



Research report

Effects of 5-HT_{2A} and 5-HT_{2C} receptor antagonists on acute and chronic dyskinetic effects induced by haloperidol in ratsMeaghan Creed-Carson^{a,b}, Alhan O raha^{a,b}, José N. Nobrega^{a,b,c,d,*}^a Neuroimaging Research Section, Centre for Addiction and Mental Health, Toronto, Ontario, Canada^b Department of Pharmacology, University of Toronto, Ontario, Canada^c Department of Psychiatry, University of Toronto, Ontario, Canada^d Department of Psychology, University of Toronto, Ontario, Canada

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ABSTRACT

An important limitation of classical antipsychotic drugs such as haloperidol (HAL) is their liability to induce extrapyramidal motor symptoms acutely and tardive dyskinetic syndromes when given chronically. These effects are less likely to occur with newer antipsychotic drugs, an attribute that is often thought to result from their serotonin-2 (5-HT₂) receptor antagonistic properties. In the present study, we used selected doses of the 5-HT_{2A} antagonist M100,907, the 5-HT_{2C} antagonist SB242,084 and the mixed 5-HT_{2A/C} antagonist ketanserin to re-examine the respective roles of 2A vs. 2C 5-HT₂ receptor subtypes in both acute and chronic motor effects induced by HAL. Acutely, SB242,084 (0.5 mg/kg) reduced HAL-induced catalepsy, while M100,907 (0.5 mg/kg) and ketanserin (1 mg/kg) were without effect. None of the drugs reduced HAL-induced Fos expression in the striatum or frontal cortex, and M100,907 actually potentiated HAL-induced Fos expression in the n. accumbens. In rats chronically treated with HAL, both ketanserin and SB242,084 attenuated vacuous chewing movements, while M100,907 had no effect. In addition, 5-HT_{2C} but not 5-HT_{2A} mRNA levels were altered in several brain regions after chronic HAL. These results highlight the importance of 5-HT_{2C} receptors in both acute and chronic motoric side effects of HAL, and suggest that 5-HT_{2C} antagonism could be targeted as a key property in the development of new antipsychotic medications.

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1. Introduction

Treatment with classical antipsychotic drugs (APDs) such as haloperidol (HAL) is associated with both acute and chronic motor side effects. Acutely, these drugs may induce extrapyramidal symptoms (EPS), including akathisia, rigidity, tremor and bradykinesia, while prolonged treatment may result in tardive dyskinesia (TD) or tardive dystonia. Acute EPS and tardive syndromes differ in time of onset, clinical manifestation, persistence, and response to pharmacological agents [14,16]. While both acute and chronic motor side effects are less likely to occur with so called atypical APDs, the latter are not completely devoid of acute EPS effects [8] and, more importantly, are associated with other health-threatening side effects such as excessive weight gain and potentially fatal metabolic complications [35]. Renewed interest in mechanisms involved in motor side effects of classical APDs is also due in part to the need to

develop effective treatments for patients that have developed persistent TD before the advent of atypical APDs [40] and to inform the development of new medications devoid of such effects.

A prevailing hypothesis for the decreased incidence of EPS associated with atypical antipsychotics centers on serotonin-2 (5-HT₂) receptor antagonism [18,19,24–26,41]. In vivo brain imaging has revealed low occupancy of 5-HT₂ receptors by HAL at therapeutic doses, while the atypical APDs, olanzapine, sertindole, risperidone and clozapine occupy 80–100% of these receptors at therapeutic doses [44]. Given the inhibitory role of 5-HT on dopamine (DA) release from axon terminals, it has been suggested that antagonism of 5-HT₂ receptors would increase DA release and this might potentially reverse the effects of D₂ receptor blockade selectively in the nigrostriatal pathway [6].

5-HT_{2A} agonists positively modulate DA release under basal conditions, and 5-HT_{2A} antagonism decreases evoked DA release [1]. Conversely, 5-HT_{2C} receptors phasically and tonically inhibit DA release in the nucleus accumbens and caudate-putamen, while 5-HT_{2C} antagonism disinhibits DA release throughout the mesostriatal system [9,10]. There is evidence to suggest a differential involvement of 2A vs. 2C subtypes in the development of EPS [12,24,25,33]. A particularly relevant possibility relates to evidence

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that 2A and 2C antagonism may in several cases lead to opposite functional effects [15].

Previous work addressing the role of 5-HT receptors in HAL motoric side effects often failed to distinguish acute from long-term effects and used test compounds with limited specificity. In the present study, we re-examined the respective contributions of 5-HT_{2A} and 5-HT_{2C} receptor subtypes in both acute and chronic motor effects induced by HAL by using potent and selective 5-HT_{2A} and 5-HT_{2C} antagonists, namely M100,907 (previously MDL-100,907) and SB242,084, respectively. For comparative purposes we also tested the effects of the mixed 5-HT_{2A/2C} antagonist ketanserin. In acute studies catalepsy was used as a prototypical behavioural index of HAL-induced EPS, and patterns of brain Fos expression were chosen as a typical brain response. We were particularly interested in the possibility that addition of 5-HT₂ antagonists could convert the characteristic Fos pattern induced by HAL into a pattern similar to the one obtained with atypical APDs such as clozapine [36,37]. In chronic HAL studies we tested the effects of 5-HT₂ antagonists on vacuous chewing movements (VCMs), a well-documented behavioural effect of prolonged classical APD use [14,43,47]. We also examined chronic HAL effects on 5-HT_{2A} and 5-HT_{2C} gene expression in brain, in an effort to further ascertain the contribution of each of these receptor subtypes to the pathophysiology of chronic HAL-induced motor side effects.

