January 2014

# Olanzapine reduced brown adipose tissue thermogenesis and locomotor activity in female rats

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## Abstract

Excessive weight gain has been identified as a serious metabolic side-effect of second-generation antipsychotics (SGAs), including olanzapine. While hyperphagia has been suggested to be the main contributor for this side-effect in the short term, reduced energy expenditure, in particular thermogenesis and locomotor activity, has been considered to contribute to the maintenance of heavy weight under long-term SGA treatments. Recent studies have identified metabolically active brown adipose tissues (BAT) in adult humans, suggesting potential clinical significance for the involvement of BAT thermogenesis in SGA-induced weight gain. However, to date there has been little research elucidating the central neuronal pathways affecting BAT thermogenesis or the morphological changes of the BAT. The present study aimed to investigate the role of BAT thermogenesis and locomotor activity in olanzapine-induced weight gain during the prolonged time courses of olanzapine treatment in an established female rat model. Although short- to mid-term olanzapine treatment had no effect on BAT temperature, we observed that long-term olanzapine treatment (from day 18 to 34) induced a significant reduction in BAT temperature, with an acute effect being observed between 45 and 150 min post-treatment in the long-term cohort. Additionally, in the long-term olanzapine group, the reduced BAT temperature was accompanied by decreased UCP1 and PGC-1α expressions in the BAT. Moreover, TH mRNA expressions in both hypothalamus and brainstem were also downregulated after mid- to long-term olanzapine treatment. Further, olanzapine led to reduced percentage of brown adipocytes in BAT during mid- to long-term treatments. Finally, locomotor activity was reduced throughout the three treatment cohorts. In summary, our results suggest that the reduction of BAT thermogenesis plays an important role during the long-term of olanzapine-induced weight gain, which was accompanied by an earlier onset of BAT adipocyte morphological changes and biochemical changes in the hypothalamus and the brainstem, while locomotor activity contributes to the entire olanzapine treatment courses.



## Keywords

Antipsychotics, brown adipose tissue, PGC-1α, thermogenesis, weight gain


## Disciplines

Medicine and Health Sciences


## Publication Details

Zhang, Q., Lian, J., He, M., Deng, C., Wang, H. & Huang, X. (2014). Olanzapine reduced brown adipose tissue thermogenesis and locomotor activity in female rats. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 51 172-180.



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Word count of abstract: 299

Word count of body of the manuscript: 4143

Number of references: 30

Number of Figures: 7

**Abstract**

Excessive weight gain has been identified as a serious metabolic side-effect of second-generation antipsychotics (SGAs), including olanzapine. While hyperphagia has been suggested to be the main contributor for this side-effect in the short term, reduced energy expenditure, in particular thermogenesis and locomotor activity, has been considered to contribute to the maintenance of heavy weight under long-term SGA treatments. Recent studies have identified metabolically active brown adipose tissues (BAT) in adult humans, suggesting potential clinical significance for the involvement of BAT thermogenesis in SGA-induced weight gain. However, to date there has been little research elucidating the central neuronal pathways affecting BAT thermogenesis or the morphological changes of the BAT. The present study aimed to investigate the role of BAT thermogenesis and locomotor activity in olanzapine-induced weight gain during the prolonged time courses of olanzapine treatment in an established female rat model. Although short- to mid-term olanzapine treatment had no effect on BAT temperature, we observed long-term olanzapine treatment (from day 18-34) induced a significant reduction in BAT temperature, with an acute effect being observed between 45-150 minutes post-treatment in the long-term cohort. Additionally, in the long-term olanzapine group, the reduced BAT temperature was accompanied by decreased UCP1 and PGC-1α expressions in the BAT. Moreover, TH mRNA expressions in both hypothalamus and brainstem were also down-regulated after mid- to long-term olanzapine treatment. Further, olanzapine led to reduced percentage of brown adipocytes in BAT during mid- to long-term treatments. Finally, locomotor activity was reduced throughout the three treatment cohorts. In summary, our results suggest that the reduction of BAT thermogenesis plays an important role during the long-term of olanzapine-induced weight gain, which was accompanied by an earlier onset of BAT adipocyte morphological changes and biochemical changes in the hypothalamus and the brainstem, while locomotor activity contributes to the entire olanzapine treatment courses.

