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Chronic betahistine co-treatment reverses olanzapine's effects on dopamine D2 but not 5-HT_{2A/2C} bindings in rat brains

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Abstract

Olanzapine is widely prescribed for treating schizophrenia and other mental disorders, although it leads to severe body weight gain/obesity. Chronic co-treatment with betahistine has been found to significantly decrease olanzapine-induced weight gain; however, it is not clear whether this co-treatment affects the therapeutic effects of olanzapine. This study investigated the effects of chronic treatment of olanzapine and/or betahistine on the binding density of the serotonergic 5-HT_{2A} (5-HT_{2AR}) and 5-HT_{2C} (5-HT_{2CR}) receptors, 5-HT transporter (5-HTT), and dopaminergic D₂ receptors (D_{2R}) in the brain regions involved in antipsychotic efficacy, including the prefrontal cortex (PFC), cingulate cortex (Cg), nucleus accumbens (NAc), and caudate putamen (CPu). Rats were treated with olanzapine (1 mg/kg, t.i.d.) or vehicle for 3.5 weeks, and then olanzapine treatment was withdrawn for 19 days. From week 6, the two groups were divided into 4 groups (n = 6) for 5 weeks' treatment: (1) olanzapine-only (1 mg/kg, t.i.d.), (2) betahistine-only (9.6 mg/kg, t.i.d.), (3) olanzapine and betahistine co-treatment (O + B), and (4) vehicle. Compared to the control, the olanzapine-only treatment significantly decreased the bindings of 5-HT_{2AR}, 5-HT_{2CR}, and 5-HTT in the PFC, Cg, and NAc. Similar changes were observed in the rats receiving the O + B co-treatment. The olanzapine-only treatment significantly increased the D_{2R} binding in the Cg, NAc, and CPu, while the betahistine-only treatment reduced D_{2R} binding. The co-treatment of betahistine reversed the D_{2R} bindings in the NAc and CPu that were increased by olanzapine. Therefore, chronic O + B co-treatment has similar effects on serotonin transmission as the olanzapine-only treatment, but reverses the D_{2R} that is up-regulated by chronic olanzapine treatment. The co-treatment maintains the therapeutic effects of olanzapine but decreases/prevents the excess weight gain.

Keywords

Betahistine, Dopamine receptor, Olanzapine, Receptor binding, Serotonin receptor

Disciplines

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Chronic betahistine co-treatment reverses olanzapine's effects on dopamine D₂ but not 5-HT_{2A/2C} bindings in rat brains

Running title: Chronic betahistine and olanzapine co-treatment affects serotonin and dopamine receptor binding

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Abstract

Olanzapine is widely prescribed for treating schizophrenia and other mental disorders although it leads to severe body weight gain/obesity. Chronic co-treatment with betahistine has been found to significantly decrease olanzapine-induced weight gain; however it is not clear whether this co-treatment affects the therapeutic effects of olanzapine. This study investigated the effects of chronic treatment of olanzapine and/or betahistine on the binding density of the serotonergic 5-HT_{2A} (5-HT_{2A}R) and 5-HT_{2C} (5-HT_{2C}R) receptors, 5-HT transporter (5-HTT), and dopaminergic D₂ receptors (D₂R) in the brain regions involved in antipsychotic efficacy, including the prefrontal cortex (PFC), cingulate cortex (Cg), nucleus accumbens (NAc), and caudate putamen (CPu). Rats were treated with olanzapine (1 mg/kg, t.i.d.) or vehicle for 3.5 weeks, and then olanzapine treatment was withdrawn for 19 days. From week 6, the two groups were divided into 4 groups (n=6) for 5 weeks' treatment: (1) olanzapine-only (1 mg/kg, t.i.d.), (2) betahistine-only (9.6 mg/kg, t.i.d.), (3) olanzapine and betahistine co-treatment (O+B), and (4) vehicle. Compared to the control, the olanzapine-only treatment significantly decreased the bindings of 5-HT_{2A}R, 5-HT_{2C}R, and 5-HTT in the PFC, Cg and NAc. Similar changes were observed in the rats receiving the O+B co-treatment. The olanzapine-only treatment significantly increased the D₂R binding in the Cg, NAc, and CPu, while the betahistine-only treatment reduced D₂R binding. The co-treatment of betahistine reversed the D₂R bindings in the NAc and CPu that were increased by olanzapine. Therefore, the chronic O+B co-treatment has similar effects on serotonin transmission as the olanzapine-only treatment, but reverses the D₂R that is up-regulated by chronic olanzapine treatment. The co-treatment maintains the therapeutic effects of olanzapine but decreases / prevents the excess weight gain.

Key Words: olanzapine; betahistine; dopamine receptor; serotonin receptor; receptor binding

LIST OF ABBREVIATIONS

5-HT_{2A}R, serotonin 5-HT_{2A} receptor

5-HT_{2C}R, serotonin 5-HT_{2C} receptor

5-HTT, serotonin 5-HT transporter

ANOVA, analysis of variance

CAFE, comparison of atypical anti-psychotics for first episode

CATIE, the clinical anti-psychotic trials of intervention effectiveness

Cg, cingulate cortex

CPu, caudate putamen

D₂R, dopamine D₂ receptor

EPS, extrapyramidal symptoms

FGAs, first generation antipsychotic drugs

HIP, hippocampus

H₁R, histamine H₁ receptor

NAc, nucleus accumbens

NAcC, nucleus accumbens core

NAcS, nucleus accumbens shell

PFC, prefrontal cortex

SGAs, second generation antipsychotic drugs

SN, substantia nigra

VTa, ventral tegmental area

1. Introduction

Second generation antipsychotic drugs (SGAs) such as olanzapine have improved tolerability compared with first generation antipsychotic drugs (FGAs) as they produce fewer extrapyramidal symptoms (EPS) (Leucht et al., 2009). However, SGAs, such as olanzapine, can induce severe body weight gain and obesity side-effects (Lambert, 2011, Deng, 2013). For example, the CATIE (The Clinical Anti-psychotic Trials of Intervention Effectiveness) study reported that olanzapine caused significant weight gain (>7% from baseline) in highest proportion of chronic schizophrenia patients (30%) and amount of weight gain (average 0.9kg/month) over an 18 months period, compared with quetiapine (16% and 0.23kg/month respectively), risperidone (14% and 0.18kg/month respectively) and ziprisidone (7% and 0.14 kg/month respectively) (Lieberman et al., 2005). Furthermore, the CAFE (Comparison of Atypical Anti-psychotics for First Episode) study showed that the 80% of olanzapine-treated patients had significant weight gain (average 1.76 kg/month), compared with 57.6% of risperidone (average 1.28 kg/month), and 50% of quetiapine (average 1.29 kg/month) (Patel et al., 2009) after 52 weeks' treatment (Patel et al., 2009). Compared with FGAs, olanzapine is a less potent antagonist at the dopamine D₂ receptor (D₂R), having a pharmacological binding profile by binding at a wide range of non-dopaminergic G-protein-coupled receptors including serotonin 5-HT_{2A} (5-HT_{2A}R) and 5-HT_{2C} (5-HT_{2C}R), histamine H₁ (H₁R) and muscarinic M₁ receptors (Nasrallah, 2008, Lian et al., 2013). Among these receptors, the dopamine D₂ and 5-HT₂ receptors play critical roles in the therapeutic effects of olanzapine and other SGAs (Meltzer and Massey, 2011, Ginovart and Kapur, 2012).

