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Risperidone-induced weight gain and reduced locomotor activity in juvenile female rats: the role of histaminergic and NPY pathways

Jiamei Lian

University of Wollongong, jlian@uow.edu.au

Michael De Santis

University of Wollongong, mds953@uowmail.edu.au

Meng He

University of Wollongong, mh688@uowmail.edu.au

Chao Deng

University of Wollongong, chao@uow.edu.au

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Abstract

Second generation antipsychotic drugs (SGAs) such as risperidone are increasingly prescribed (mostly for off-label use) to children and adolescents for treating various mental disorders. SGAs cause serious weight gain/obesity and other metabolic side-effects. This study aimed to establish an animal model of risperidone-induced weight gain in female juvenile rats, and to investigate the effects of risperidone on the expression of hypothalamic histaminergic H1 receptors (H1R) and neuropeptides, and their association with weight gain. Female Sprague Dawley rats were treated orally with risperidone (0.3 mg/kg, 3 times/day) or vehicle (control) starting from postnatal day (PD) 23 (± 1 day) for 3 weeks (a period corresponding to the childhood-adolescent period in humans). In the female juvenile rats, risperidone treatment increased food intake and body weight gain, which started to appear after 12 days' treatment. Risperidone also significantly decreased the locomotor activity of the female rats. Consistently, risperidone significantly elevated mRNA expression of hypothalamic H1R, neuropeptide Y (NPY), and agouti-related peptide (AgRP) compared to controls, and H1R and NPY levels were correlated with risperidone enhanced weight gain and food intake in the female juvenile rats. However, risperidone did not affect hypothalamic proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) mRNA expression. Therefore, these results suggested that risperidone elevated appetite and body weight gain in juveniles via regulation of the hypothalamic H1R, NPY and AgRP pathways, as well as by reducing activity.

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Running title: Risperidone-induced weight gain in juvenile rats

Authors: Jiamei Lian, Michael De Santis, Meng He, Chao Deng*

Address: Antipsychotic Research Laboratory, Illawarra Health and Medical Research Institute, and School of Medicine, University of Wollongong, Wollongong, 2522, NSW, Australia

***Corresponding author:**

Associate Professor Chao Deng, 32.330, IHMRI, University of Wollongong, NSW, 2522, Australia. E-mail: chao@uow.edu.au, Tel: (+61 2) 4221 4934, Fax: (+61 2) 4221 8130

Abstract

Second Generation Antipsychotic drugs (SGAs) such as risperidone are increasingly prescribed (mostly for off-label use) to children and adolescents for treating various mental disorders. SGAs cause serious weight gain/obesity and other metabolic side-effects. This study aimed to establish an animal model of risperidone-induced weight gain in female juvenile rats, and to investigate the effects of risperidone on the expression of hypothalamic histaminergic H₁ receptors (H₁R) and neuropeptides, and their association with weight gain. Female Sprague Dawley rats were treated orally with risperidone (0.3 mg/kg, 3 times/day) or vehicle (control) starting from postnatal day (PD) 23 (± 1 day) for 3 weeks (a period corresponding to the childhood-adolescent period in humans). In the female juvenile rats, risperidone treatment increased food intake and body weight gain, which started to appear after 12 days' treatment. Risperidone also significantly decreased the locomotor activity of the female rats. Consistently, risperidone significantly elevated mRNA expression of hypothalamic H₁R, neuropeptide Y (NPY), and agouti-related peptide (AgRP) compared to controls, and H₁R and NPY levels were correlated with risperidone enhanced weight gain and food intake in the female juvenile rats. However, risperidone did not affect hypothalamic proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) mRNA expression. Therefore, these results suggested that risperidone elevated appetite and body weight gain in juveniles via regulation of the hypothalamic H₁R, NPY and AgRP pathways, as well as by reducing activity.

Key Words: risperidone; weight gain; juvenile rats; histamine receptor; neuropeptide Y; locomotor activity

LIST OF ABBREVIATIONS

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

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

AgRP, agouti-related peptide

Arc, arcuate nuclei

CART, cocaine- and amphetamine-regulated transcript

D₂R, dopaminergic D₂ receptor

H₁R, histaminergic H₁ receptor

NPY, neuropeptide Y

PD, postnatal day

POMC, proopiomelanocortin

qRT-PCR, quantitative real-time polymerase chain reaction

SGAs, second generation antipsychotics

VMH, ventromedial nucleus

1. Introduction

Over recent decades, antipsychotic prescriptions have sharply increased, not only to treat childhood-onset schizophrenia, but have also been widely used in children and adolescents to treat mental disorders such as bipolar disorder, autism and attention deficit hyperactivity disorder [1-4]. The majority of antipsychotic drugs prescribed in children and adolescents are second generation antipsychotics (SGAs) including risperidone and olanzapine, which have serious weight gain and other metabolic side-effects [5-10]. Of even greater concern, however, is evidence clearly demonstrating that the paediatric population appears to be at greater risk than adults for SGA-induced weight gain and metabolic side-effects [11].

Risperidone is the most commonly prescribed antipsychotic drug in paediatric patients [4, 12, 13]. It has been repeatedly reported that risperidone is associated with much higher weight gain in children and adolescents than those in adults [14-17]. For example, a meta-analysis of placebo controlled trials over 3000 paediatric and adult subjects using risperidone found that it can cause over three times more body weight gain (by percentage of weight changes) in youths than in adults [18]. A clinical trial also confirmed that after an average of 2.9 years' of risperidone treatment, 99 children and adolescent patients suffered a significant weight gain and increased body mass index (BMI) [19].

In contrast to first generation antipsychotics (such as haloperidol) that are largely potent and selective dopamine D₂ receptor (D₂R) antagonists, SGAs have binding affinities for various neurotransmitter receptors, such as D₂R, serotonin 5-HT_{2A} (5-HT_{2AR}) and 5-HT_{2C} receptors (5-HT_{2CR}) and histamine H₁ receptors (H_{1R}) [20]. Accumulated evidence from our laboratory and other laboratories has revealed that dopamine D₂R and 5-HT_{2AR} play a critical role in the therapeutic effects of SGAs including risperidone, while 5-HT_{2CR} and H_{1R}

contribute to weight gain/obesity side-effects [21-24]. In particular, using an adult rat model for olanzapine-induced weight gain, we previously identified that hypothalamic H₁R is a key factor for the olanzapine-induced weight gain side-effect [25, 26]. Since risperidone is also a potent antagonist for H₁R [20], H₁R may also play a key role in risperidone-induced weight gain.