**Keywords: Brown adipose tissue; Thermogenesis; Antipsychotics; Weight gain; PGC-1α**

## 1. Introduction

While second-generation antipsychotics (SGAs) have been widely prescribed as the primary treatment for patients with schizophrenia and other psychiatric diseases, excessive body weight gain associated with the use of SGAs, in particular olanzapine and clozapine, has attracted increasing concerns from patients, clinicians, and medical researchers (Zhang et al. , 2013a). This weight gain side-effect of SGAs, along with other metabolic side-effects, could lead to increased morbidity and mortality, and poor compliance to the antipsychotic drug for patients (Deng, 2013).

Both clinical and animal studies have suggested that along the time course of olanzapine-induced weight gain, there are three typical stages: the initial stage with rapid increase of body weight accompanied with elevated food intake, the middle stage with slow body weight gain and no elevation of food intake, and the late stage with maintenance of the heavy body weight without elevated food intake (Huang et al. , 2006, Pai et al. , 2012). In recent years, researchers have identified multiple contributing factors for the weight gain side-effect induced by SGAs (Kroeze et al. , 2003, Nasrallah, 2008, Reynolds et al. , 2010). For example, the roles of antagonism on the histaminergic H1 and H3 receptors (Deng et al. , 2010), serotoninergic 5HT2c receptor (Panariello et al. , 2011), dopaminergic D2 receptor (Lian et al. , 2013) and α-adrenergic receptor (Nasrallah, 2008), and central ghrelin signalling (Zhang et al. , 2013b) have been indicated. However, the exact mechanism of SGA-induced weight gain was not fully understood. One of the unsolved questions of these studies is with maintenance of the heavy body weight during the long-term of SGA treatments when elevated food intake is absent. Reduced energy expenditure, in particular thermogenesis, has been suggested to play an important role in antipsychotic-induced weight gain (Arjona et al. , 2004, Stefanidis et al. , 2009, van der Zwaal et al. , 2012).

Metabolically active brown adipose tissue (BAT) has been identified in adult humans (Nedergaard et al. , 2007, 2010), with its functional thermogenic protein, uncoupling protein1

(UCP1) found in humans (Cinti, 2006), suggesting the involvement of BAT thermogenesis in SGA-induced weight gain observed in rodents may have clinical relevance in humans. In addition, olanzapine treatment has been reported to reduce locomotor activity in rodents (Deng et al. , 2012, van der Zwaal et al. , 2010), although the time-dependent effect has not been investigated. The aim of this study is to investigate the effect of olanzapine on BAT thermogenesis and locomotor activity during the prolonged time courses of olanzapine treatment, in an established female rat model of olanzapine-induced weight gain. Besides the physiological changes in BAT thermogenesis, biochemistry changes in the central sympathetic network for BAT thermogenic innervations, as well as morphological changes of the interscapular brown adipose tissue (IBAT), were also examined through the time courses of olanzapine-induced weight gain.