Dopamine receptor occupation (which for most antipsychotics is within the range of 65-78%), especially at the D₂R, is crucial to achieve the optimal therapeutic effects of most antipsychotics with minimal EPS (Seeman, 2011, Ginovart and Kapur, 2012). Three major

dopaminergic pathways involved in the actions of antipsychotics, which are the mesolimbic, mesocortical, and nigrostriatal pathways (Kapur and Mamo, 2003). D₂R blockade of the mesolimbic pathway, in which dopaminergic neurons project from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is the major mechanism by which antipsychotics control positive schizophrenia symptoms (Seeman, 2011, Ginovart and Kapur, 2012). The mesocortical pathway, in which the dopaminergic neurons project from the VTA to the cortex, including the prefrontal cortex (PFC) and cingulate cortex (Cg), is involved in the cognitive deficits and the negative symptoms of schizophrenia (Volk, 2010, Ginovart and Kapur, 2012); By contrast, EPS are related to the D₂R blockade in the nigrostriatal pathway, in which dopaminergic neurons project from the substantia nigra (SN) to the caudate putamen (CPu) (Ginovart and Kapur, 2012). However, recent evidence indicates that the nigrostriatal pathway is also involved in the pathophysiology of schizophrenia and antipsychotic treatment (Howes et al., 2012).

Serotonin receptors such as 5-HT_{2A}R and 5-HT_{2C}R are G protein-coupled receptors which are linked to an intracellular molecular signal-transduction cascade, and which have been implicated in the therapeutics of SGAs and various neuropsychiatric disorders (Horacek et al., 2006, Meltzer and Massey, 2011). It has been hypothesized that a relatively high 5-HT_{2A}R antagonistic affinity compared to the D₂R is the basis for the difference between the majority of SGAs and FGAs (Meltzer and Massey, 2011), although some of SGAs (such as Amisulpride) have low affinity for 5-HT_{2A}R (Leucht et al., 2009). Additionally, the high 5-HT_{2A}R and 5-HT_{2C}R affinity of most SGAs including olanzapine has been demonstrated to facilitate dopamine release in the PFC and hippocampus (Kuroki et al., 2008). The 5-HT_{2C}R antagonists may increase dopamine levels in the NAc and PFC (Di Matteo et al., 2001). There is evidence that a combination of 5-HT_{2A}R and 5-HT_{2C}R blockades is more efficient

than the 5-HT_{2A}R blockade alone at increasing dopamine release in the NAc and PFC, which would improve cognitive deficits (Horacek et al., 2006). Therefore, the functional combination of the 5-HT₂ and D₂ receptors is crucial to the therapeutic efficacy of olanzapine.

The histaminergic H₁R is involved in the regulation of body weight, food intake and energy expenditure, which is a key contributor for body weight gain induced by olanzapine (a potent H₁R antagonist) (Kim et al., 2007, Deng et al., 2010). Our previous study targeting the H₁R showed that short-term (2 weeks) co-treatment of betahistine (an H₁R agonist and H₃R antagonist) and olanzapine can reduce about 45% the body weight gain induced by olanzapine treatment in drug-naïve rats (Deng et al., 2012). Consistently, the co-treatment of olanzapine, betahistine and reboxetine (a selective norepinephrine reuptake inhibitor) was found to reduce the olanzapine-induced weight gain following short-term (6 weeks) treatment in first-episode schizophrenia patients (Poyurovsky et al., 2013). Importantly, patients with schizophrenia or other mental disorders (such as bipolar disorder) often face chronic and repeated treatments with antipsychotics. Antipsychotic treatments induce severe weight gain not only in drug-naïve patients, but also in chronic patients with previous antipsychotic exposure and repeated treatments (Lieberman et al., 2005). In a chronic rat model with repeated olanzapine exposure, we recently found that chronic (5 weeks) co-treatment with betahistine is also effective in reducing olanzapine-induced weight gain (Lian et al., 2014).

A key issue is whether chronic betahistine co-treatment affects the therapeutic efficacy of olanzapine. Our previous study found that a short-term (2 weeks) co-treatment of olanzapine and betahistine does not influence the effect of olanzapine on dopamine D₂ and 5-HT_{2A}R bindings in the PFC, Cg, NAc, CPu, SN, and VTA in drug naïve rats (Lian et al., 2013). This may explain why betahistine does not affect the therapeutic efficacy of olanzapine observed

in short-term therapy in first-episode, drug-naïve schizophrenia patients (Poyurovsky et al., 2005, Lian et al., 2013). However, in the clinic, chronic schizophrenia patients normally have previously experienced antipsychotic exposure when they are prescribed an antipsychotic treatment. Therefore, we mimicked the repeated treatment condition in which rats had an early olanzapine exposure and was followed by chronic treatment of olanzapine and/or betahistine. Since it is not clear whether chronic co-treatment with betahistine affects the therapeutic effects in chronic patients with repeated second generation antipsychotics (SGAs) treatments, or affects the D₂R and 5-HT₂R neurotransmission, this study investigated the effects of chronic co-treatment of olanzapine and betahistine on the 5-HT_{2A}R, 5-HT_{2C}R and D₂R binding in a rat model with repeated olanzapine treatment.