2. Materials and methods

2.1. Subjects

Adult male Sprague-Dawley rats (Charles River, Quebec) were pair-housed and maintained on a 12-h light/dark cycle (lights on at 8:00 AM), with ad libitum access to food and water. All tests and treatments were conducted according to the guidelines of the Canadian Council on Animal Care and were approved by the Centre for Addiction and Mental Health Animal Care Committee. All behavioural tests were performed by a trained observer blind to treatment conditions.

2.2. Test drugs

Haloperidol and haloperidol decanoate were obtained from Sabex (Quebec, Canada). Ketanserin was purchased from Tocris (Burlington, Canada) and M100,907 ((R)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]-4-piperidyl]methanol) and SB242,084 (6-chloro-5-methyl-1-[2-(2-methylpyrid-5-yl carbonyl)] indoline) from Sigma-Aldrich (Oakville, Canada). Since the study involved several drugs in both acute and chronic regimens, a decision was made to carefully select a single appropriate dose for each test compound. Thus acute HAL effects were induced by 0.5 mg/kg given s.c. as in previous work [29,30] and HAL chronic effects were evaluated after 12 weeks of treatment with HAL decanoate (21 mg/kg given i.m. once every three weeks), as in previous work [45–47]. Likewise, doses for M100,907 (0.5 mg/kg) and SB242,084 (0.5 mg/kg) were carefully chosen on the basis of previous work demonstrating their respective ability to effectively block 5-HT_{2A}- or 2C-dependent behaviour [15,34] while minimizing effects at non-target 5-HT₂ subtype in each case.

2.3. Acute drug administration and catalepsy

Rats weighing 214–275 g on the day of the experiment were used. Fifty-four animals were administered HAL (0.5 mg/kg s.c.) and 22 received saline vehicle. This was immediately followed by administration of M100,907 (0.5 mg/kg dissolved in 0.9% saline containing 0.3% Tween), ketanserin (1.0 mg/kg, i.p. in 0.9% saline), SB242,084 (0.5 mg/kg i.p. in 8% cyclodextrin and 0.52% citric acid) or their respective vehicles. In all experiments drug doses were calculated as the base. Catalepsy was assessed 110 min after the drug injections in a quiet room. The rat's forepaws were gently placed over the edge of a platform raised 7 cm above the working surface and the animal was slowly released. Catalepsy was scored as the latency to remove both forepaws from this position or to climb onto the platform. If this did not occur within 3 min, the test was terminated and a latency of 180 s was recorded.

2.4. Fos immunocytochemistry

Animals were injected subcutaneously with HAL (0.5 mg/kg; $n = 33$) or vehicle and then immediately received an i.p. injection of either M100,907 (0.5 mg/kg), ketanserin (1 mg/kg), SB242,084 (0.5 mg/kg), or vehicle. Two hours after the injections, rats were sacrificed with an overdose of sodium pentobarbital and perfused with saline and 10% formaldehyde. The brains were post-fixed in 4% paraformaldehyde, transferred to sucrose solutions (10% for 2 h, 20% for 12 h, and 30% for 24 h),

and then dried and stored at -80°C until processing. Fos immunoreactive nuclei, labeled with antiserum raised in rabbits against the Fos peptide 4–17 amino acids of human Fos (Oncogene Research Products, Cambridge, MA), were counted within a $400\ \mu\text{m} \times 400\ \mu\text{m}$ grid at a magnification of $\times 100$ using an MCID Elite system (InterFocus Imaging, Linton, UK). Cell counts were obtained from the shell and core of the nucleus accumbens, dorsolateral caudate-putamen, and prefrontal cortex [32,37] from at least three separate brain sections for each brain in at least 5 subjects per group.

2.5. Vacuous chewing movements (VCMs) induced by chronic haloperidol

Twenty-four rats were treated with HAL decanoate (21 mg/kg, i.m.) once every three weeks, the equivalent of approximately 1 mg/kg/day. Another sixteen rats received i.m. injections of the sesame oil vehicle. This regimen was maintained for 17 weeks, with VCM assessments occurring once a week. For VCM assessments, rats were placed on a cylindrical platform (height: 50 cm; diameter: 26 cm) and allowed 2 min to acclimate. Over the subsequent 2 min, VCMs were quantified by a trained observer blind to treatment condition. VCMs were defined as jaw movements in the vertical plane not directed at any object. Starting on week 13 HAL-treated rats were challenged with each of the 3 test drugs before the weekly VCM observations in a counterbalanced within-subject design.