## 2. Material and methods

### 2.1. Animals and oral drug treatments

The animal model of olanzapine-induced weight gain has been well established and validated in female rats in our and other laboratories (Deng, 2012, Lian, 2013, Stefanidis, 2009, Weston-Green et al. , 2011). Rats were closely housed in a room occupied by only female rats, which ensures that the ovarian cycles of all female rats are synchronized (Lian, 2013). Briefly, female Sprague-Dawley rats (201-225g) were obtained from the Animal Resource Centre (Perth, WA, Australia). Rats were individually housed at 22°C, 12-h light-dark cycle with lights on at 07:00h. All animals had *ad libitum* access to water and a standard laboratory chow diet (3.9 kcal/g; 10% fat, 74% carbohydrate and 16% protein). After one week of acclimatization, a Bio-Thermo microchip "LifeChip" (Destron Fearing, South St. Paul, MN, USA) was inserted into the interscapular brown adipose tissue with independent packaged disposable sterilized needles. The positions of the microchips were visually confirmed post-euthanasia (Supplementary Fig. S1). After one week of recovery, animals were trained to self-administer the placebo sweet cookie-dough. Rats were randomised into either olanzapine (O) or control (C) treatment groups, with

three treatment duration cohorts: short-term (8 days), mid-term (16 days) and long-term (36 days) (6 groups; n=12/group). However, for the subsequent biochemistry (Western-blot and qRT-PCR) and histology measurements, only 6 rats were randomly selected from each group (n=6/group). All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia, and were complied with the 'Australian Code of Practice for the Care and Use of Animals for Scientific Purposes' (Australian Government National Health and Medical Research Council, 2004).

A cookie-dough (62% carbohydrate, 22% protein, 6% fibre, 10% vitamins and minerals) method was employed as previously reported (Deng, 2012, Han et al. , 2008, Weston-Green, 2011). Briefly, a mixture of cornstarch (30.9%), sucrose (30.9%), gelatine (6.3%), casein (15.5%), fibre (6.4%), minerals (8.4%) and vitamins (1.6%) was produced. Three times per day, a cookie-dough (0.3g; 3.36kcal/g) mixed with either olanzapine (1 mg/kg BW) (Eli Lilly, Indianapolis, IN, USA) or placebo were served to the corresponding animals. Animals were observed during the administration period to ensure complete consumption of the pellets. The dosage of olanzapine (1 mg/kg BW, three times per day) were based on our prior studies (Deng, 2012, Han, 2008, Weston-Green, 2011), which were clinical relevant calculated based on D2 receptor occupancy (Kapur et al. , 2003). Body weight was measured every second day.

**2.2. BAT Temperature Measurements**

The instant BAT temperature data was detected by the microchip and received by a remote pocket reader "Pocket Reader EX" (Digital Angel Corp., South St. Paul, MN, USA). BAT temperature measurements were conducted 2 hours and 6 hours after the 7am and 11pm drug treatments (Supplementary Fig.S2) every second day. To determine whether there is a circadian effect over the effect of olanzapine on BAT temperature, BAT temperature up to 3 hours immediately post treatment was measured after 7am (light phase) and 11pm (dark phase), two days before euthanasia.

**2.3. Open Field Test**

To determine the effect of olanzapine on locomotor activity during the three different treatment courses, open field tests were performed three days before euthanization for each cohort (Day 5, Day 13 and Day 31 for the short-, mid- and long-term cohorts, respectively). The open field test protocol was described previously (Deng, 2012, Weston-Green, 2011). Briefly, each rat was placed in the centre of a black rectangular arena (60x60cm$^2$, 40cm high) with an average light exposure of 25 lux. The behaviour of the rats was recorded by a video camera from the top for 30 minutes. Locomotor activity was analysed using EthoVision Color-Pro software (Noldus Information Technology, Wageningen, The Netherlands). Total distance moved (cm) and average velocity (cm/s) were measured.

**2.4. Euthanasia and tissue collection**

Two hours after the last 7am treatment (on Day 8, Day 16 and Day 36, for short-, mid-, and long-term cohorts, respectively), rats were euthanized by fast $CO_2$ infusion (Deng, 2012, Han, 2008, Weston-Green, 2011). Brains were dissected on an ice plate immediately after euthanasia, snap-frozen in liquid nitrogen and stored in -80$^o$C. IBAT was dissected and cut into two halves: one half was snap-frozen and stored in -80$^o$C, and the other half was fixed overnight by immersion at 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The fixed samples were then dehydrated, cleared, and embedded in paraffin.