2. Experimental Procedures

2.1. Animal treatment and administration

Female Sprague Dawley rats (201-225g) were obtained from the Animal Resources Centre (Perth, WA, Australia). After one week of habituation, they were housed individually with *ad-libitum* access to water and standard laboratory chow (3.9 kcal/g; 10% fat, 74% carbohydrate, 16% protein) at 22°C under a 12 hour light-dark cycle (light cycle from 07:00-19:00 and dark cycle from 19:00-07:00) throughout the study (Deng et al., 2012, Lian et al., 2013). The animals were then trained for one week to self-administer specially prepared cookie-dough pellets by metal spoon without the drug (0.3 g) and handled to minimise stress throughout the experiment. The drug powder containing cornstarch (30.9%), sucrose (30.9%), gelatine (6.3%), casein (15.5%), fibre (6.4%), minerals (8.4%) and vitamins (1.6%), and then mixed with water (Deng et al., 2012). Rats were randomly administered different drugs 3 times per day orally (07:00, 15:00, 23:00 h). Briefly (Figure 1), rats were treated with olanzapine (1 mg/kg, t.i.d., n=12) or vehicle (n=12) for 3.5 weeks, and the drug treatment

was then withdrawn from Day 23 for 19 days. From week 6, the two groups were divided into 4 groups (n=6) for further treatment for 5 weeks: (1) olanzapine (1 mg/kg, t.i.d.), (2) betahistine (9.6 mg/kg, t.i.d.), (3) co-treatment of olanzapine and betahistine (same doses as above), and (4) control (vehicle). The olanzapine dosage used is equivalent to the recommended dosage for treating schizophrenia patients, and it was translated based on body surface area according to the FDA guidelines for clinical trials (FDA, 2005, Reagan-Shaw et al., 2008). This dosage is behavioural and pharmacologically effective (Weston-Green et al., 2011, Deng et al., 2012, Lian et al., 2013). The betahistine dosage can effectively decrease body weight gain (Szelag et al., 2001, Tarricone et al., 2010). In the rat, the half-lives of olanzapine are 2.5 hours and 5.1 hours in the plasma and brain, respectively, and they are maintained at a high level for 8 hours (Aravagiri et al., 1999). However, in humans, the half-life of olanzapine in plasma is 24.2 hours, compared to 72 hours in the brain (Tauscher et al., 2002). Betahistine has 3-4 hours of plasma half-life in humans with one day of urine excretion, but no data have shown the half-life of betahistine in rats (Botta et al., 2001). All of the experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia (AE11/10); and complied with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (2004).

2.2. Histology

Forty eight hours after the last drug treatment, and the following euthanasia, the brain tissue was immediately removed, frozen in liquid nitrogen, and stored at -80°C until analysis. Six brains were randomly selected from each treatment group and sectioned coronally at -18 °C into 14 µm using a cryostat (Leica CM1850, Leica Microsystems, Germany) for receptor autoradiography. Sections were thaw-mounted onto Polysine™ Microscope Slides (Menzel GmbH & Co. KG, Braunschweig, Germany) and stored at -20 °C. A set of sections from each

animal was stained with 0.5% cresyl violet solution (Nissl staining) and used to confirm of anatomical structures.

2.3. Receptors autoradiography and quantification

2.3.1. Receptors autoradiography

Procedures for [³H]ketanserin binding to the 5-HT_{2A}R has been successfully performed in our laboratory (Kang et al., 2009, Lian et al., 2013). Briefly, brain sections were thawed and then pre-incubated for 15 minutes in 170 mM Tris buffer (pH 7.4) at room temperature. For total binding, sections were incubated with 2 nM [³H]ketanserin (Specific activity: 67 Ci/mmol; PerkinElmer, Waltham, MA, USA) in 170 mM Tris buffer for 2 hours at room temperature. Non-specific binding was determined by incubating subsequent sections with 2 nM [³H]ketanserin and 2 μM spiperone (Sigma Pharmaceuticals, Melbourne, VIC, Australia). Sections were washed in Tris buffer (pH 7.4) four times for 2 minutes at 4°C, dipped in ice-cold milliQ H₂O water to remove buffer salts, and then gently air dried under a stream of cool air.

The procedures for bindings of [³H]mesulergine (to 5-HT_{2C}R and 5-HT_{2A}R), [³H]paroxetine (to 5-HTT) and [³H]raclopride (to D₂R) autoradiography are well established in our laboratory and are similar to the 5-HT_{2A}R binding procedures as described above. In brief, the brain sections were incubated with 5 nM [³H]mesulergine (84.5 Ci/mmol), 0.6 nM [³H]paroxetine (20.8 Ci/mmol) and 5nM [³H]raclopride (60.1 Ci/mmol) (PerkinElmer), respectively. Non-specific binding are detected at the presence of 100 nM spiperone and 10 μM mianserin for [³H]mesulergine binding, 10 μM fluoxetine for [³H]paroxetine binding, and 10 μM butaclamol for [³H]raclopride binding (du Bois et al., 2006, du Bois et al., 2008, Lian et al., 2013).

2.3.2. Quantitative analysis of autoradiography

All of the receptor binding slides were exposed to Kodak BioMax MR film for 2-3 months, together with autoradiographic standards ($[^3\text{H}]$ microscales from Amersham), in X-ray film cassettes. This was followed by the analysis of binding images using the Multi-Analyst image analysis system (Bio-Rad, USA), connected to a GS-800 Imaging Densitometer (Bio-Rad, USA). The specific binding was calculated by subtracting non-specific binding from total binding. A set of sections from each animal were stained with 0.5% cresyl violet solution (Nissl staining) and used to confirm anatomical structures. Specific brain regions in this project were identified by reference to the Nissl-stained sections and a standard rat brain atlas (Paxinos and Watson, 2007).

2.4. Statistical analysis

Statistical analysis was performed using SPSS (IBM version 19.0, SPSS Inc., NY, USA). The Kolmogorov-Smirnov test was used to examine whether data from all experiments are normally distributed. Two-way ANOVAs (OLANZAPINE \times BETAHISTINE) were used to analyse receptor binding density in the relevant rat brain regions. The *post-hoc* Dunnett-T test was used to analyse the relationships among the measurements. The Mann-Whitney U test was applied to the data without abnormal distribution. All data were expressed as mean \pm SEM, and statistical significance was accepted when $p < 0.05$.

3. Results

Body weight and energy data have been reported previously (Lian et al., 2014). Briefly, the olanzapine-only treatment significantly increased weight gain and food intake/feeding

efficiency, while the co-treatment of olanzapine and betahistine significantly reduced about 51% weight gain and feeding efficiency compared to the olanzapine-only treatment (Lian et al., 2014).

3.1. [³H]ketanserin binding to 5-HT_{2A}R

Two-way ANOVAs revealed the significant effects of the OLANZAPINE factor on 5-HT_{2A}R in the PFC ($F_{1,20}=36.028$, $p<0.001$), Cg ($F_{1,20}=10.691$, $p=0.004$), NAc core (NAcC) ($F_{1,20}=15.663$, $p=0.005$), NAc shell (NAcS) ($F_{1,20}=6.224$, $p=0.021$), and SN ($F_{1,20}=7.046$, $p=0.015$). However, there was no significant effect of the BETAHISTINE factor in these brain nuclei, and, there was no significant interaction between the two factors (all $p>0.05$).