Using the adult female rat model for antipsychotic-induced weight gain, it has been repeatedly revealed that olanzapine elevated the expression of appetite stimulating neuropeptides in the hypothalamus, including neuropeptide Y (NPY) and agouti-related peptide (AgRP), while decreasing appetite inhibiting neuropeptides such as proopiomelanocortin (POMC) [25, 27-29]. On the other hand, H₁R is independent of POMC regulation [30], but links to NPY expression [31]. Evidence has also shown that increased activities of NPY and AgRP expression by olanzapine were through blocking the H₁R [25, 26, 32]. There have been two previous studies investigating the effects of risperidone on expression of hypothalamic neuropeptides in male rats, however the results were contradictory and puzzling [33, 34]. Ota and his colleagues found that 3-week treatment of risperidone in adult male rats did not have any effect on the expression of hypothalamic NPY, AgRP, POMC and CART, even though treatment increased food intake [33, 35]. The other study reported some puzzling results in four-week old male rats; although food intake increase and weight gain were observed, four weeks' treatment of risperidone significantly decreased both appetite stimulating neuropeptides (NPY and AgRP) and appetite inhibiting neuropeptide POMC, while the other appetite inhibiting neuropeptide CART mRNA expression was increased [34]. Gothelf and colleagues revealed that after 4 weeks' of antipsychotic administration to male adolescent schizophrenic inpatients, BMI was significantly enhanced in those treated with olanzapine, but not in those treated with

haloperidol, which was associated with an increase in caloric intake [36]. Another study found that first-attack psychotic patients had reduced plasma levels of NPY and α -melanocyte melanocytostimulating hormone (α -MSH), but an increased plasma CART level compared to the control; four weeks' risperidone treatment did not change circulating NPY, α -MSH, and CART levels [8]. A recent review paper demonstrated that the effect of SGAs including risperidone is associated with food intake, physical activity and energy expenditure in both children and adult patients [37].

To date, there has been no study investigating the hypothalamic regulatory mechanisms underlying risperidone-induced weight gain in female juvenile rats. Therefore, in this study, we investigated the effects of risperidone on body weight and food intake in female juvenile rats and further investigated its effects on expression of hypothalamic H_1R , NPY, POMC, AgRP and CART mRNA expression, and their relationships with changes in body weight gain and food intake. Since our recent studies in adult rat models have demonstrated that olanzapine-induced weight gain is associated with a significant decrease in locomotor activity [38-40], and decreased activity was also observed after acute administration of antipsychotics during early development [41], the locomotor activity of female juvenile rats was also examined in this study.

2. Materials and Methods

2.1 Animals Housing, Administration and Drug Treatment

Timed pregnant Sprague Dawley rats (at gestation day 16) were obtained from the Animal Resources Centre (Perth, WA, Australia). They were housed in individual cages and allowed *ad-libitum* access to standard laboratory chow diet (3.9 kcal/g: 10% fat, 74% carbohydrate,

16% protein) and water under a light (07:00 to 19:00) and dark (19:00 to 7:00) cycle and temperature control (22°C) throughout the experiment [32, 38]. Day of birth was recognised as postnatal day (PD) 0. Pups were sexed on PD14, and 24 female rats were weaned on PD21 and housed in individual cages.

Before the treatment procedures, rats were trained for self-administration of the drug by feeding them using 0.3 g cookie dough (including 30.9 % cornstarch, 30.9% sucrose, 6.3% gelatine, 15.5% casein, 6.4% fibre, 8.4% minerals and 1.6% vitamins) without drug twice a day during PD18-21. The postnatal female rats (PD23±1) were then randomly assigned to one of two treatment groups as follows: (1) risperidone (0.3 mg/kg, t.i.d., Janssen, USA; n=12) or (2) vehicle (control; n=11) for 3 weeks (a period corresponding to the childhood-adolescent period in humans) [42]. Drugs were prepared in advance by mixing with cookie dough pellets and droplets of water, and were administered 3 times per day (8±1 hours, interval) orally for 3 weeks [32, 38]. The rats in the control group received an equivalent pellet without drug. Rats were observed throughout the experiment to ensure all cookie dough pellets were consumed. Body weight, food intake and water intake were measured and recorded once every two days. This study was approved by the Animal Ethics Committee, University of Wollongong, Australia (AE12/20); and all the procedures complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

After completing treatment, all rats were sacrificed (without fasting) by carbon dioxide asphyxiation. Brain samples were collected and stored in a -80 °C freezer until analysis. The hypothalamic nuclei were dissected based on the rat brain atlas [43]. In brief, rat brains were cut at $-10.5\text{ }^{\circ}\text{C} \pm 1.5\text{ }^{\circ}\text{C}$ into 500µm coronal sections. The nuclei were dissected using micro-punches (57401, Stoelting Co, USA), targeted at the arcuate nuclei (Arc). Since the Arc is

small, the punched tissue primarily contained Arc, but included adjacent ventromedial nucleus (VMH), therefore the punched tissue was labelled as the mediobasal hypothalamus. The dissected tissue was placed into 1.5 ml Eppendorf tubes, and stored at -80°C until use for qRT-PCR [25].

2.2 Open Field Test

An open field test was performed on $\text{PD36} \pm 1$ day in order to determine whether risperidone could affect the locomotor activity of rats. A rat was placed in the centre of a black rectangular arena ($60 \times 60 \text{ cm}^2$, 40 cm high) that was exposed to lighting of 25 lux across the entire arena. A video camera was used to record the behaviour of the rats for 30 minutes from the central top of the arena. The locomotor activity of the rats was analysed using EthoVision Color-Pro software (Noldus Information Technology, Wageningen, The Netherlands) [38] and the distance moved (cm) and velocity (cm/s) were measured.

2.3 Quantitative real-time PCR

Quantitative real-time polymerase chain reaction (qRT-PCR) was applied to detect mRNA expression of H_1R , NPY, POMC, AgRP and CART in the mediobasal hypothalamus. Total RNA was isolated using a PureLinkTM RNA extraction kit (Invitrogen Life Technologies, Carlsbad, CA, USA). RNA quality was then assessed by Nanodrop (ThermoFisher Scientific, MA, USA). Aliquots of total RNA were digested within a spin column RNase-free DNase I kit (MO BIO Laboratories, Inc., CA, USA) to remove residual genomic DNA. The digested RNA was purified by ethanol precipitation, dried and dissolved in RNA Storage Solution (Ambion, Inc., Austin, TX). Aliquots of RNA were subjected to reverse transcription using super-script II RNase H-Reverse Transcriptase. To ensure the total RNA was free from genomic DNA after DNase digestion, aliquots of total RNA were subjected to PCR using a

pair RT-control. First-strand cDNA was synthesised from RNA with Superscript® VILO™ cDNA synthesis kit (Life Technologies, NSW, Australia) with 20 µL reaction volume containing 9 µL cDNA, 10 µL SYBR Green PCR Master Mix (Applied Biosystems, Boston, MA), 1 µL each of primers.