**2.5. Western-blot**

The western-blot protocol was adopted from our previous report (Zhang, 2013b). Briefly, the BAT were homogenised in 10vol (v/w) homogenising buffer (containing NP40, Protease Inhibitor Cocktail, 1mM PMSF and 0.5mM β-glycerophosphate). Total protein concentrations were determined by DC-Assay (Bio-Rad, Hercules, USA), detected by SpectraMax Plus384 absorbance microplate reader (Molecular Devices, USA). Samples were heat-treated in Laemmli buffer at 95$^o$C, loaded to 8% SDS-PAGE gels for fractionation, and then transferred onto Immun-BlotTM PVDF membranes (Bio-Rad, Hercules, CA, USA). The block consists of 5% BSA in TBST. The membranes were then incubated with UCP1 or

peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) (Santa Cruz Biotechnologies; dilution factor 1:500) antibody in TBST containing 1% BSA overnight at 4$^{o}$C. Secondary antibodies were anti-rabbit (for PGC-1α) or anti-goat (for UCP1) IgG conjugated with horseradish peroxidase (Santa Cruz Biotechnologies, USA; dilution factor 1:3000). For visualization, ECL detection reagents were used and films were exposed on the AGFA CP1000 Tabletop Processor (COD Medical, USA). Films were then analysed using the Quantity One software, connected to GS-690 Imaging Densitometer (Bio-Rad, Hercules, USA).

**2.6. Quantitative Real-time PCR (qRT-PCR)**

The qRT-PCR protocol was adopted from our previous report (Zhang, 2013b). Briefly, total RNA was extracted from the hypothalamus and brainstem with PureLink RNA extraction kit (Life Technologies, NSW, Australia) according to the manufacturer's protocol. First-strand cDNA was synthesized with VILO cDNA synthesis kit (Life Technologies, NSW, Australia) with 20μL reaction volume. qRT-PCR was carried out in triplicates using TaqMan Gene Expression Assays (Life Technologies, NSW, Australia) on LightCycler480$^{+}$ (Roche, Penzberg, Germany). The results were normalized to β-actin (cat. no. 4252640E; Life Technologies, NSW, Australia), and were expressed as folds different from control. The assay identification of the target gene was: Rat tyrosine hydroxylase (TH) (Rn00562500_m1) (Life Technologies, NSW, Australia).

**2.7. Histology (H & E Staining)**

To access the morphology of the adipocytes, the paraffin-embedded IBAT tissue was section-sliced (4μm/section) and treated with hematoxylin and eosin-staining immediately after mounting (n=6/group). Ten areas per section (per rat) containing mixed brown and white adipocytes were randomly captured with a Syntec STK1160 CCD camera (Syntec Semiconductor Co. Ltd., Taipei, Taiwan) at 10x objective. The percentage area of the

multilocular brown adipocytes over the total area was measured by the software ImageJ 1.44p (Wayne Rasband, National Institutes of Health, USA).

### 2.8. Statistics

The statistical software, SPSS (version 19, SPSS, Chicago, IL, USA) was used to perform all analysis. Repeated measures ANOVA were used to access comparative differences in BAT temperature, with post-hoc Turkey's test for multiple comparisons. Independent sample t-test was used for BAT UCP1 and PGC-1α expressions, BAT TH mRNA expressions, and percentage of the multilocular brown adipocyte area at IBAT, for the three time point of interest (short-, mid- and long-term cohorts). Correlations were identified by Pearson's correlation. Data were expressed as mean ± SEM, and $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Olanzapine treatment induced elevation of food intake in the short-term only, but a sustained body weight gain

In the long-term treatment cohort, rats treated with olanzapine had an increased food intake compared to control from Day 4 through Day 12 of treatment ($P < 0.01$; Figure 1A). From Day 14 onwards, this elevation effect of olanzapine on daily food intake weaned off. Daily food intake for rats in the short- and mid- term cohorts had a similar pattern (i.e. elevated from Day 4 to Day 12; data not shown). However, olanzapine treatment led to an elevation of body weight compared to control since day 4 of treatment, which sustained throughout the rest of treatment periods (Fig. 1B).