The olanzapine-only treatment significantly influenced the levels of 5-HT_{2A}R binding density (Table 1). *Post-hoc* analysis identified a significant decrease in 5-HT_{2A}R binding in the PFC, Cg, NAcC, NAcS (all $p<0.01$), and SN ($p<0.05$) in the rats receiving the olanzapine-only treatment (Table 1). The olanzapine-only treatment did not significantly decrease 5-HT_{2A}R binding in the VTA ($p>0.05$; Table 1). Compared to the control, the levels of 5-HT_{2A}R binding density were also significantly decreased by O+B co-treatment in the PFC, Cg, NAcC and NAcS (all $p<0.01$), but not in the VTA and SN ($p>0.05$; Table 1). However, there were no significant differences in 5-HT_{2A}R binding between the olanzapine-only and O+B co-treatment groups in these brain regions (all $p>0.05$, Table 1). In addition, the betahistine-only treatment did not affect 5HT_{2A}R binding compared to the control (all $p>0.05$; Table 1).

3.2. [³H]mesulergine binding to 5-HT_{2C}R and 5-HT_{2A}R

The altered of [³H]mesulergine binding density induced by olanzapine was observed in several brain regions (Table 2). Two-way ANOVAs showed a significant effect of the

OLANZAPINE factor on [³H]mesulergine binding density in the PFC ($F_{1,20}=17.918$, $p<0.001$), Cg ($F_{1,20}=64.387$, $p<0.001$), NAcC ($F_{1,20}=15.576$, $p=0.001$), and NAcS ($F_{1,20}=17.384$, $p<0.001$). There was a tendency to significance of the OLANZAPINE factor on [³H]mesulergine binding in the CPu ($F_{1,20}=4.145$, $p=0.055$). However, there were no significant effects of the BETAHISTINE factor and no interaction between the two factors (all $p>0.05$).

Post-hoc analysis revealed that significant attenuation of the [³H]mesulergine binding density was caused by the olanzapine-only treatment in the PFC, Cg, NAcC, and NAcS (all $p<0.01$) compared with the control, but not in the CPu and SN ($p>0.05$) (Table 2). Furthermore, compared with the control, the [³H]mesulergine binding level was also significantly decreased by the co-treatment with O+B in the PFC and CPu ($p<0.05$), and the Cg, NAcC, and NAcS ($p<0.01$) (Table 2). However, there was no significant difference in the [³H]mesulergine binding between the co-treatment of O+B and the olanzapine-only treatment, or between the control and betahistine-only groups (Table 2).

3.3. [³H]paroxetine binding to 5-HTT binding

Two-way ANOVAs revealed the significant effects of the OLANZAPINE factor in the Cg ($F_{1,20}=18.189$, $p<0.001$), NAcC ($F_{1,20}=9.862$, $p=0.005$) and NAcS ($F_{1,20}=22.853$, $p<0.001$). There was borderline significance for the OLANZAPINE factor in the PFC ($F_{1,20}=4.076$, $p=0.057$). However, there was no significant effect of the BETAHISTINE factor, and no interaction between the two factors.

The 5-HTT binding density was significantly lower in the olanzapine-only group than in the controls in the PFC and NAcC ($p<0.05$), as well as the Cg and NAcS (all $p<0.01$). Similar to

the olanzapine-only treatment, the co-treatment of O+B also significantly decreased 5-HTT binding in the Cg ($p<0.01$) and NAcS ($p<0.05$) compared with the control, and tended to decrease 5HTT binding in the PFC ($p=0.059$) and NAcC ($p=0.089$) (Table 3). However, the olanzapine-only and O+B co-treatment had no effect on the 5-HTT binding in the CPu and VTA (Table 3). Furthermore, there was no significant difference between the olanzapine-only treatment and the co-treatment of O+B. Also, the betahistine-only treatment did not affect the 5-HTT binding in any of the brain region (Table 3).

3.4. [³H]raclopride binding to D₂R

Two-way ANOVAs (OLANZAPINE \times BETAHISTINE) revealed significant effects of the OLANZAPINE factor on the PFC ($F_{1,20}=5.857$, $p=0.025$), NAcC ($F_{1,20}=20.631$, $p<0.001$), NAcS ($F_{1,20}=19.065$, $p<0.001$), Cg ($F_{1,20}=10.924$, $p=0.004$) and CPu ($F_{1,20}=21.025$, $p<0.001$), as well as significant effects of the BETAHISTINE factor on the NAcC ($F_{1,20}=6.438$, $p=0.020$), NAcS ($F_{1,20}=4.802$, $p=0.040$) and CPu ($F_{1,20}=8.690$, $p=0.008$). However, no significant interaction was detected between the two factors.

Post-hoc tests showed that the olanzapine-only treatment significantly increased D₂R binding density in the NAcC, NAcS and CPu (all $p<0.01$), as well as the Cg ($p<0.05$), while it tended to increase D₂R binding in the PFC ($p=0.076$). The betahistine-only treatment significantly decreased D₂R binding density in the CPu and NAcC ($p<0.05$) compared to the control (Table 4). The co-treatment of the O+B group had a significantly lower D₂R binding levels in the NAcC ($p<0.01$) and NAcS ($p<0.05$) than the olanzapine-only treatment group. The O+B co-treatment group had no significant change in D₂R binding in the CPu compared with the olanzapine-only group ($p=0.107$) (Table 4).

4. Discussion

The present study using female rats provides the first evidence of the effects of chronic olanzapine and/or betahistine treatment on the 5-HT_{2A}R, 5-HT_{2C}R, 5-HTT and D₂R bindings in the brain regions involved in the therapeutic efficacy of olanzapine. Olanzapine-only and O+B co-treatment had similar effects in down-regulating the 5-HT_{2A}R, 5-HT_{2C}R and 5-HTT binding density in various brain regions, particularly the PFC, NAcC, NAcS and Cg. In contrast, the D₂R binding density was significantly up-regulated by the olanzapine-only treatment in the Cg, NAcC, NAcS and CPu, while it was down-regulated by the betahistine-only treatment in the NAcC and NAcS. Importantly, the betahistine co-treatment reversed the D₂R up-regulation induced by olanzapine in the NAcC and NAcS.

The female rats were used in this study, because the olanzapine-induced weight gain model has been well established and validated in female rats in our and other laboratories (Choi et al., 2007, Weston-Green et al., 2011, Deng et al., 2012). Clinically, it is also a common observation that female patients have a much higher risk than males for SGA-induced weight gain side-effects (Gebhardt et al., 2009, Seeman, 2009, Weston-Green et al., 2010, Treuer et al., 2011). Furthermore, in this study, rats were closely housed in a room occupied by only female rats. Our pre-experiments have shown that, under this rearing condition, the estrus cycles of all female rats are synchronized (Lian et al., 2013).