2.6. In situ hybridization (ISH)

Hybridization was performed using ³⁵S-UTP labeled riboprobes complementary to regions of 5-HT_{2A} or 5-HT_{2C} receptor mRNA [5-HT_{2C} (GenBank Accession # NM.012765) (ACGT Corp) left primer: 5'-attaggtgacactatagaacagaaaaagcctctgttc-3', and 5-HT_{2C} right primer: 5'-taatacgactcactatagggatcctctgctgaccacat-3'; 5-HT_{2A} (GenBank Accession # NM.017254) (ACGT Corp) left primer: 5'-attaggtgacactatagaacagggagcagaaaaaga-3', and 5-HT_{2A} right primer: 5'-taatacgactcactatagggatcctctgacgctcctatcc-3']. Using the NCBI BLAST Tool, the sequences were checked for homology with the rat genome and found to be specific for their respective transcripts. Probes were diluted to a concentration of 200,000 cpm/ μl in hybridization solution containing: 50% formamide, 35% Denhardt's solution, 10% dextran sulfate, $0.1 \times \text{SSC}$, salmon sperm DNA (300 $\mu\text{g}/\text{ml}$), yeast tRNA (100 $\mu\text{g}/\text{ml}$), and DTT (40 μM). Slides were incubated in plastic mailers overnight at 60°C . After hybridization, sections were rinsed in $4 \times \text{SSC}$ at 60°C , treated in RNase A (20 $\mu\text{g}/\text{ml}$) solution at 45°C for 40 min, washed with agitation in decreasing concentrations of SSC containing 25 g/ml sodium thiosulfate, dipped in water, dehydrated in 70% ethanol, and air-dried. The slides were exposed to Hyperfilm β -Max film (Amersham, Quebec) for 4 weeks at 4°C along with calibrated radioactivity standards. Probe specificity was confirmed by testing labeled sense and scrambled probes, both of which produced no measurable signal on film.

2.7. Statistical analyses

Analyses were done with SPSS version 12.0 (Chicago, IL). The statistical significance of differences among treatment groups was first determined by analyses of variance followed by Bonferroni-adjusted tests. $p \leq 0.05$ was considered to be statistically significant.

3. Results

3.1. Catalepsy induced by acute haloperidol

A two-way ANOVA indicated significant main effects of HAL ($F_{1,63} = 139.2$, $p < 0.001$), 5-HT₂ antagonist ($F_{3,63} = 4.97$, $p < 0.004$) and their interaction ($F_{3,63} = 4.65$, $p < 0.005$). As expected, rats receiving a single injection of haloperidol (0.5 mg/kg s.c.) showed significantly higher catalepsy scores than their vehicle-treated counterparts ($p < 0.001$). As shown in Fig. 1, co-administration of M100,907 or ketanserin had no effect on HAL-induced catalepsy, while SB242,084 reduced the HAL effect by more than 50% ($p < 0.02$). A lower dose of SB242,084 (0.25 mg/kg) and a higher dose of ketanserin (1.5 mg/kg) did not affect catalepsy (data not shown). Vehicle-treated rats did not display catalepsy scores significantly different from zero and these scores were not affected by any of the test compounds (Fig. 1).

3.2. Fos expression induced by acute haloperidol

Separate ANOVAs for each brain region revealed significant HAL main effects in the dorsolateral caudate-putamen (CPu) ($F_{1,52} = 140.97$, $p < 0.001$), medial prefrontal cortex (PFC)

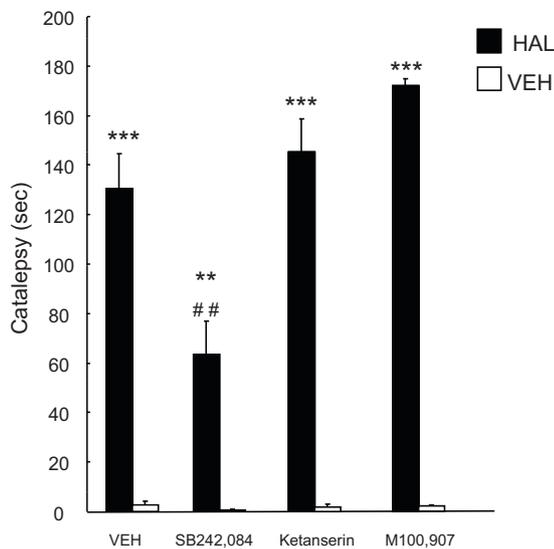


Fig. 1. Effect of 5-HT_{2A} or 5-HT_{2C} blockade on HAL-induced catalepsy. Each bar represents mean catalepsy score (\pm SEM) of 6–12 rats per group. Separate groups were given vehicle or HAL (0.5 mg/kg s.c.) followed by i.p. injections of the appropriate test compound or vehicle. Ketanserin (1.0 mg/kg) and M100,907 (0.5 mg/kg) had no effect on HAL-induced catalepsy ($p > 0.05$). SB242,084 (0.5 mg/kg) significantly attenuated HAL-induced catalepsy. ** $p < 0.02$, *** $p < 0.001$ in comparison to the VEH group receiving the same 5-HT₂ blocker. $p < 0.02$ in comparison to the HAL-VEH group (Bonferroni comparisons).

