight.

*Experimental protocol*

Twenty four animals were divided into two groups (i) saline injected (ii) haloperidol injected. The animals received respective treatment twice a day for 5 weeks as described by Merchese *et al.*, 2002 [8]. VCMs were scored weekly and after a wash out period of two days. Effects of m-CPP were monitored after a wash out period of 2 days.

Saline or haloperidol injected groups were further divided into saline and m-CPP injected groups. The animals were sacrificed 5 hrs after the drug administration to collect the striatum. The samples were stored at -70 °C for the neurochemical analysis by HPLC-EC as described before by Haleem and Khan, 2003 [10].

*Vacuous Chewing Movements (VCMs) quantification*

Animals were placed individually in a activity box (26 x 26 x 26 cm) with sawdust-covered floor and were allowed to adapt the observation cage for period of 15 minutes. VCMs monitored during 10 minutes observation period. For calculation purposes, each burst of purposeless chewing was counted as once only, if its duration was at least 3 seconds [8].

*Dissection of Striatum*

The dissection procedure was essentially same as described before [26]. A fresh brain was dipped in ice cold saline and placed with its ventral site up in molded cavity of a brain slicer. Fine fishing line wire was inserted into the slots of the slicer to give slices of 2 mm thickness. The slices containing striatum was transferred to a slide kept on ice. Punches of 2.5 mm diameter were made bilaterally in the striatum to collect the brain region.

*HPLC-EC determination of serotonin, dopamine and their metabolites:*

HPLC-EC determination was carried out as described before [10]. A 5  $\mu$  Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used. Separation was achieved by mobile phase containing methanol (14 %), octyl sodium sulfate (0.023 %) and EDTA (0.0035 %) in 0.1 M phosphate buffer of pH 2.9 at an operating pressure 2000-3000 psi on Shimadzu HPLC pump. Electrochemical detection was achieved on Shimadzu LEC 6A detector at an operating potential of 0.8 V.

*Statistical analysis:*

Data on effect of haloperidol withdrawal on VCMs analyzed by t-test. Data on effect of haloperidol on intensity of VCMs and neurochemistry analyzed by two-way ANOVA. Post-hoc comparison was done by Newman-Keuls test:  $p < 0.05$  taken as significant. Source: [31].

**Conclusions**

The present study was designed to monitor the responsiveness of 5-HT-2C receptor agonist m-CPP in rats exhibiting VCMs. The results illustrate that serotonergic responses of the drug were attenuated, while dopaminergic responses enhanced in rats treated with haloperidol for 5 weeks. The results are consistent with the notion that a decrease in the serotonergic influence on the activity of dopaminergic neurons is one of the major contributing factors in the pathophysiology of TD.

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