SGAs such as olanzapine and clozapine have been reported to attenuate 5-HT_{2A}R binding and mRNA expression, which are involved in the therapeutic effects of SGAs (Tarazi et al., 2002, Lian et al., 2013). Coinciding with these findings, this study revealed that chronic olanzapine treatment significantly reduced 5-HT_{2A}R binding density in the PFC, Cg, NAcC, NAcS and SN, strongly implicated in the therapeutics of antipsychotics (Kuroki et al., 2008, Meltzer and

Massey, 2011). Notably, the chronic O+B co-treatment had similar effects in down-regulating 5-HT_{2A}R binding as the olanzapine-only treatment in rats on chronic and repeated olanzapine treatment, although the chronic betahistine-only treatment did not affect 5-HT_{2A}R binding. Consistent with findings in this study, our previous study found that short-term O+B co-treatment had similar down regulatory effects as the olanzapine-only treatment on 5-HT_{2A}R binding density in drug-naïve rats (Lian et al., 2013). These results suggest that both early olanzapine exposure and chronic betahistine co-treatment do not influence olanzapine's actions on 5-HT_{2A}R.

Besides 5-HT_{2A}R, most of SGAs have a high binding affinity with 5-HT_{2C}R which is involved in the antipsychotic drug action (Meltzer and Massey, 2011). Several studies have revealed the association between the polymorphism of the 5-HT_{2C}R gene/its promoter region and clinical response to antipsychotic (including olanzapine) treatment (Reynolds et al., 2005). Similar to 5-HT_{2A}R binding, the 5-HT_{2C}R binding is down-regulated in response to olanzapine and other SGAs treatment (Tarazi et al., 2002, Zhang and Malhotra, 2011). In this study, [³H]mesulergine was used to examine 5-HT_{2C}R density, although we should consider the limitation that [³H]mesulergine has also high affinity with 5-HT_{2A}R (Abbas et al., 2009). Consistent with previous reports, this study revealed that decreased [³H]mesulergine binding density in the PFC, Cg, NAcC, and NAcS after chronic olanzapine treatment. It should also be noted that chronic O+B co-treatment had a similar attenuation effect as olanzapine-only treatment on the [³H]mesulergine binding, while the betahistine-only treatment had no significant effects on the [³H]mesulergine binding in these brain regions. Consequently, this study provided further evidence that chronic O+B co-treatment would have similar effects on 5-HT translations through 5-HT_{2A}R and 5-HT_{2C}R.

As an integral membrane protein, 5-HTT terminates the action of serotonin by transporting serotonin from synaptic spaces into presynaptic neurons (Zhang and Malhotra, 2011, Lian et al., 2013). Variations in several 5-HTT gene polymorphisms have been associated with the response to olanzapine and other SGAs (Zhang and Malhotra, 2011). For example, a repeat length polymorphism 5-HTT-LPR (a 44bp insertion/deletion in the promoter region) has shown that the short allele of 5-HTT-LPR is associated with poor response to olanzapine, clozapine, and risperidone treatment (Vazquez-Bourgon et al., 2010, Zhang and Malhotra, 2011). However, the exact role of 5-HTT in the olanzapine treatment of schizophrenia symptoms is still not clear. Our previous study revealed that short-term treatment of olanzapine and co-treatment of O+B down-regulated 5-HTT bindings in the SN and VTA of drug-naïve rats (Lian et al., 2013). In this study, chronic olanzapine treatment significantly decreased the 5-HTT binding density in the PFC, Cg, NAcC, and NAcS. The present results suggest that the 5-HT uptake in the PFC, Cg and NAc may contribute to therapeutic effects of chronic olanzapine treatment. In addition, a similar decrease of 5-HTT binding density was also revealed in rats with O+B co-treatment. Thus co-treatment with betahistine should not affect olanzapine's action on 5-HTT. However, although 5-HTT binding was down-regulated in both short-term treatment in drug-naïve subjects and chronic olanzapine treatment, this effect occurred in different brain regions. Further study is necessary to determine the contributing factors (short-term *vs.* chronic or drug-naïve *vs.* repeated treatment) for these differences.

In terms of D₂R binding, in this study chronic olanzapine treatment (3 mg/kg/day, t.i.d.) up-regulated the D₂R binding levels in the Cg, NAcC, NAcS and CPu. This result corresponds to a previous report that chronic treatment of olanzapine (5mg/kg/day *via* osmotic minipumps) caused an increase in D₂R binding in the PFC, CPu, and NAc (Tarazi et al., 2001). However,

olanzapine was found only having a tendency to increase D₂R binding density in the PFC, but not significance in this study. It is worth noting that a previous study reported a significant increase of D₂R binding in the PFC following 4 weeks treatment of olanzapine (Tarazi et al., 2001). This discrepancy could be due to different radioactive ligands used in this ([³H]Raclopride) and Tarazi et al. ([³H]nemonapride) studies, or most possibly a higher olanzapine dosage (5 mg/kg/day *via* osmotic minipump) used in Tarazi et al than in this study (3 mg/kg/day, t.i.d. *via* orally feeding in cookie dough) (Tarazi et al., 2001, Lian et al., 2014). In fact, some studies using olanzapine treatment at lower doses (1-2mg/kg/day) or short-term treatment (~2 weeks) did not affect D₂R binding (Han et al., 2009, Lian et al., 2013). It has been well documented in both humans and laboratory animals that D₂R up-regulation induced by chronic antipsychotic (including olanzapine) treatment may lead to a state of “dopaminergic supersensitivity” (Samaha et al., 2007, Seeman, 2011). This “dopaminergic supersensitivity” is characterized by an increased vulnerability to psychosis (termed “antipsychotic-induced supersensitivity psychosis” in humans), and to psychomotor activating effects of the dopamine agonist, which may lead to treatment failure over time (Samaha et al., 2007). Furthermore, it is worth noting that chronic olanzapine treatment is still associated with some risks for EPS, although at a much lower rate than FGAs (Kantrowitz and Citrome, 2008). The D₂R binding up-regulation in the CPu observed in the chronic olanzapine treatment in this and other studies may partially explain its risk for the development of EPS (Tarazi et al., 2001). It is interesting that this is the first study to reveal the effect of chronic betahistine treatment in decreasing D₂R binding in the NAc and CPu, which was not observed in our previous study with a short-term (2-weeks) treatment at a lower dose (2.67mg/kg *vs.* 9.6mg/kg in this study) (Lian et al., 2013). The betahistine dosage (9.6 mg/kg) applied in this present study is equal to about 93 mg in human (60 kg body weight) based on dosage translation between species according to body surface area followed

the FDA guideline (FDA, 2005, Reagan-Shaw et al., 2008). Betahistine has 3-4 hours of plasma half-life in humans with one day of urine excretion (Botta et al., 2001). Although there is no data available for the half-life of betahistine in rats, it is reasonable to suppose that betahistine is most likely to have a shorter half-life in rats than in humans. This dosage (9.6 mg/kg, t.i.d) should be relevant to the human dosage (48 mg, t.i.d.) used to attenuate olanzapine-induced weight gain in the clinical trials (Poyurovsky et al., 2005, Poyurovsky et al., 2013). Therefore, 9.6 mg/kg was used in this study, which should be appropriate for investigating the effect of co-treatment with betahistine in relieving olanzapine-induced body weight gain in rats. More importantly, co-treatment with betahistine significantly reversed D₂R binding up-regulation in the NAc and there was a trend towards reversing it in the CPu. Therefore, co-treatment with betahistine may be able to reduce the “dopaminergic supersensitivity” and EPS caused by chronic antipsychotic (olanzapine) treatment, although further clinical studies are necessary. On the other hand, betahistine may affect olanzapine’s action on dopamine D₂R. Notably, that histamine H₃R can form intramembrane interactions with D₂R (Ferrada et al., 2008), and negatively modulate dopaminergic transmission via interactions with D₂R (Panula and Nuutinen, 2013). Since betahistine is a potent H₃R antagonist (and also a H₁R agonist), it is probable that the H₃R antagonist property of betahistine plays a key role in attenuating the D₂R binding density observed in this study.