### 3.2. Olanzapine treatment decreased BAT temperature in the long-term only

In the long-term cohort, olanzapine treatment had a significant main effect on IBAT temperature measured 2 hours post-treatment ($F_{(1, 22)} = 8.240$, $P = 0.009$), as did time ($F_{(17, 374)} = 17.046$, $P < 0.001$). There was a significant interaction between time and olanzapine

treatment ($F_{(17, 374)} = 2.946$, $P < 0.001$). IBAT temperature measured 2 hours post-treatment was significantly reduced by olanzapine in the long-term cohort from day 18 through day 34 compared to control ($P<0.05$; Fig. 2A). Interestingly, IBAT temperature measured 6 hours post-treatment was not changed by olanzapine treatment compared to control (Fig. 2B). Additionally, in the short- or mid-term cohort, olanzapine had no effect on IBAT temperature (measured either 2 hour or 6 hours post-treatment; data not shown).

Consistently, in the long-term cohort, olanzapine significantly reduced IBAT temperature between 45 minutes and 150 minutes post-treatment, for temperature measured in both light phase and dark phase ($P < 0.05$; Fig. 3E, F). However, this effect was diminished at 180 minutes post-treatment (Fig. 3E, F), which explains our finding that olanzapine had no effect on the IBAT temperature measured 6 hours post-treatment. There was no significant difference in IBAT temperatures at any time point measured up to 180 minutes post-treatment between olanzapine group and control group in the mid-term or short-term cohorts (Fig. 3A-D). IBAT temperature was generally higher during the dark phase compared to the light phase ($P < 0.05$); however, there was no difference in terms of the effect of olanzapine on IBAT temperature between the light and dark phases (Fig. 3A-F).

### 3.3. Time-dependent downregulation of the BAT UCP1 and PGC-1α protein expressions under olanzapine treatment

By uncoupling the mitochondrial respiration and the ATP synthesis process, UCP1 is well-known as a biomarker for BAT thermogenesis. PGC-1α is responsible for mitochondrial biogenesis, BAT thermogenesis, and white-to-brown transdifferentiation of adipocytes (Barbatelli et al. , 2010). We measured the protein expressions of UCP1 and PGC-1α at the IBAT during the three courses of olanzapine treatments by Western-blot. Olanzapine significantly down-regulated IBAT UCP1 protein expressions in the mid- and long-term cohorts by 22% ($P < 0.05$) and 30% ($P < 0.01$), respectively (Fig. 4A, C). Similarly, IBAT PGC-1α protein expression was downregulated by 21% ($P < 0.05$) and 27% ($P < 0.01$) under

mid- and long-term olanzapine treatments, respectively (Fig. 4B, C). Interestingly, olanzapine had no effect on either BAT UCP1 or PGC-1α expressions in the short-term treatment cohort (Fig. 4A-C). Additionally, in the long-term cohort, both UCP1 and PGC-1 α expressions in the BAT were positively correlated with BAT temperature (measured 2 hours post-treatment) ($r = 0.816$, $P < 0.01$; $r = 0.937$, $P < 0.001$, respectively; Fig. 4D, E).