($F_{1,52} = 4.78$, $p < 0.03$), nucleus accumbens (NAc) shell ($F_{1,52} = 39.79$, $p < 0.001$) and core ($F_{1,52} = 18.99$, $p < 0.001$). The main effect of 5-HT₂ blockers was significant only in the nucleus accumbens core ($F_{3,52} = 6.53$, $p < 0.001$) and the HAL \times 5-HT₂ blocker interaction was not significant in any of the four regions examined.

Bonferroni-adjusted comparisons confirmed that acute HAL administration increased Fos expression in the dorsolateral CPu ($p < 0.0001$), NAc core ($p = 0.004$) and shell ($p = 0.001$) but not in the PFC ($p > 0.05$) (Fig. 2). Fig. 3 illustrates the typical effect of acute HAL on Fos levels in the dorsolateral CPu. As illustrated in Fig. 2, this basic HAL effect was not modified by ketanserin or SB242,084 in any of the four brain regions studied. In contrast, M100,907 significantly potentiated HAL-induced Fos expression in the NAc core and shell ($p < 0.05$). In vehicle-treated rats ketanserin tended to reduce Fos levels as compared to the vehicle-vehicle group in the NAc and in the PFC but these effects did not reach statistical significance ($p > 0.05$, Fig. 2).

For comparison purposes, a separate group of animals received acute clozapine, the prototype atypical antipsychotic drug. As expected, Fos levels were significantly increased by clozapine in the NAc shell and core and PFC ($p < 0.05$), but not in the dorsolateral CPu (Fig. 2). None of the test compounds, when administered concurrently with HAL, were able to transform the pattern of Fos induction into a pattern similar to that induced by clozapine (Fig. 2).

3.3. Vacuous chewing movements induced by chronic haloperidol

As expected, HAL-treated animals exhibited significantly more VCMs than vehicle-treated animals at all time points after the onset of HAL treatment (Fig. 4). After 12 weeks of HAL treatment, the effects of acute administration of the three 5-HT₂ blockers were assessed by a repeated measures ANOVA ($F_{4,80} = 6.54$, $p < 0.001$) followed by Bonferroni-adjusted paired t tests. As illustrated in Fig. 5, SB242,084 ($p < 0.006$) and ketanserin ($p < 0.02$) but not M100,907 significantly reduced VCMs in HAL-treated rats in comparison to acute vehicle.

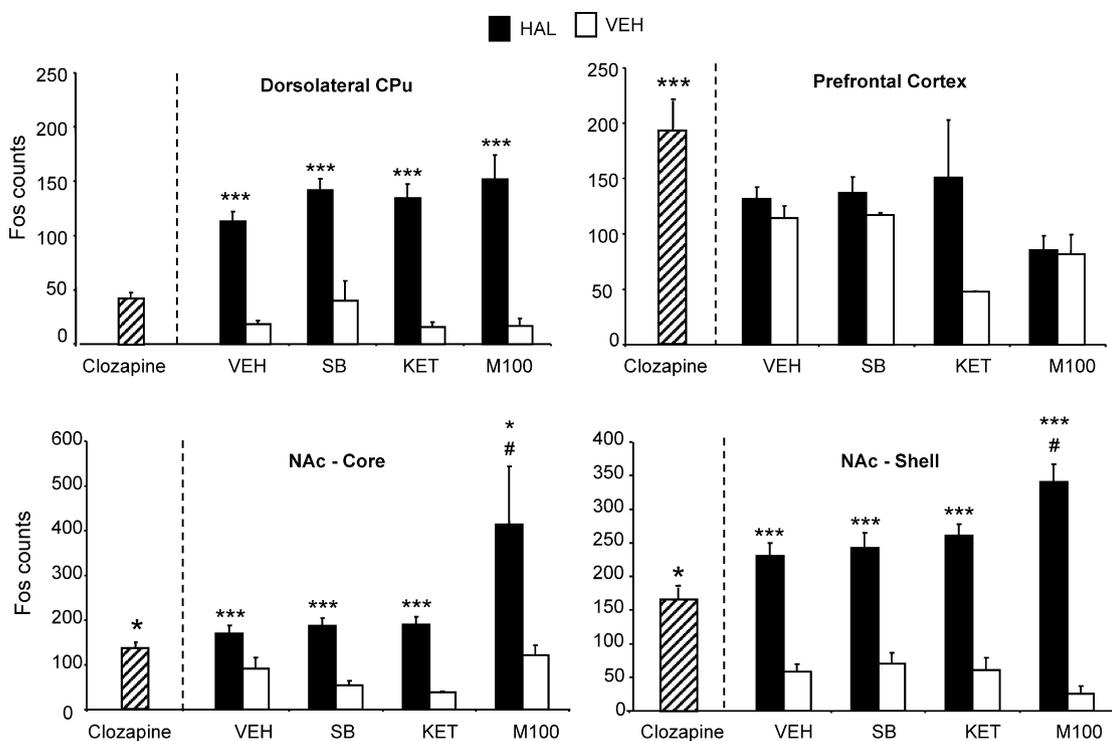


Fig. 2. Effect of 5-HT_{2A} or 5-HT_{2C} blockade on HAL-induced Fos expression. Each bar represents mean Fos counts \pm SEM of 3–6 rats per group. Abbreviations: CPu, caudate putamen, Acb, accumbens; SB, SB242,804; KET, ketanserin; M100, M100,907. Compared to vehicle (open bars), HAL (filled bars) induced significant increases in Fos levels in all regions, except for the prefrontal cortex. None of the 5-HT₂ antagonists attenuated this basic HAL effect. M100,907 significantly potentiated HAL effects in the n. accumbens (# $p < 0.05$ compared to the HAL-VEH group).