In summary, this study revealed the effects of chronic olanzapine and/or betahistine treatment on the 5-HT_{2A}R, 5-HT_{2C}R, 5-HTT and D₂R bindings in the brain regions involved in the therapeutic effects of antipsychotics (Kuroki et al., 2008). The effects of co-treatment with O+B on 5-HT_{2A}R binding is in line with our previous short-term study showed that both olanzapine-only and co-treatment of O+B have similar effects in decreasing 5-HT_{2A}R binding density (Lian et al., 2013). The present study also showed that both olanzapine-only and

O+B co-treatment has similar effects in attenuating 5-HT_{2C}R and 5-HTT levels. Therefore, these results suggested that chronic O+B co-treatment does not affect olanzapine's actions on 5-HT neurotransmission. While up-regulation of D₂R binding in the Cg, NAc, and CPu was observed in rats treated with chronic olanzapine treatment, chronic betahistine treatment decreased D₂R binding density. Since the up-regulation of D₂R binding is associated with “dopaminergic supersensitivity” (or “antipsychotic-induced supersensitivity psychosis”) that may lead to treatment failure over time (Samaha et al., 2007), the observation that O+B co-treatment tends to reverse the up-regulation of D₂R binding caused by chronic olanzapine treatment is promising for further investigation as whether co-treatment with betahistine can prevent the “dopaminergic supersensitivity” caused by chronic antipsychotic treatment.

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References

- Abbas AI, Yadav PN, Yao WD, Arbuckle MI, Grant SG, Caron MG, Roth BL (2009) PSD-95 is essential for hallucinogen and atypical antipsychotic drug actions at serotonin receptors. *J Neurosci* 29:7124-7136.
- Aravagiri M, Teper Y, Marder SR (1999) Pharmacokinetics and tissue distribution of olanzapine in rats. *Biopharm Drug Dispos* 20:369-377.
- Botta L, Mira E, Valli S, Zucca G, Benvenuti C, Fossati A, Soto E, Guth P, Valli P (2001) Effects of betahistine and of its metabolites on vestibular sensory organs. *Acta Otorhinolaryngol Ital* 21:24-30.
- Choi S, DiSilvio B, Unangst J, Fernstrom JD (2007) Effect of chronic infusion of olanzapine and clozapine on food intake and body weight gain in male and female rats. *Life Sci* 81:1024-1030.
- Deng C (2013) Effects of antipsychotic medications on appetite, weight, and insulin resistance. *Endocrinology and metabolism clinics of North America* 42:545-563.
- Deng C, Lian J, Pai N, Huang XF (2012) Reducing olanzapine-induced weight gain side-effect by betahistine: a study in the rat model. *J Psychopharmacol (Oxf)* 26:1291-1279.
- Deng C, Weston-Green K, Huang X-F (2010) The role of histaminergic H₁ and H₃ receptors in food intake: a mechanism for atypical antipsychotic-induced weight gain? *Prog Neuropsychopharmacol Biol Psychiatry* 34:1-4.
- Di Matteo V, De Blasi A, Di Giulio C, Esposito E (2001) Role of 5-HT_{2C} receptors in the control of central dopamine function. *Trends in Pharmacological Sciences* 22:229-232.
- du Bois TM, Deng C, Bell W, Huang XF (2006) Fatty acids differentially affect serotonin receptor and transporter binding in the rat brain. *Neuroscience* 139:1397-1403.
- du Bois TM, Hsu CW, Li Y, Tan YY, Deng C, Huang XF (2008) Altered dopamine receptor and dopamine transporter binding and tyrosine hydroxylase mRNA expression following perinatal NMDA receptor blockade. *Neurochem Res* 33:1224-1231.
- FDA (2005) Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers. U.S. FDA Center for Drug Evaluation and Research.
- Ferrada C, Ferre S, Casado V, Cortes A, Justinova Z, Barnes C, Canela EI, Goldberg SR, Leurs R, Lluís C, Franco R (2008) Interactions between histamine H₃ and dopamine D₂ receptors and the implications for striatal function. *Neuropharmacology* 55:190-197.
- Gebhardt S, Haberhausen M, Heinzl-Gutenbrunner M, Gebhardt N, Remschmidt H, Krieg J-C, Hebebrand J, Theisen FM (2009) Antipsychotic-induced body weight gain: predictors and a systematic categorization of the long-term weight course. *J Psychiatr Res* 43:620-626.
- Ginovart N, Kapur S (2012) Role of dopamine D(2) receptors for antipsychotic activity. *Handb Exp Pharmacol* 27-52.
- Han M, Huang XF, du Bois TM, Deng C (2009) The effects of antipsychotic drugs administration on 5-HT_{1A} receptor expression in the limbic system of the rat brain. *Neuroscience* 164:1754-1763.
- Horacek J, Bubenikova-Valesova V, Kopecek M, Palenicek T, Dockery C, Mohr P, Hoschl C (2006) Mechanism of action of atypical antipsychotic drugs and the neurobiology of schizophrenia. *CNS Drugs* 20:389-409.
- Howes O, Kambeitz J, Kim E, Stahl D, Slifstein M, Abi-Dargham A, Kapur S (2012) The nature of dopamine dysfunction in schizophrenia and what this means for treatment: Meta-analysis of imaging studies. *Arch Gen Psychiatry* 69:776-786.