### 3.4. Olanzapine time-dependently downregulates TH mRNA expressions at hypothalamus and brainstem

Expressions of TH mRNA at the hypothalamic paraventricular nucleus and the locus coerulus at the brainstem have been suggested to be responsible for the sympathetic innervation and thermogenic activity of the IBAT (Shi et al. , 2013). We measured the mRNA expressions of TH at hypothalamus and brainstem during the three courses of olanzapine treatments using qRT-PCR. At the hypothalamus, olanzapine treatment produced 29% ($P < 0.05$) and 31% ($P < 0.05$) reductions in TH mRNA expressions in the mid- and long-term cohorts, respectively, but had no effect on the short-term cohort (Fig. 5A). Similar results were observed at the brainstem, where mid- and long-term olanzapine treatment induced 32% ($P < 0.05$) and 35% ($P < 0.05$) reductions in TH mRNA expressions, respectively, while short-term treatment had no effect (Fig. 5B). Finally, in the long-term cohort, TH mRNA expressions at both hypothalamus and brainstem were positively correlated with BAT temperature (measured 2 hours post-treatment) ($r = 0.812$, $P < 0.01$; $r = 0.828$, $P < 0.01$, respectively; Fig. 5C, D).

### 3.5. Olanzapine time-dependently decrease the percentage of multilocular brown adipocytes at IBAT

In the short-term olanzapine treatment group, no significant IBAT morphology changes were observed compared to the control (Fig. 6A). However, in the mid-term olanzapine treated rats, an increase of the amount of paucilocular adipocytes were visually evidenced at the IBAT (Fig. 6A). Similarly, in the long-term olanzapine treatment group, increased amount of unilocular white adipocytes were observed at the IBAT compared to the control (Fig. 6A). A

quantitative analysis of the percentage of the area of multilocular brown adipocytes in 10 randomly captured areas for each rat (n=6/group) revealed that in the IBAT, olanzapine induced significant reductions in the percentage of multilocular brown adipocytes in the mid-term and long-term cohorts (-11%, $P < 0.05$; -20%, $P < 0.01$, respectively; Fig. 6B).

### 3.6. Olanzapine decreased locomotor activity throughout treatment periods

Compared to control, rats treated with olanzapine had reduced locomotor activity measured by both total distance moved (-34.4%, $P < 0.001$; -22.4%%, $P < 0.01$; -15.0%, $P < 0.05$ for short-, mid- and long-term cohorts, respectively) and velocity (-29.3%, $P < 0.01$; -23.6%, $P < 0.001$; -15.0%, $P < 0.05$ for short-, mid- and long-term cohorts, respectively) during all the three treatment periods (Fig. 7A, B). There was no time effect of olanzapine treatment over either total distance moved or velocity ($P > 0.05$). Finally, there was no significant correlation between either total distance moved or velocity and BAT temperature ($P > 0.05$).

## 4. Discussion

The present study provide physiology, molecular biochemistry and morphology evidence that prolonged olanzapine treatment can lead to reduction of thermogenesis from IBAT in female Sprague-Dawley rats, which contributes at least partly to the weight gain side-effect induced by olanzapine, especially in the long-term.

Daily food intake was elevated by olanzapine treatment in the short-term only, while body weight gain sustained throughout the rest of the treatment periods. This discrepancy is consistent with previous reports by our group and others (Huang, 2006, Stefanidis, 2009, Zhang, 2013b), which indicated an effect of reduced energy expenditure driving the continuous body weight gain in the mid- to long-term of olanzapine treatment.

Specifically, our IBAT temperature data shows that long-term olanzapine treatment (1mg/kg BW, three times per day for over 18 days) can reduce IBAT temperature in female rats shortly after olanzapine administration (between 45 minutes and 150 minutes post-treatment).