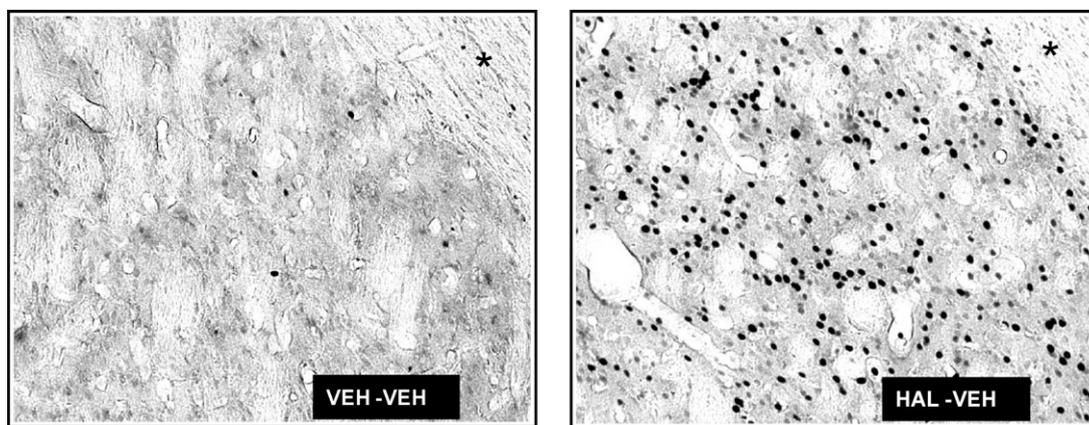


Fig. 3. Representative photomicrograph illustrating pattern of Fos induction by HAL in dorsolateral caudate putamen. Rats were sacrificed 2 h after vehicle or HAL (0.5 mg/kg). The asterisk indicates the location of the corpus callosum on both panels.

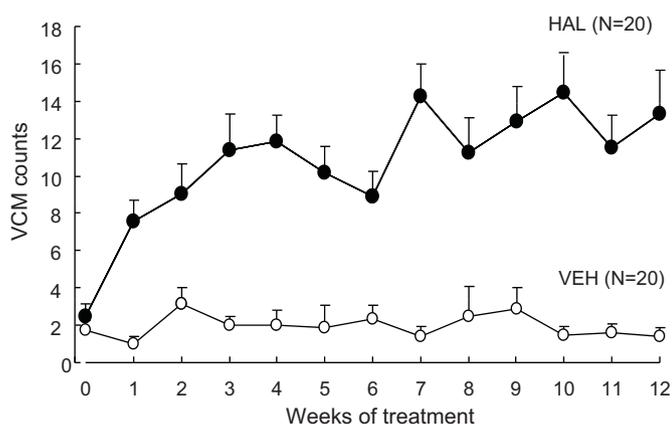


Fig. 4. Development of VCMs during chronic HAL treatment. Rats received 21 mg/kg haloperidol decanoate once every 3 weeks and were assessed once a week. Except for baseline (week 0) all points are statistically different between HAL and VEH groups ($p < 0.01$).

3.4. Changes in 5-HT₂ receptor mRNA expression after chronic haloperidol

Several brain regions known to express 5-HT₂ receptors were examined for mRNA levels (Fig. 6). Eighteen weeks of HAL treat-

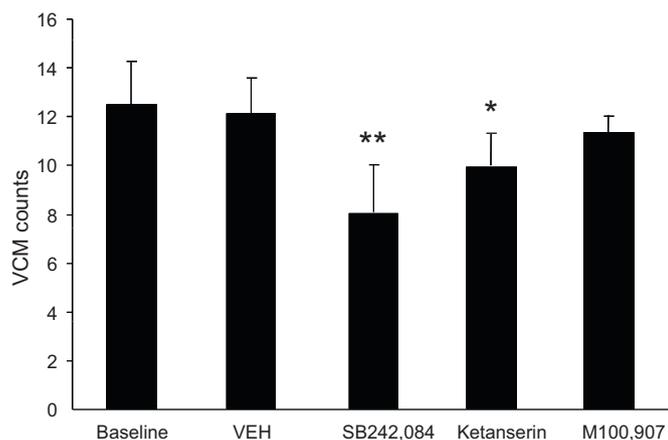


Fig. 5. Effects of 5-HT_{2A} or 5-HT_{2C} blockade on HAL-induced VCMs. Each bar represents mean VCM counts \pm SEM in 3 separate determinations. Compared to vehicle, SB242,084 (SB, 0.5 mg/kg) and ketanserin (1 mg/kg, i.p.) significantly reduced VCMs, while M100,907 (0.5 mg/kg) had no effect. * $p < 0.02$; ** $p < 0.006$ compared to vehicle (Bonferroni-adjusted paired t tests).

ment had no effect on levels of 5-HT_{2A} receptor mRNA in any brain region examined (Table 1). In contrast, chronic HAL treatment caused significant decreases in 5-HT_{2C} mRNA levels in the dorsal ($p = 0.02$) and ventral ($p = 0.03$) CPU, and a significant increase in the ventral ($p = 0.046$) and dorsal aspects of the and dorsomedial thalamus ($p = 0.006$) (independent t tests, Table 2).