Real-time quantitative PCR was performed in triplicate using TaqMan® Gene Expression Assays (Applied Biosystems, Foster City, USA) in combination with continuous SYBR Green detection (Applied Biosystems) on LightCycler®480 (Roche, Penzberg, Germany). The assay (Life Technologies, NSW, Australia) identifications of the target genes were H₁ receptor (Rn00566691 s1); NPY (Rn01410145 m1); POMC (Rn00595020 m1); AgRP (Rn01431703 g1), and CART (Rn01645174 m1). β-actin (Rn00667869 m1) and GAPDH (Rn01775763 g1) were expressed as endogenous controls. The RT-PCR plates were run by polymerase amplification for 10 mins at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15s, and annealing and extending at 60 °C for 1 min. The $2^{-\Delta\Delta T}$ method was used to calculate the results [44].

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 the distribution of data from all experiments. Weight gain, food intake and water intake data were analysed by two-way repeated ANOVAs (Treatment × Days as repeated measures). Behavior and mRNA expression were analyzed by independent-samples T test to compare the risperidone and control groups. Pearson's correlation test was used to analyse the relationships among the measurements. All data were expressed as mean ± SEM, and statistical significance was accepted when $p < 0.05$.

3. Results

3.1 Effects of risperidone on body weight gain, food intake and water intake

Risperidone had no effects on weight gain in the first 12 days of drug treatment (Figure 1A), however it was shown that body weight gain was significantly greater in the risperidone group compared to the controls from day 14 (Figure 1A). Two-way repeated ANOVAs (Treatment \times Days as repeated measures) showed significant main effects of Days ($F_{3,63}=193.915$, $p<0.001$), Treatment ($F_{1,21}=8.939$, $p<0.01$), and significant interaction between the two factors ($F_{3,63}=5.484$, $p<0.01$) on accumulated body weight gain. Risperidone significantly increased the accumulated body weight gain on treatment day 14 ($t=-2.535$, $df=21$, $p=0.019$), day 16 ($t=-2.712$, $df=21$, $p=0.013$), day 18 ($t=-3.345$, $df=21$, $p=0.003$) and day 20 ($t=-2.534$, $df=21$, $p=0.018$) (Figure 1A). There was no significant difference between risperidone and control groups in leg length (Risperidone, 4.55 ± 0.04 cm vs. Control, 4.43 ± 0.06 cm; $t=-1.562$, $df=21$, $p=0.133$).

Consistent with the weight gain data, there was no difference in food intake on the first 12 days' of drug treatment, while risperidone-treated juvenile rats showed significant enhanced accumulated food intake compared with controls during treatment days 14-20 (Figure 1B). There were significant main effects of Treatment ($F_{1,21}=6.222$, $p<0.05$) and Days ($F_{3,63}=865.998$, $p<0.001$), and significant interaction between the two factors ($F_{3,63}=3.895$, $p<0.05$) (Figure 1B). Risperidone-treated juvenile rats also had higher average feeding efficiency (accumulated body weight gain/accumulated food intake during treatment days 14-20) than the control ($t=-2.707$, $df=21$, $p=0.013$) (Figure 1C). Moreover, there were highly positive correlations between total body weight gain and total food intake ($r=0.581$, $p<0.01$), and with feeding efficiency ($r=0.614$, $p<0.01$).

There was also no change of water intake in the first 12 days' of treatment. From treatment day 14 (Figure 1D), the two-way repeated ANOVAs (Treatment \times Days as repeated measures) also showed the significant main effects of Treatment ($F_{1,21}=5.503$, $p<0.05$) and Days ($F_{3,63}=642.025$, $p<0.001$), but there was no significant interaction between the two factors ($F_{3,63}=0.738$, $p=0.533$). There was a significant difference of water intake between risperidone treatment and control on treatment day 14 ($t=-2.376$, $df=21$, $p=0.027$), day 16 ($t=-2.457$, $df=21$, $p=0.023$), day 18 ($t=-2.451$, $df=21$, $p=0.023$), and day 20 ($t=-2.447$, $df=21$, $p=0.023$) (Figure 1D).

3.2 Effects of risperidone treatment on locomotor activity

Figure 2A shows examples of locomotor activity of rats treated with risperidone and vehicle (controls). Compared to the control, the rats treated with risperidone had significantly decreased total distance moved ($t=2.951$, $df=21$, $p<0.01$) and total lower velocity ($t=3.310$, $df=21$, $p<0.01$) (Figures 2B and C). Furthermore, it is important to note that total distance moved was negatively correlated with total body weight gain ($r=-0.569$, $p<0.01$), and feeding efficiency ($r=-0.444$, $p<0.05$). The velocity also negatively correlated with total body weight gain ($r=-0.610$, $p<0.01$), and feeding efficiency ($r=-0.474$, $p<0.05$).

3.3 Effects of risperidone treatment on expression of hypothalamic H₁R, NPY, POMC, AgRP and CART

Figure 3 presents the effects of risperidone treatment on the mRNA expression of hypothalamic H₁R, NPY, POMC, AgRP and CART in female juvenile rats compared with the control. Risperidone treatment significantly upregulated mRNA expression of H₁R (+23%, $t=-3.784$, $df=9$, $p=0.004$) and AgRP (+155%, $t=-2.917$, $df=9$, $p=0.021$), and a tendency to elevate NPY ($t=-2.124$, $df=9$, $p=0.055$), compared with the control (Figure 3). Hypothalamic

H₁R mRNA expression was positively correlated to NPY ($r=0.579$, $p<0.05$) and AgRP ($r=0.565$, $p<0.05$) levels. There were positive correlations between hypothalamic NPY expression and body weight gain ($r=0.678$, $p<0.05$), and feeding efficiency ($r=0.560$, $p<0.05$).

4. Discussion

This study revealed the effects of risperidone treatment on body weight, food and water intake, locomotor activity, and mRNA expression of H₁R and hypothalamic neuropeptides POMC, NPY, AgRP and CART in juvenile (PD23-44) female rats. To our knowledge, this is the first study into the effects of risperidone on hypothalamic appetite signalling in a young female rat model of risperidone-induced weight gain. Our results showed that risperidone treatment at 0.3 mg/kg, t.i.d. (equivalent to the recommended dosage in the clinic) significantly increased body weight gain in female juvenile rats. We found that risperidone-induced weight gain and food intake was associated with increased histamine H₁R and NPY levels in the hypothalamus. Furthermore, our results showed that risperidone-induced weight gain is associated with a significant decrease in locomotor activity in juvenile female rats. Our findings coincide with previous reports on children/adolescents [17, 45-48].

4.1 Choice of female rats

Female rats were chosen in this study, both because risperidone-induced weight gain has been reported in a previous study using female juvenile rats [49] and also because, while SGA-induced weight gain has been consistently established in females using various SGAs including risperidone and olanzapine [27, 32, 38, 40, 50-53], it could not be consistently modelled in male rodents. While risperidone-induced weight gain has been well modelled in female rodents [49, 52-54], it has been established in male rats in some studies [34] but not in others [35, 54, 55].