- Kang K, Huang XF, Wang Q, Deng C (2009) Decreased density of serotonin 2A receptors in the superior temporal gyrus in schizophrenia--a postmortem study. *Prog Neuropsychopharmacol Biol Psychiatry* 33:867-871.
- Kantrowitz JT, Citrome L (2008) Olanzapine: review of safety 2008. *Expert Opin Drug Saf* 7:761-769.
- Kapur S, Mamo D (2003) Half a century of antipsychotics and still a central role for dopamine D₂ receptors. *Prog Neuropsychopharmacol Biol Psychiatry* 27:1081-1090.
- Kim SF, Huang AS, Snowman AM, Teuscher C, Snyder SH (2007) From the Cover: Antipsychotic drug-induced weight gain mediated by histamine H₁ receptor-linked activation of hypothalamic AMP-kinase. *Proceedings of the National Academy of Sciences of the United States of America* 104:3456-3459.
- Kuroki T, Nagao N, Nakahara T (2008) Neuropharmacology of second-generation antipsychotic drugs: a validity of the serotonin-dopamine hypothesis. In: *Prog Brain Res*, vol. 172 (Giuseppe Di Giovanni, V. D. M. and Ennio, E., eds), pp 199-212: Elsevier.
- Lambert T (2011) Managing the metabolic adverse effects of antipsychotic drugs in patients with psychosis. *Aust Prescr* 34:97-99.
- Leucht S, Corves C, Arbter D, Engel RR, Li C, Davis JM (2009) Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. *Lancet* 373:31-41.
- Lian J, Huang X-F, Pai N, Deng C (2013) Effects of olanzapine and betahistine co-treatment on serotonin transporter, 5-HT_{2A} and dopamine D₂ receptor binding density. *Prog Neuropsychopharmacol Biol Psychiatry* 47:62-68.
- Lian J, Huang X-F, Pai N, Deng C (2014) Preventing Olanzapine-Induced Weight Gain Using Betahistine: A Study in a Rat Model with Chronic Olanzapine Treatment. *PLoS ONE* 9:e104160.
- Lieberman J, Stroup T, Swartz M (2005) Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N Engl J Med* 353:1209-1223.
- Meltzer H, Massey B (2011) The role of serotonin receptors in the action of atypical antipsychotic drugs. *Curr Opin Pharmacol* 11:59-67.
- Nasrallah (2008) Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles. *Mol Psychiatry* 13:27-35.
- Panula P, Nuutinen S (2013) The histaminergic network in the brain: basic organization and role in disease. *Nat Rev Neurosci* 14:472-487.
- Patel JK, Buckley PF, Woolson S, Hamer RM, McEvoy JP, Perkins DO, Lieberman JA, for the Ci (2009) Metabolic profiles of second-generation antipsychotics in early psychosis: Findings from the CAFE study. *Schizophr Res* 111:9-16.
- Paxinos G, Watson C (2007) *The rat brain in stereotaxic coordinates*: Academic Press.
- Poyurovsky M, Fuchs C, Pashinian A, Levi A, Weizman R, Weizman A (2013) Reducing antipsychotic-induced weight gain in schizophrenia: a double-blind placebo-controlled study of reboxetine–betahistine combination. *Psychopharmacology (Berl)* 226:615-622.
- Poyurovsky M, Pashinian A, Levi A, Weizman R, Weizman A (2005) The effect of betahistine, a histamine H₁ receptor agonist/H₃ antagonist, on olanzapine-induced weight gain in first-episode schizophrenia patients. *Int Clin Psychopharmacol* 20:101-103.
- Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. *The FASEB Journal* 22:659-661.

- Reynolds GP, Templeman LA, Zhang ZJ (2005) The role of 5-HT_{2C} receptor polymorphisms in the pharmacogenetics of antipsychotic drug treatment. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 29:1021-1028.
- Samaha A-N, Seeman P, Stewart J, Rajabi H, Kapur S (2007) "Breakthrough" dopamine supersensitivity during ongoing antipsychotic treatment leads to treatment failure over time. *J Neurosci* 27:2979-2986.
- Seeman MV (2009) Secondary effects of antipsychotics: women at greater risk than men. *Schizophr Bull* 35:937-948.
- Seeman P (2011) All roads to schizophrenia lead to dopamine supersensitivity and elevated dopamine D₂(high) receptors. *CNS Neurosci Ther* 17:118-132.
- Szelag A, Trocha M, Merwid-Lad A (2001) Betahistine inhibits food intake in rats. *Pol J Pharmacol* 53:701-707.
- Tarazi FI, Zhang K, Baldessarini RJ (2001) Long-term effects of olanzapine, risperidone, and quetiapine on dopamine receptor types in regions of rat brain: implications for antipsychotic drug treatment. *J Pharmacol Exp Ther* 297:711-717.
- Tarazi FI, Zhang K, Baldessarini RJ (2002) Long-term effects of olanzapine, risperidone, and quetiapine on serotonin 1A, 2A and 2C receptors in rat forebrain regions. *Psychopharmacology (Berl)* 161:263-270.
- Tarricone I, Ferrari Gozzi B, Serretti A, Grieco D, Berardi D (2010) Weight gain in antipsychotic-naïve patients: a review and meta-analysis. *Psychol Med* 40:187-200.
- Tauscher J, Jones C, Remington G, Zipursky RB, Kapur S (2002) Significant dissociation of brain and plasma kinetics with antipsychotics. *Molecular Psychiatry* 7:317-321.
- Treuer T, Pendlebury J, Lockman H, Bushe C, Karagianis J, Raskin J, Lipkovich I (2011) Weight Gain Risk Factor assessment checklist: overview and recommendation for use. *Neuroendocrinol Lett* 32:199-205.
- Vazquez-Bourgon J, Arranz MJ, Mata I, Pelayo-Teran JM, Perez-Iglesias R, Medina-Gonzalez L, Carrasco-Marin E, Vazquez-Barquero JL, Crespo-Facorro B (2010) Serotonin transporter polymorphisms and early response to antipsychotic treatment in first episode of psychosis. *Psychiatry Res* 175:189-194.
- Volk D (2010) Prefrontal cortical circuits in schizophrenia. *Curr Top Behav Neurosci* 4:485-508.
- Weston-Green K, Huang X-F, Deng C (2010) Sensitivity of the female rat to olanzapine-induced weight gain--Far from the clinic? *Schizophr Res* 116:299-300.
- Weston-Green K, Huang X-F, Deng C (2011) Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats. *Behav Brain Res* 217:337-346.
- Zhang JP, Malhotra AK (2011) Pharmacogenetics and antipsychotics: therapeutic efficacy and side effects prediction. *Expert Opin Drug Metab Toxicol* 7:9-37.