4. Discussion

The aim of the present study was to assess the role of 5-HT_{2A} and 5-HT_{2C} receptors in both acute and chronic motoric effects induced by haloperidol. It was found that 5-HT_{2C}, but not 5-HT_{2A} antagonism decreased catalepsy induced by acute HAL. However, none of the 5-HT₂ antagonists significantly modified the typical pattern of brain Fos expression induced by HAL, thus suggesting a dissociation between Fos induction and acute catalepsy induced by haloperidol. After chronic HAL, both the mixed 5-HT_{2A/2C} antagonist ketanserin and the 5-HT_{2C} antagonist SB242,084, but not the 5-HT_{2A} antagonist M100,907, significantly attenuated VCMs. Chronic HAL treatment did not appreciably affect 5-HT_{2A} mRNA levels, but did alter 5-HT_{2C} mRNA levels in dorsal striatum and thalamic nuclei.

4.1. Effects of acute haloperidol

We found that SB242,084, but not ketanserin or MDL-100,907, attenuated HAL-induced catalepsy. Catalepsy is thought to result

Table 1
5-HT_{2A} mRNA levels after chronic HAL.^a

	VEH (N=7)	HAL (N=5)
Cortex		
Prefrontal	26.27 \pm 1.14	25.88 \pm 0.47
Prefrontal, layer 2	17.89 \pm 1.50	16.87 \pm 1.15
Cingulate	29.12 \pm 0.99	28.74 \pm 1.19
Cingulate, layer 2	18.23 \pm 1.59	17.74 \pm 1.07
Piriform	33.44 \pm 1.78	32.53 \pm 1.81
n. Accumbens—shell	14.04 \pm 1.62	11.99 \pm 1.53
n. Accumbens—core	11.63 \pm 1.35	10.89 \pm 1.29
Caudate-putamen		
Anterior	9.20 \pm 1.06	9.50 \pm 1.55
Dorsomedial	8.49 \pm 0.98	8.67 \pm 0.80
Dorsolateral	8.83 \pm 1.07	9.11 \pm 0.81
Ventrolateral	8.95 \pm 1.04	9.17 \pm 0.81
Ventromedial	8.62 \pm 1.04	8.28 \pm 0.74
Posterior, dorsal	9.00 \pm 0.82	11.02 \pm 1.09
Posterior, ventral	9.46 \pm 0.95	12.08 \pm 1.12

^a Values are means \pm SEM in μ ci/gT. No significant differences were found.

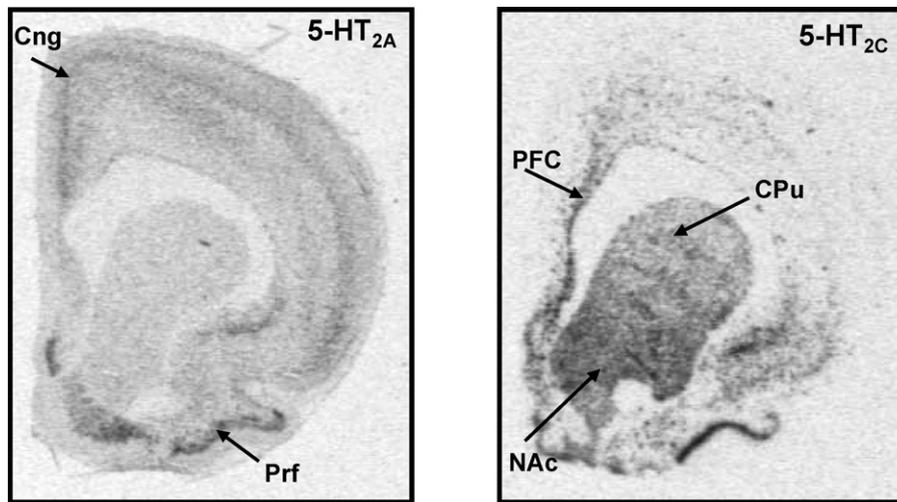


Fig. 6. Representative photomicrograph illustrating 5-HT_{2A} and 5-HT_{2C} gene expression. Abbreviations: Prf, piriform cortex; Cng, anterior cingulate cortex; PFC, prefrontal cortex; NAc, nucleus accumbens; CPu, caudate-putamen.

from blocked signaling through post-synaptic D₂ receptors [52], an effect that may be modulated by 5-HT activity. SB242,084 has been shown to increase midbrain dopaminergic transmission [11] and this may account in part for its beneficial effects on HAL-induced catalepsy.