4.2 Late onset of weight gain

The increase in weight gain and food intake caused by risperidone treatment in this study occurred after 12 days' treatment. This was unexpected, since previous studies in adult models often showed that risperidone and olanzapine increased food intake and weight gain after 3-4 days' treatment [38, 52, 54, 56]. Risperidone was also reported to increase food intake and body weight after 3 days' treatment in female rats at 7-weeks old [49]. In this study, however, much younger female animals (23-days old) were used. These results suggest a possible age difference in the onset of increased weight gain and food intake caused by risperidone treatment, although we could not fully explain it at this stage. Further study is needed to address this issue.

4.3 Dosage

According to dosage translation between humans and rats based on body surface following the FDA guideline for clinical trials [57, 58], the risperidone dosage in this study (0.3 mg/kg) is equivalent to the human dosage (1.8 mg in human children at 25 kg body weight, or 2.8 mg in human children at 40 kg body weight). The half-life of active moiety (including risperidone and 9-hydroxyrisperidone) is 8.6 hours in the rat brain and 11.4 hours in rat plasma after an oral dose risperidone [59], compared to an average 20 hours in human plasma (up to 30 hours in poor metabolisers) [60]. This drug was therefore delivered orally 3 times/day (at 8 hour intervals) in this study to ensure a consistently high concentration that better mirrors the human scenario of oral administration [38]. The risperidone (0.3 mg/kg, t.i.d.) used in this study is therefore within the range of recommended dosage used in paediatric patients (0.5-3 mg/day) [1, 4, 60], and does not reach the maximum indicated paediatric dosage (6 mg/day) [61]. At this dosage, the risperidone drug treatment reaches

between 65-90% dopamine D₂ receptor occupancy [62, 63]. Risperidone treatment at 0.5 mg/kg (once daily, intraperitoneal or subcutaneous injection) has been reported to significantly increase weight gain and food intake in female adult/7-week old rats [49, 52, 54] and in female adult mice risperidone (2 mg/kg, orally twice per day) for 3 weeks led to significantly increased body weight gain and food intake compared to controls [53].

4.4 Hypothalamic H₁R mRNA expression

Histamine H₁R is highly expressed in the hypothalamic Arc and VMH, which play significant roles in body weight regulation, food intake and energy expenditure [64, 65]. Two meta-analyses of clinical data have found that the H₁R antagonistic property of antipsychotics is strongly correlated with their body weight gain side-effect [66, 67]. Studies in animal models have further evidenced that hypothalamic H₁R is a major contributor for olanzapine/clozapine-induced weight gain and obesity [25, 26, 32, 68, 69]. Risperidone has high binding affinity with H₁R [20], and our examination of mRNA expression of hypothalamic H₁R found a significant increase in hypothalamic H₁R mRNA expression in the risperidone treatment group. This result is consistent with previous findings that olanzapine (a potent H₁R antagonist) increased hypothalamic H₁R mRNA expression associated with olanzapine-induced weight gain [25, 26].

4.5 mRNA expressions of hypothalamic neuropeptides

In this study, we revealed that hypothalamic NPY and AgRP mRNA expression were elevated by risperidone treatment in young female rats, which were correlated with risperidone-induced food intake and weight gain. This is also consistent with previous reports in female rat models for olanzapine-induced weight gain with a significant increase of NPY mRNA expression [25, 27-29, 70]. In addition, it has previously been found that acute ICV

olanzapine treatment led to an upregulated AgRP level in male adult rats [17]. Furthermore, our recent study showed an elevated AgRP mRNA expression after two weeks' treatment with olanzapine in female adult rats [25]. Corresponding with our findings, Gruber and colleagues reported that NPY-like immunoreactivity was increased in the hypothalamic region but decreased in the ventral striatum of male Wistar rats (weighing 130 g at the beginning of the experiment) after 4-weeks' risperidone treatment [71]. However, results contradictory to this study were reported in one recent study, where 4 weeks' treatment of risperidone in 4-week old male rats caused a decrease in hypothalamic NPY/AgRP expression; instead, there was a significant decrease in hypothalamic POMC mRNA expression induced by risperidone treatment, while CART mRNA expression increased [34]. In view of the fact that hypothalamic NPY and AgRP are appetite stimulating neuropeptides, and POMC and CART are appetite inhibiting neuropeptides, these results are puzzling and hard to explain, because a significant increase in weight gain and food intake was observed in these risperidone treated male rats [34]. In this study, we did not find any changes in POMC and CART mRNA expression after risperidone treatment in female juvenile rats. This was different from previous findings in the olanzapine-induced weight gain model, in which olanzapine treatment was found to decrease POMC mRNA expression in adult female rats [25, 27-29, 70]. However, although first-attack psychotic patients had higher plasma NPY and α -MSH, but lower CART levels than the control, four weeks' risperidone treatment did not change NPY, α -MSH, and CART levels [8]. Therefore, further studies are necessary to investigate the mechanisms underlying risperidone-induced weight gain, particularly in juveniles.

4.6 Reduced locomotor activity

It is worth noting that weight gain induced by antipsychotics is not only attributed to increased energy intake; reduced energy expenditure may also play an important role as proven in olanzapine-induced weight gain rat models [38, 39, 56, 72]. In fact, this study found that risperidone reduced locomotor activity in juvenile female rats, which is consistent with a number of studies in which risperidone was proven to reduce locomotor activity in rats and mice [53, 73, 74]. Clinical studies have also reported that risperidone treatment reduced hyperactivity in children with attention-deficit and bipolar disorder [37, 75, 76]. Therefore, the decreased activity exhibited in the present study may also play a role in risperidone-induced body weight gain. Furthermore, this study found that risperidone treatment also increased water intake, which might reflect the dry mouth side-effects following risperidone treatment often observed in clinics [77].

5. Conclusion

Overall, this study has shown that oral risperidone treatment induced significantly increased weight gain and food intake in juvenile female rats. Risperidone-induced body weight gain and hyperphagia could be through regulating histamine H₁R, NPY and AgRP signalling in the hypothalamus, as well as reducing locomotor activity. Since weight gain and other metabolic abnormalities during childhood could strongly predict adult obesity and metabolic syndromes, paediatric subjects treated with antipsychotics such as risperidone face a much greater risk of obesity and other metabolic disorders in adulthood, which pose a major risk of premature cardiovascular morbidity and mortality in adults [78]. Therefore, further studies using the animal model are important for developing strategies to ameliorate risperidone-induced weight gain in juvenile patients, and to reveal its underlying mechanisms.

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Authors' Contributions

JL and CD conceived and designed the experiments. JL, MDS and MH performed the experiments. JL did the statistical analysis and drafted the manuscript. JL and CD made significant contributions to revise the manuscript.

Conflict of Interest

All authors have no conflict of interest.