These findings indicate that during the initial (short-term) olanzapine treatment period, thermogenesis is not the main reason for the rapid weight gain induced by olanzapine, but rather, other factors, such as elevated food intake should be playing a major role (as we have shown in our previous studies); however, during the prolonged period of treatment, when the elevated food intake has dissipated, the reduction of thermogenesis may contribute to the maintenance of body weight gain. Interestingly, it appears that our results are at odd with those reported by Stafanidies and colleagues, which showed that the reduction of BAT temperature started from the first day throughout the 23 days of treatment period (Stefanidis, 2009, Zhang, 2013b). One possible explanation for this discrepancy is the higher olanzapine dosage used in their study (3mg/kg, bid) compared to our present study (1mg/kg, tid). In fact, another study using an even higher olanzapine dosage (5mg/kg, bid) found that olanzapine reduced BAT temperature from day 1 to day 14 during the dark phase but increased BAT temperature during the light phase (Evers et al. , 2010). However, in the study by Evers and colleagues, olanzapine was administered via intraperitoneal injection, which may lead to different physiological reactions compared to the study by Stefanidis and colleagues and our present study. Intriguingly, an earlier study showed that a one-off intraperitoneal olanzapine (10mg/kg) injection produced no acute effect on IBAT temperature compared to saline (Monda et al. , 2008). In addition, we have also shown that the effect of olanzapine on IBAT temperature was present in both light and dark phases 2 hours post-treatment. This seems controversial with the results reported by Stafanidies and colleagues (2009), who showed a reduction effect of olanzapine on BAT temperature during the dark phase but no effect during the light phase; and the study by Evers and colleagues (2010) showed a reduction effect during the dark phase but an elevation effect during the light phase. However, in both of these studies, olanzapine was only administered at the start of dark phase and in the middle of dark phase: the lack of olanzapine treatment during the light phase may contribute to the absence of effect or the inverse effect shown during the light phase of these studies. In fact, in the hands of Evers and colleagues (2010), the reduction effect of olanzapine on BAT temperature

only present up to approximately two hours post-treatment. Similar results were also evidenced by another study measuring the effect of olanzapine on core temperature, which showed that olanzapine (1mg/kg) reduced core temperature in male rats only up to approximately three hours post-treatment (van der Zwaal, 2012).

We demonstrated for the first time that the percentage of multilocular brown adipocytes at IBAT were significantly reduced by mid- to long-term olanzapine treatment, which supports the notion that BAT thermogenic activity is inhibited under long-term olanzapine treatment, contributing to the long-term heavy weight maintenance in olanzapine-induced obese patients. The brown-to white transdifferentiation of adipocytes has been evidenced during sympathetic activation by either β adrenergic agonist or cold stimulation (Chao et al. , 2012, Nagase et al. , 1996). Our results suggest that a reversed morphological change may happen in the IBAT under long-term olanzapine treatment, which contribute to the reduced BAT thermogenesis in this time point. Interestingly, during the mid-term of olanzapine treatment, the histologically intermediate stages of the transdifferentiation, the paucilocular adipocytes, were observed, indicating a gradual transformation of the brown adipocytes into white adipocytes in the IBAT along the time courses of olanzapine treatment, possibly through the chronic inhibition of the β3-adrenoceptors (Barbatelli, 2010). Further studies are required to confirm this hypothesis.

Recent studies have suggested that the sympathetic pathway is critical in the control of BAT thermogenesis (Chao, 2012, Shi, 2013). In particular, the expressions of TH mRNA at the paraventricular nucleus of the hypothalamus and the locus coerulus at the brainstem have been suggested to be responsible for sympathetic innervation of the thermogenic activity of the BAT (Shi, 2013). UCP1 is a well-known biomarker for BAT thermogenesis as it uncouples the mitochondrial respiration and ATP synthesis. Further, PGC-1α at the BAT has also been reported to be responsible for mitochondrial biogenesis and white-to-brown transdifferentiation of adipocytes, both contributing to BAT thermogenesis (Barbatelli, 2010). Our qRT-PCR results showed a time-dependent downregulation effect of olanzapine on the

hypothalamic and brainstem TH mRNA expressions, suggesting a time-dependent inhibition of the SNS activity, which controls the thermogenic activity of the BAT. In fact, the expressions of thermogenic activity biomarkers UCP1 and PGC-1α in IBAT were time-dependently downregulated by olanzapine treatment in the present study, supporting the notion that SNS inhibition is responsible for the time-dependent reduction of BAT thermogenesis under olanzapine treatment.