4.1.1. Fos effects

It is well established that HAL, but not atypical APDs such as clozapine, increase Fos expression in the dorsolateral CPu, which agrees with our present observations [36,37]. Further, the favorable profile of motor side effects exhibited by clozapine has been attributed to its antagonism of 5-HT_{2A} receptors. In the present study, however, either 5-HT_{2A} or 5-HT_{2C} antagonism modified the HAL-induced Fos expression pattern. In fact the 5-HT_{2A} antagonist

M100,907 enhanced HAL-induced Fos expression in the NAc core and shell. Since NAc neurons activated by HAL have been shown to express 5-HT_{2A} receptors [21], it is conceivable that concurrent administration of HAL and M100,907 could produce a synergistic activation effect in these cells.

Irrespective of exact mechanisms involved, Fos induction in the dorsolateral CPu has been shown to correlate well with severity of cataleptic symptoms [7,27,37–39]. We found however that the attenuation of HAL-induced catalepsy by SB242,084 was not accompanied by changes in HAL-induced Fos levels in the dorsolateral CPu. Our findings therefore suggest an interesting dissociation between Fos induction and acute cataleptic behaviour induced by haloperidol.

4.2. Effects of chronic haloperidol

4.2.1. Oral dyskinesias

Previous studies examining the role of 5-HT₂ receptors in vacuous chewing movements (VCMs) have produced inconsistent results. Takeuchi et al. [42] reported that acute doses of the 5-HT_{2A/2C} antagonist ritanserin did not attenuate VCMs, but did prevent the development of VCMs when administered concurrently with HAL over 4 weeks; paradoxically however VCMs did emerge despite co-treatment with ritanserin by 6 weeks [42]. Other studies have reported that acute and chronic administration of the 5-HT_{2A/2C} receptor antagonists, seganserin, ketanserin and ritanserin, over 3 weeks reduced HAL-induced VCMs in a dose-dependent manner [28]. However, when administered acutely at very high doses (1.0 mg/kg), ritanserin increased VCMs, ketanserin decreased VCMs, while seganserin had no effect on VCM levels [28].

Two factors that may explain these inconsistencies are failure to distinguish “early” from “late” VCMs and poor specificity of 5-HT₂ antagonists. It has been shown that VCMs emerging in the first 3 weeks of HAL treatment (so called “early VCMs”) are pharmacologically distinct from late VCMs, which may reflect morphological and functional changes associated with prolonged treatment [13,22,23]. Moreover, the use of poorly selective 5-HT₂ antagonists in previous studies failed to distinguish between the effects of 2A and 2C subtypes, while evidence suggests differential involvement of 2A and 2C subtypes in the development of EPS [12,24,25].

In the present study, both the mixed 5-HT_{2A/C} antagonist ketanserin and the 5-HT_{2C} antagonist SB242,084 significantly attenuated VCMs after chronic HAL treatment, although the effects were more pronounced with 5-HT_{2C} antagonist treatment. This is

Table 2
5-HT_{2C} mRNA levels after chronic HAL.^a

	VEH (N = 7)	HAL (N = 5)
Cortex		
Prefrontal	24.97 ± 1.86	21.41 ± 1.74
Cingulate	16.73 ± 1.30	17.96 ± 0.78
Piriform	63.34 ± 3.89	54.08 ± 2.87
n. Accumbens shell	32.6 ± 0.79	32.18 ± 2.39
n. Accumbens core	36.80 ± 1.42	36.63 ± 1.00
Caudate-putamen		
Anterior	19.71 ± 0.80	17.47 ± 0.94
Dorsomedial	14.65 ± 0.16	13.09 ± 0.67*
Dorsolateral	8.16 ± 0.29	8.08 ± 0.52
Ventrolateral	11.36 ± 0.31	11.14 ± 0.39
Ventromedial	19.03 ± 0.45	17.13 ± 0.58*
Posterior, dorsal	9.27 ± 0.35	8.42 ± 0.37
Posterior, ventral	7.91 ± 0.26	7.87 ± 0.32
Choroid plexus	198.59 ± 8.55	192.33 ± 6.31
Thalamus		
Laterodorsal, lateroventral	15.04 ± 0.91	19.09 ± 1.74*
Laterodorsal, ventromedial	10.41 ± 1.14	15.48 ± 1.70*
Central n., ventral	27.26 ± 1.18	30.51 ± 2.37
Central n., medial	30.85 ± 2.04	32.30 ± 3.90
Lateroposterior n.	20.06 ± 2.48	25.84 ± 3.14
Posterior n.	6.24 ± 1.46	9.73 ± 1.71
Parafascicular n.	25.80 ± 0.80	28.61 ± 3.78
Subthalamic nucleus	113.61 ± 3.26	125.69 ± 7.11
Subst. nigra pars compacta	44.56 ± 5.23	48.94 ± 3.47
Subst. nigra pars reticulata	19.24 ± 3.71	19.22 ± 1.45

^a Values are means ± SEM in $\mu\text{ci/gT}$.