References

- [1] Zuddas A, Zanni R, and Usala T. Second generation antipsychotics (SGAs) for non-psychotic disorders in children and adolescents: A review of the randomized controlled studies. *Eur Neuropsychopharmacol* 2011; 21:600-20.
- [2] Fraguas D, Correll CU, Merchán-Naranjo J, Rapado-Castro M, Parellada M, Moreno C, et al. Efficacy and safety of second-generation antipsychotics in children and adolescents with psychotic and bipolar spectrum disorders: comprehensive review of prospective head-to-head and placebo-controlled comparisons. *Eur Neuropsychopharmacol* 2011; 21:621-45.
- [3] Piontkewitz Y, Arad M, and Weiner I. Risperidone administered during asymptomatic period of adolescence prevents the emergence of brain structural pathology and behavioral abnormalities in an animal model of schizophrenia. *Schizophrenia Bulletin* 2011; 37:1257-69.
- [4] Caccia S. Safety and pharmacokinetics of atypical antipsychotics in children and adolescents. *Paediatr Drugs* 2013; 15:217-33.
- [5] Remschmidt H, Hennighausen K, Clement HW, Heiser P, and Schulz E. Atypical neuroleptics in child and adolescent psychiatry. *Eur Child Adolesc Psychiatry* 2000; 9 Suppl 1:19-19.
- [6] Panagiotopoulos C, Ronsley R, Elbe D, Davidson J, and Smith DH. First do no Harm: Promoting an Evidence-Based Approach to Atypical Antipsychotic use in Children and Adolescents. *J Can Acad Child Adolesc Psychiatry* 2010; 19:124-37.
- [7] De Hert M, Schreurs V, Vancampfort D, and Van Winkel R. Metabolic syndrome in people with schizophrenia: a review. *World Psychiatry* 2009; 8:15-22.
- [8] Yanik T, Kursungoz C, Sutçigil L, and Ak M. Weight Gain in risperidone therapy: Investigation of peripheral hypothalamic neurohormone levels in psychotic patients. *J Clin Psychopharmacol* 2013; 33:608-13.
- [9] Margari L, Matera E, Craig F, Petruzzelli MG, Palmieri VO, Pastore A, et al. Tolerability and safety profile of risperidone in a sample of children and adolescents. *Int Clin Psychopharmacol* 2013; 28:177-83.
- [10] Leucht S, Cipriani A, Spineli L, Mavridis D, Orey D, Richter F, et al. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *Lancet* 2013; 382:951-62.
- [11] Correll CU. Addressing adverse effects of antipsychotic treatment in young patients with schizophrenia. *J Clin Psychiatry* 2011; 72:e01.
- [12] Walter G, DeLaroche A, Soh N, Hunt G, Cleary M, Malhi G, et al. Side effects of second-generation antipsychotics: the experiences, views and monitoring practices of Australian child psychiatrists. *Australas* 2008; 16:253-62.
- [13] Olfson M, Crystal S, Huang C, and Gerhard T. Trends in antipsychotic drug use by very young, privately insured children. *J Am Acad Child Adolesc Psychiatry* 2010; 49:13-23.
- [14] Maayan LA and Vakhrusheva J. Risperidone associated weight, leptin, and anthropometric changes in children and adolescents with psychotic disorders in early treatment. *Hum* 2010; 25:133-38.
- [15] Demb H, Valicenti-McDermott M, Navarro A, and Ayoob KT. The effect of long-term use of risperidone on body weight of children with an autism spectrum disorder. *J Clin Psychopharmacol* 2011; 31:669-70.
- [16] Hoekstra PJ, Troost PW, Lahuis BE, Mulder H, Mulder EJ, Franke B, et al. Risperidone-induced weight gain in referred children with autism spectrum disorders

- is associated with a common polymorphism in the 5-hydroxytryptamine 2C receptor gene. *J Child Adolesc Psychopharmacol* 2010; 20:473-77.
- [17] Martin A, Landau J, Leebens P, Ulizio K, Cicchetti D, Scahill L, et al. Risperidone-associated weight gain in children and adolescents: a retrospective chart review. *J Child Adolesc Psychopharmacol* 2000; 10:259-68.
 - [18] Maayan Land Correll CU. Weight gain and metabolic risks associated with antipsychotic medications in children and adolescents. *J Child Adolesc Psychopharmacol* 2011; 21:517-35.
 - [19] Calarge CA, Acion L, Kuperman S, Tansey M, and Schlechte JA. Weight Gain and Metabolic Abnormalities During Extended Risperidone Treatment in Children and Adolescents. *J Child Adolesc Psychopharmacol* 2009; 19:101-09.
 - [20] Correll CU. From receptor pharmacology to improved outcomes: individualising the selection, dosing, and switching of antipsychotics. *Eur Psychiatry* 2010; 25 Suppl 2:S12-21.
 - [21] Meltzer Hand Massey B. The role of serotonin receptors in the action of atypical antipsychotic drugs. *Curr Opin Pharmacol* 2011; 11:59-67.
 - [22] Ginovart Nand Kapur S. Role of dopamine D(2) receptors for antipsychotic activity. *Handb* 2012:27-52.
 - [23] van Bellinghen Mand de Troch C. Risperidone in the Treatment of Behavioral Disturbances in Children and Adolescents with Borderline Intellectual Functioning: A Double-Blind, Placebo-Controlled Pilot Trial. *J Child Adolesc Psychopharmacol* 2001; 11:5-13.
 - [24] Deng C, Weston-Green K, and Huang X-F. The role of histaminergic H₁ and H₃ receptors in food intake: a mechanism for atypical antipsychotic-induced weight gain? *Prog Neuropsychopharmacol Biol Psychiatry* 2010; 34:1-4.
 - [25] Lian J, Huang X-F, Pai N, and Deng C. Betahistidine ameliorates olanzapine-induced weight gain through modulation of histaminergic, NPY and AMPK pathways. *Psychoneuroendocrinology* 2014; 48:77-86.
 - [26] He M, Zhang Q, Deng C, Wang H, Lian J, and Huang X-F. Hypothalamic histamine H₁ receptor-AMPK signaling time-dependently mediates olanzapine-induced hyperphagia and weight gain in female rats. *Psychoneuroendocrinology* 2014; 42:153-64.
 - [27] Fernø J, Varela L, Skrede S, Vázquez MJ, Nogueiras R, Diéguez C, et al. Olanzapine-induced hyperphagia and weight gain associate with orexigenic hypothalamic neuropeptide signaling without concomitant AMPK phosphorylation. *PLoS ONE* 2011; 6:e20571.
 - [28] Weston-Green K, Huang XF, and Deng C. Alterations to Melanocortinergic GABAergic and Cannabinoid Neurotransmission Associated with Olanzapine. *PLoS ONE* 2012; 7:e33548.
 - [29] Zhang Q, He M, Deng C, Wang H, Lian J, and Huang XF. Hypothalamic ghrelin signalling mediates olanzapine induced hyperphagia and weight gain in female rats. *Int J Neuropsychopharmacol* 2014; 17:807-18.
 - [30] Yoshimatsu H. The neuronal histamine H(1) and pro-opiomelanocortin-melanocortin 4 receptors: independent regulation of food intake and energy expenditure. *Peptides* 2006; 27:326-32.
 - [31] Jorgensen EA, Knigge U, Watanabe T, Warberg J, and Kjaer A. Histaminergic neurons are involved in the orexigenic effect of orexin-A. *Neuroendocrinology* 2005; 82:70-77.