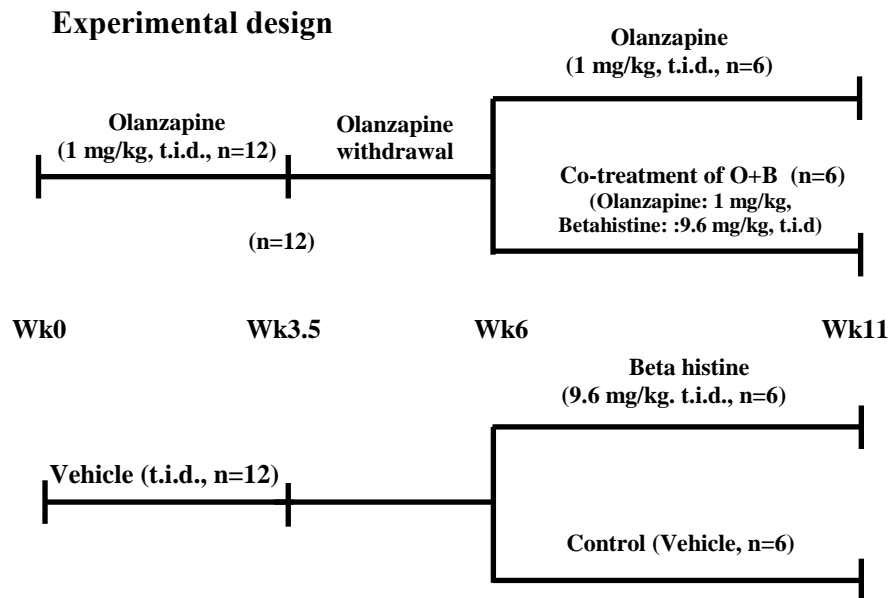


Figure 1. Outline of the experimental design.

Table 1

The effects of chronic olanzapine and/or betahistine treatment ($n = 6/\text{group}$) on [^3H] ketanserin binding to the 5-HT_{2A} receptors (fmole/mg tissue).

Group	Control	Olanzapine	Betahistine	O + B
PFC	126.3 ± 13.2	55.1 ± 3.9**	115.9 ± 7.6	72.3 ± 10.9**
Cg	89.0 ± 14.3	45.2 ± 4.4**	80.6 ± 11.0	54.2 ± 10.9**
NAcC	62.0 ± 9.5	28.0 ± 4.1**	50.2 ± 8.1	27.9 ± 5.5**
NAcS	70.4 ± 10.7	32.8 ± 4.1**	57.3 ± 14.0	42.2 ± 10.9**
VTA	48.6 ± 11.9	26.8 ± 6.0	50.1 ± 10.9	41.9 ± 6.7
SN	54.8 ± 11.3	23.6 ± 8.1*	59.9 ± 8.6	44.0 ± 7.0

Abbreviations: Cg, cingulate cortex; NAcC, nucleus accumbens, core; NAcS, nucleus accumbens, shell; O + B, olanzapine and betahistine co-treatment; PFC, prefrontal cortex; SN, substantia nigra; VTA, ventral tegmental area. Data shown are the mean values ± SEM.

* $p < 0.05$, ** $p < 0.01$ vs. control.

Table 2

The effects of chronic olanzapine and/or betahistine treatment ($n = 6/\text{group}$) on [^3H] mesulergine binding to the 5-HT_{2C/2A} receptors (fmole/mg tissue).

Group	Control	Olanzapine	Betahistine	O + B
PFC	75.2 ± 9.9	30.3 ± 6.8**	70.5 ± 8.7	43.5 ± 8.3*
Cg	58.8 ± 4.9	17.0 ± 3.2**	54.2 ± 7.2	19.3 ± 2.4**
NAcC	51.4 ± 1.7	25.4 ± 6.8**	53.4 ± 10.4	20.6 ± 8.0**
NAcS	50.5 ± 3.9	25.6 ± 6.6**	56.0 ± 9.6	21.8 ± 7.0**
CPu	30.4 ± 4.1	21.0 ± 6.3	34.1 ± 10.2	16.9 ± 3.2*
SN	29.1 ± 10.7	28.2 ± 4.8	28.3 ± 4.3	30.7 ± 1.5

Abbreviations: Cg, cingulate cortex; CPu, caudate putamen; NAcC, nucleus accumbens, core; NAcS, nucleus accumbens, shell; O + B, olanzapine and betahistine co-treatment; PFC, prefrontal cortex; SN, substantia nigra. Data shown are the mean values ± SEM.

* $p < 0.05$, ** $p < 0.01$ vs. control.

Table 3

The effects of chronic olanzapine and/or betahistine treatment ($n = 6/\text{group}$) on [^3H]paroxetine binding to 5-HTT (fmole/mg tissue).

Group	Control	Olanzapine	Betahistine	O + B
PFC	312.9 \pm 23.8	234.9 \pm 30.9*	266.9 \pm 19.4	222.6 \pm 42.2
Cg	200.1 \pm 30.0	101.8 \pm 18.7**	194.9 \pm 20.8	97.6 \pm 20.6**
NAcC	149.7 \pm 15.0	95.7 \pm 13.5*	182.4 \pm 30.0	86.2 \pm 31.3
NAcS	169.4 \pm 10.4	64.2 \pm 13.0**	193.5 \pm 28.4	89.0 \pm 29.0*
CPu	120.2 \pm 13.7	102.1 \pm 18.4	121.8 \pm 26.8	116.4 \pm 13.4
VTA	118.3 \pm 10.6	114.4 \pm 10.7	120.8 \pm 10.5	105.5 \pm 14.8

Abbreviations: Cg, cingulate cortex; CPu, caudate putamen; NAcC, nucleus accumbens, core; NAcS, nucleus accumbens, shell; O + B, olanzapine and betahistine co-treatment; PFC, prefrontal cortex; VTA, ventral tegmental area. Data shown are the mean values \pm SEM. * $p < 0.05$, ** $p < 0.01$ vs. control.

Table 4

The effects of chronic olanzapine and/or betahistine treatment ($n = 6/\text{group}$) on [^3H]raclopride binding to D₂R (fmole/mg tissue).

Group	Control	Olanzapine	Betahistine	O + B
PFC	12.9 \pm 1.9	24.0 \pm 5.8	10.6 \pm 2.0	23.9 \pm 7.7
Cg	15.9 \pm 1.6	25.4 \pm 3.9*	11.5 \pm 3.3	24.7 \pm 4.3
NAcC	21.6 \pm 3.8	38.0 \pm 3.3**	17.2 \pm 2.7*	27.5 \pm 1.5##
NAcS	22.8 \pm 3.7	38.8 \pm 3.1**	19.5 \pm 2.8	29.3 \pm 1.7#
CPu	29.1 \pm 1.3	41.6 \pm 4.1**	19.3 \pm 2.2*	33.9 \pm 3.4
SN	11.1 \pm 4.6	20.3 \pm 4.5	13.2 \pm 5.1	12.4 \pm 2.9

Abbreviations: Cg, cingulate cortex; CPu, caudate putamen; NAcC, nucleus accumbens, core; NAcS, nucleus accumbens, shell; O + B, olanzapine and betahistine co-treatment; PFC, prefrontal cortex; SN, substantia nigra. Data shown are the mean values \pm SEM. * $p < 0.05$, ** $p < 0.01$ vs. control. # $p < 0.05$, ## $p < 0.01$ vs. olanzapine.