* $p < 0.05$.

in good agreement with previous findings in HAL-treated rats that had undergone neonatal 6-OHDA lesions [20]. In addition to being expressed in the striatum, 5-HT_{2C} receptors are located in the subthalamic nucleus (STN) on glutamatergic projection neurons to the globus pallidus internus (GPI) and substantia nigra pars reticulata (SNr) [9,12]. Intra-subthalamic infusion of the 5-HT_{2C} agonist mCPP is sufficient to elicit VCMs, implicating these receptors in orofacial dyskinesias [12]. 5-HT_{2C} receptors are also located on GABA interneurons in the SNr which project to the pars compacta (SNc) [12]. This provides a potential mechanism whereby 5-HT_{2C} could mediate inhibition of DA cellular activity in the SNc, which is the main source of DA projections to the striatum [50]. Antagonism of these 5-HT_{2C} receptors would be expected to increase DA release to the striatum, where HAL exerts its motor effects.

The mixed 5-HT_{2A/2C} antagonist ketanserin but not the highly selective 5-HT_{2A} antagonist M100,907 also reduced VCMs. It is conceivable that the ketanserin effects reflected activity at 5-HT_{2C} receptors. While ketanserin exhibits a three-fold higher affinity for the 5-HT_{2A} receptor subtype over the 5-HT_{2C} subtype, it may have still affected 5-HT_{2C} receptors

4.2.2. Effects on 5-HT_{2A} and 5-HT_{2C} mRNA

Results from previous studies examining the effects of HAL on 5-HT receptor mRNA have been inconsistent. In agreement with previous studies in normal drug-naïve rats, we report 5-HT_{2A} in the prefrontal, piriform and cingulate cortices, with lower expression in the NAc core and shell and even lower expression throughout the CPu. 5-HT_{2C} mRNA was detected in the same regions, in addition to the choroid plexus, thalamus, SNc, SNr and STN.

Consistent with most previous studies [2,4,5,17,51], we report that HAL had minimal effects on levels of 5-HT_{2A} mRNA in all regions tested. Previously, a decrease in 5-HT_{2A} mRNA in the hippocampus and midbrain after 32 days of HAL treatment was reported [3]. In all previous studies, however, the duration of HAL treatment ranged between 14 and 36 days, whereas here we examined effects of HAL administered for 18 weeks. While 5-HT_{2A} is a unique GPCR in that chronic antagonism paradoxically leads to pronounced down-regulation rather than up-regulation [48], the failure of HAL to alter levels of 5-HT_{2A} mRNA is not unexpected given the weak affinity of HAL for the 5-HT_{2A} receptor [24]. However, it must be noted that 5-HT_{2A} binding and activity do not necessarily change in parallel with mRNA levels [3,5].

In contrast, we found significant decreases in 5-HT_{2C} mRNA levels in the ventromedial and dorsomedial CPu after chronic HAL. Consistent with this, Burnet et al. [5] reported a small, albeit non-significant decrease in 5-HT_{2C} mRNA in the striatum after 14 days of HAL treatment, and a concomitant decrease in 5-HT and 5-HIAA levels in the striatum only. Other groups have reported 32–41% decreases in 5-HT_{2C} mRNA levels in the cerebellum after 32 days HAL and a 42% decrease in the SNc after 36 days of HAL [3,17]. Discrepancies between studies could again be due to shorter durations of HAL administration in previous studies.

Dopaminergic inputs to the striatum regulate 5-HT receptor gene expression. D2 receptor signaling inhibits the expression of 5-HT₂ mRNA in the striatum, although the differential regulation of 2A vs. 2C subtypes is not clear [31]. Furthermore, as in the case of 5-HT_{2A}, 5-HT_{2C} antagonism causes a paradoxical down-regulation that could also contribute to the observed decrease in 5-HT_{2C} mRNA. However, increases in 5-HT_{2C} signaling after chronic HAL administration have been reported. Wolf et al. [51] showed an adaptive increase in 5-HT_{2C} coupling to G-proteins as a result of repeated HAL administration, which was limited to the striatum. It is not clear how this increase in coupling is mediated, but it is thought to involve post-translational modifications of the 5-HT_{2C} receptor [51]. In the striatum, 5-HT_{2C} receptors are located on medium spiny interneurons, which regulate information outflow to

the STN and GPe [49]. Intra-striatal infusion of the 5-HT_{2A/2C} agonist mCPP induces orofacial dyskinetic movements [12]. In agreement with these previous observations, our present results implicate enhanced 5-HT_{2C} signaling in both acute and chronic HAL-induced motoric effects.

5. Conclusion

This may be the first examination of effects of 5-HT 2A vs. 2C antagonism on both acute and chronic brain and behavioural effects of HAL under the same laboratory conditions. We found that 5-HT_{2C} antagonism reduced motor effects induced by both acute and chronic HAL administration. 5-HT_{2A} antagonism did not affect either class of motor symptoms, whereas at the doses used, the mixed antagonist ketanserin attenuated HAL-induced VCMs but had no effect on catalepsy. None of the antagonists reduced HAL-induced Fos expression in the dorsolateral CPu, the area implicated in the cataleptic effects of HAL. Our results implicate 5-HT_{2C} receptors in the development of both acute and chronic motor effects induced by HAL, and suggest that 5-HT_{2C} antagonism could be targeted as a key property of new antipsychotic medications.

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