- [32] Lian J, Huang X-F, Pai N, and Deng C. Preventing olanzapine-induced weight gain using betahistine: a study in a rat model with chronic olanzapine treatment. *PLoS ONE* 2014; 9:e104160.
- [33] Ota M, Mori K, Nakashima A, Kaneko YS, Takami G, and Ota A. mRNA expression levels of leptin-related substances can be modified in risperidone-injected rats. *Biogenic Amines* 2005; 19:299-308.
- [34] Kursungoz C, Ak M, and Yanik T. Effects of risperidone treatment on the expression of hypothalamic neuropeptide in appetite regulation in Wistar rats. *Brain Res* 2015; 1596:146-55.
- [35] Ota M, Mori K, Nakashima A, Kaneko YS, Fujiwara K, Itoh M, et al. Peripheral injection of risperidone, an atypical antipsychotic, alters the bodyweight gain of rats. *Clin Exp Pharmacol Physiol* 2002; 29:980-89.
- [36] Gothelf D, Falk B, Singer P, Kairi M, Phillip M, Zigel L, et al. Weight gain associated with increased food intake and low habitual activity levels in male adolescent schizophrenic inpatients treated with olanzapine. *Am J Psychiatry* 2002; 159:1055-7.
- [37] Cuerda C, Velasco C, Merchan-Naranjo J, Garcia-Peris P, and Arango C. The effects of second-generation antipsychotics on food intake, resting energy expenditure and physical activity. *Eur J Clin Nutr* 2014; 68:146-52.
- [38] Deng C, Lian J, Pai N, and Huang XF. Reducing olanzapine-induced weight gain side-effect by betahistine: a study in the rat model. *J Psychopharmacol (Oxf)* 2012; 26:1291-79.
- [39] Zhang Q, Lian J, He M, Deng C, Wang H, and Huang XF. Olanzapine reduced brown adipose tissue thermogenesis and locomotor activity in female rats. *Prog Neuropsychopharmacol Biol Psychiatry* 2014; 51:172-80.
- [40] Weston-Green K, Huang X-F, and Deng C. Olanzapine treatment and metabolic dysfunction: a dose response study in female Sprague Dawley rats. *Behav Brain Res* 2011; 217:337-46.
- [41] Wiley JL. Antipsychotic-induced suppression of locomotion in juvenile, adolescent and adult rats. *Eur J Pharmacol* 2008; 578:216-21.
- [42] Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity? *Neurosci Biobehav Rev* 2003; 27:3-18.
- [43] Paxinos Gand Watson C. The rat brain in stereotaxic coordinates, Academic Press, 2007.
- [44] Schmittgen TD and Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 2008; 3:1101-08.
- [45] Snyder R, Turgay A, Aman M, Binder C, Fisman S, and Carroll A. Effects of risperidone on conduct and disruptive behavior disorders in children with subaverage IQs. *J Am Acad Child Adolesc Psychiatry* 2002; 41:1026-36.
- [46] Reyes M, Buitelaar J, Toren P, Augustyns I, and Eerdekens M. A randomized, double-blind, placebo-controlled study of risperidone maintenance treatment in children and adolescents with disruptive behavior disorders. *Am J Psychiatry* 2006; 163:402-10.
- [47] Luby J, Mrakotsky C, Stalets MM, Belden A, Heffelfinger A, Williams M, et al. Risperidone in preschool children with autistic spectrum disorders: an investigation of safety and efficacy. *J Child Adolesc Psychopharmacol* 2006; 16:575-87.
- [48] Haas M, Delbello MP, Pandina G, Kushner S, Van Hove I, Augustyns I, et al. Risperidone for the treatment of acute mania in children and adolescents with bipolar disorder: a randomized, double-blind, placebo-controlled study. *Bipolar Disord* 2009; 11:687-700.

- [49] Fell MJ, Neill JC, Rao C, and Marshall KM. Effects of sub-chronic antipsychotic drug treatment on body weight and reproductive function in juvenile female rats. *Psychopharmacology (Berl)* 2005; 182:499-507.
- [50] Pai N, Deng C, Vella SL, Castle D, and Huang XF. Are there different neural mechanisms responsible for three stages of weight gain development in anti-psychotic therapy: temporally based hypothesis. *Asian J Psychiatr* 2012; 5:315-18.
- [51] Goudie AJ, Smith J, and Halford J. Characterization of olanzapine-induced weight gain in rats. *J Psychopharmacol (Oxf)* 2002; 16:291-96.
- [52] Baptista T, de Baptista EA, Lalonde J, Plamondon J, Kin NMKNY, Beaulieu S, et al. Comparative effects of the antipsychotics sulpiride and risperidone in female rats on energy balance, body composition, fat morphology and macronutrient selection. *Prog Neuropsychopharmacol Biol Psychiatry* 2004; 28:1305-11.
- [53] Cope MB, Li X, Jumbo-Lucioni P, DiCostanzo CA, Jamison WG, Kesterson RA, et al. Risperidone alters food intake, core body temperature, and locomotor activity in mice. *Physiol Behav* 2009; 96:457-63.
- [54] Baptista T, Araujo de Baptista E, Ying Kin NMKN, Beaulieu S, Walker D, Joobar R, et al. Comparative effects of the antipsychotics sulpiride or risperidone in rats. I: bodyweight, food intake, body composition, hormones and glucose tolerance. *Brain Res* 2002; 957:144-51.
- [55] Lin E-JD, Lee NJ, Slack K, Karl T, Duffy L, O'Brien E, et al. Distinct endocrine effects of chronic haloperidol or risperidone administration in male rats. *Neuropharmacology* 2006; 51:1129-36.
- [56] Weston-Green K, Huang X-F, and Deng C. Dosage response of metabolic dysfunction to olanzapine treatment in female Sprague Dawley rats. *Behav Brain Res* 2011; 217:337-46.
- [57] FDA. Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers, U.S. FDA Center for Drug Evaluation and Research, 2005.
- [58] Reagan-Shaw S, Nihal M, and Ahmad N. Dose translation from animal to human studies revisited. *FASEB J* 2008; 22:659-61.
- [59] Aravagiri Mand Marder SR. Brain, plasma and tissue pharmacokinetics of risperidone and 9-hydroxyrisperidone after separate oral administration to rats. *Psychopharmacology (Berl)* 2002; 159:424-31.
- [60] Janssen Pharmaceuticals I. Risperdal prescribing information. Retrieved from <http://www.janssenpharmaceuticalsinc.com/assets/risperdalpdf> 2014: [retrieval date: March 3, 2015].
- [61] FDA. Atypical antipsychotics: U.S. food and drug administration-approved indications and dosages for use in pediatric patients, U.S. FDA Center for Drug Evaluation and Research, 2014.
- [62] Kapur S, VanderSpek SC, Brownlee BA, and Nobrega JN. Antipsychotic Dosing in Preclinical Models Is Often Unrepresentative of the Clinical Condition: A Suggested Solution Based on in Vivo Occupancy. *J Pharmacol Exp Ther* 2003; 305:625-31.
- [63] McCormick PN, Wilson VS, Wilson AA, and Remington GJ. Acutely administered antipsychotic drugs are highly selective for dopamine D2 over D3 receptors. *Pharmacol Res* 2013; 70:66-71.
- [64] Masaki Tand Yoshimatsu H. Neuronal histamine and its receptors: implication of the pharmacological treatment of obesity. *Curr Med Chem* 2010; 17:4587-92.
- [65] Poole SL, Lewis DI, and Deuchars SA. Histamine depolarizes neurons in the dorsal vagal complex. *Neurosci Lett* 2008; 432:19-24.

- [66] Kroeze WK, Hufeisen SJ, Popadak BA, Renock SM, Steinberg S, Ernsberger P, et al. H₁-Histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacology* 2003; 28:519-26.
- [67] Matsui-Sakata A, Ohtani H, and Sawada Y. Receptor occupancy-based analysis of the contributions of various receptors to antipsychotics-induced weight gain and diabetes mellitus. *Drug Metab Pharmacokinet* 2005; 20:368-78.
- [68] Han M, Deng C, Burne THJ, Newell KA, and Huang XF. Short- and long-term effects of antipsychotic drug treatment on weight gain and H₁ receptor expression. *Psychoneuroendocrinology* 2008; 33:569-80.
- [69] Kim SF, Huang AS, Snowman AM, Teuscher C, and Snyder SH. Antipsychotic drug-induced weight gain mediated by histamine H₁ receptor-linked activation of hypothalamic AMP-kinase. *Proc Natl Acad Sci U S A* 2007; 104:3456-59.
- [70] Sezlev D, Ak M, Yanık T, Kursungoz C, Akarsu S, and Sutçigil L. 2761–The role of central neuropeptides in weight gain caused by olanzapine. *Eur Psychiatry* 2013; 28:1.
- [71] Gruber SH and Mathe AA. Effects of typical and atypical antipsychotics on neuropeptide Y in rat brain tissue and microdialysates from ventral striatum. *J Neurosci Res* 2000; 61:458-63.
- [72] Evers SS, Calcagnoli F, van Dijk G, and Scheurink AJW. Olanzapine causes hypothermia, inactivity, a deranged feeding pattern and weight gain in female Wistar rats. *Pharmacol Biochem Behav* 2010; 97:163-69.
- [73] Richtand NM, Taylor B, Welge JA, Ahlbrand R, Ostrander MM, Burr J, et al. Risperidone pretreatment prevents elevated locomotor activity following neonatal hippocampal lesions. *Neuropsychopharmacology* 2006; 31:77-89.
- [74] Karl T, Duffy L, O'Brien E, Matsumoto I, and Dedova I. Behavioural effects of chronic haloperidol and risperidone treatment in rats. *Behav Brain Res* 2006; 171:286-94.
- [75] Malone RP, Gratz SS, Delaney MA, and Hyman SB. Advances in drug treatments for children and adolescents with autism and other pervasive developmental disorders. *CNS Drugs* 2005; 19:923-34.
- [76] Frazier JA, Meyer MC, Biederman J, Wozniak J, Wilens TE, Spencer TJ, et al. Risperidone treatment for juvenile bipolar disorder: a retrospective chart review. *J Am Acad Child Adolesc Psychiatry* 1999; 38:960-65.
- [77] Brown LT and Brown JN. Use of risperidone as augmentation treatment for major depressive disorder. *Ann Pharmacother* 2011; 45:95-100.
- [78] Almandil NB, Liu Y, Murray ML, Besag FMC, Aitchison KJ, and Wong ICK. Weight gain and other metabolic adverse effects associated with atypical antipsychotic treatment of children and adolescents: a systematic review and meta-analysis. *Paediatr Drugs* 2013; 15:139-50.

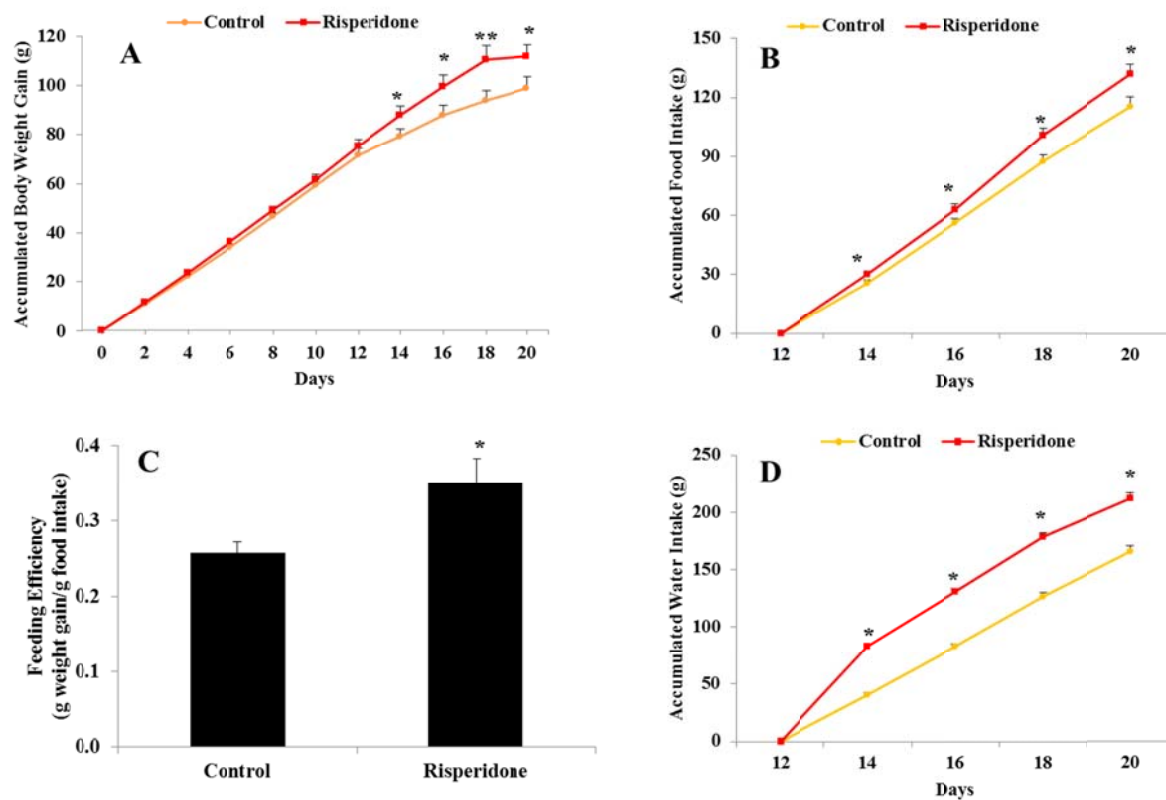


Figure 1. The effects of risperidone treatment administration on the accumulated body weight gain (A), food intake (B), feeding efficiency (C) and water intake (D) of female juvenile rats. Risperidone (0.3 mg/kg; n=12) or control (vehicle; n=11). ●: control; ■: risperidone. * $p < 0.05$, ** $p < 0.01$ vs. control.

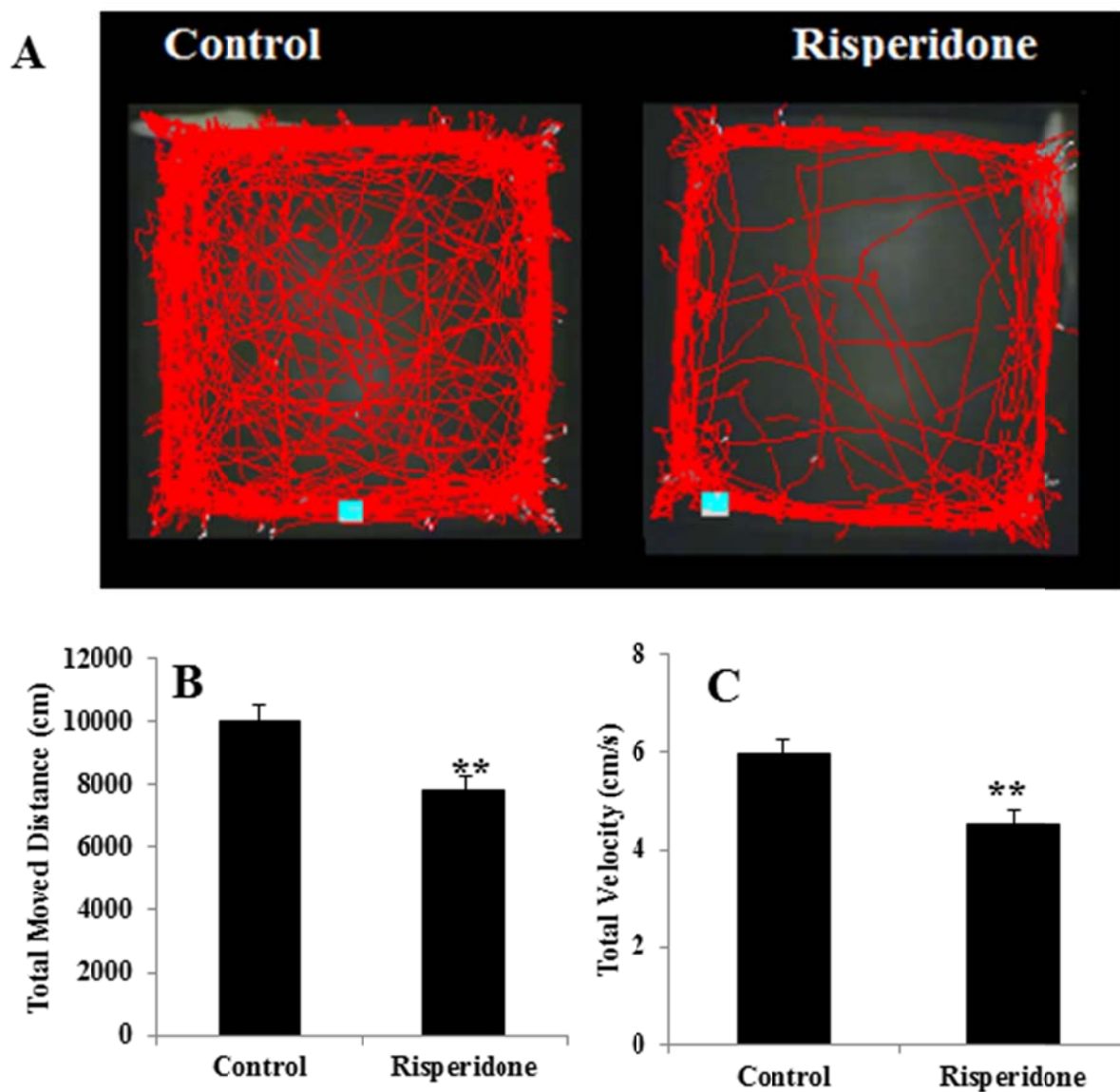


Figure 2. (A) Examples of locomotor activity from female juvenile rats treated with risperidone or control (vehicle). (B) Total distance moved and (C) Travel velocity in the open field test, ** $p < 0.01$ vs. control.

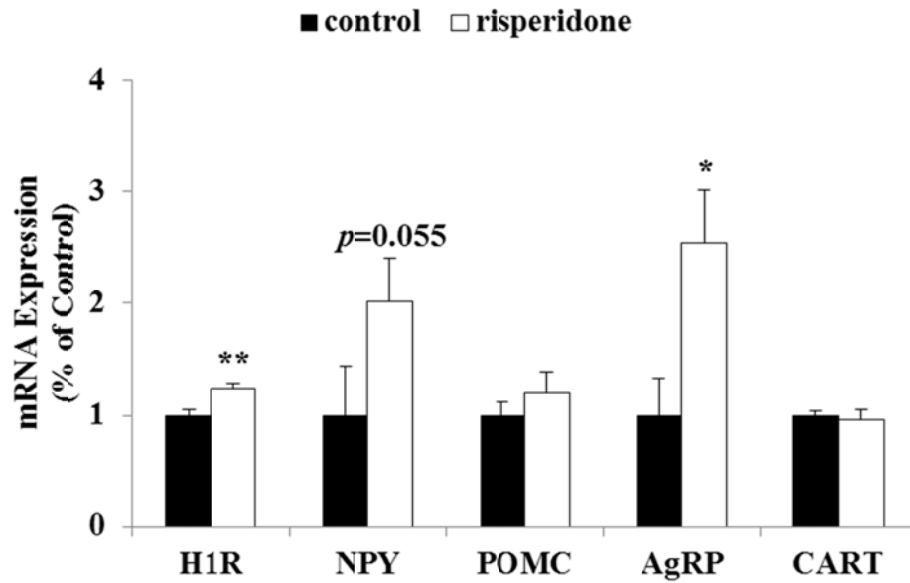


Figure 3. Effects of risperidone treatment on the hypothalamic mRNA expressions of histamine H₁R, NPY, AgRP, POMC and CART compared with controls in female juvenile rats. Abbreviations: NPY, neuropeptide Y; AgRP, agouti-related peptide; POMC, pro-opiomelanocortin; CART, cocaine- and amphetamine-regulated transcript. * $p<0.05$, ** $p<0.01$ vs. risperidone. Black bar: control; White bar: risperidone.