Interestingly, while the morphological and most of the biochemical effects of olanzapine on BAT and the brain were significant in the mid- and long-term of treatment, the hypothermic effect of olanzapine on IBAT was only evident in the long-term but not in the mid-term. This discrepancy may reflect a possible delayed effect of olanzapine on IBAT thermogenesis compared to the relevant biochemical and morphological changes. Future studies are required to confirm this hypothesis.

Finally, the reduction effect of olanzapine on locomotor activity is consistent with previous reports from both animal studies and clinical studies (which suggested a sedative effect of olanzapine) (Beasley Jr et al. , 1996, Deng, 2012, Weston-Green, 2011). This may contribute to the weight gain induced by olanzapine by reducing energy expenditure (Weston-Green, 2011). The reduction in locomotor activity revealed in this study provides evidence that physical activity may be reduced by olanzapine throughout the whole treatment periods, which contributes to the sustained body weight gain. Future studies using 24-hour activity monitoring may be conducted to provide more accurate measurements on the level of physical activity. In addition, the suppression effect of olanzapine on locomotor activity (as detected by open field test) persisted throughout the three treatment periods and was not correlated with BAT temperature. This suggests that the hypothemogenic effect of olanzapine on BAT is independent of its effect on locomotor activity. Additionally, the suppression effect of olanzapine treatment on locomotor activity was time-independent, suggesting this effect contributes, at least partly, to the whole time course of olanzapine-induced weight gain,

which is different from the effect of olanzapine on food intake or thermogenesis (both time-dependent).

## 5. Conclusions

Taken together, these findings suggest that the reduction of energy expenditure, in particular, BAT thermogenesis, plays an important role during the prolonged period of olanzapine-induced weight gain, when the elevated food intake has weaned off. The reduced BAT temperature was evident in the long-term of olanzapine treatment only, while the biochemistry and morphological changes in the BAT and the central pathways may have happened at an earlier stage (in both mid-term and long-term). Locomotor activity, on the other hand, was reduced throughout the whole period of olanzapine treatment, which also contributes to the heavy weight maintenance. These findings provide a potential mechanism of olanzapine-induced continuous weight gain, throughout the short- mid- and long-term periods of treatment. Further studies on the neurochemistry of the central sympathetic pathways (including catecholaminergic and doparminergic regulations) involved in the inhibition of BAT thermogenic activity induced by SGAs would help provide pharmacological strategies to tackle the long-term metabolic side-effect of SGAs and advance the understanding of the etiology of obesity.

## Authors' contributions

QZ and XFH conceived and designed the experiments. QZ, MH, HW and JL performed the experiments. QZ drafted the manuscript. QZ, CD and XFH made significant contributions to the revisions of the manuscript. All authors read and approved the final manuscript.

## Acknowledgements

This project was supported by the Schizophrenia Research Institute, Australia, utilizing infrastructure funding from NSW Health. This study was funded by the Australian National

Health and Medical Research Council (grant number 1027493). These funding sources had no role in study design; in data analysis and interpretation; in writing of the report; and in the decision to submit the manuscript for publication. We thank the professional editor Ms Linda Cohen who assisted with proofreading of the manuscript.

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## Figure Legends

**Fig. 1.** Olanzapine treatment increased daily food intake and percentage body weight gain. (A) Olanzapine increased daily food intake (kcal/day; incorporating the calorie contents of both lab chow and cookie-dough consumed) from Day 4 to Day 12 of treatments. (B) Olanzapine led to increased body weight since Day 4 of treatment and throughout the rest of the treatment periods. *P<0.05, **P<0.01, ***P<0.001 vs Control. C: control; O: olanzapine.

**Fig. 2.** Chronic effects of olanzapine on BAT temperature. (A) Olanzapine decreased BAT temperature measured 2 hours post-treatment from Day 18 to Day 34 of treatments. (B) Olanzapine had no effect on BAT temperature measured 6 hours post-treatment. n=12/group; *P<0.05, **P<0.01 vs Control. C: control; O: olanzapine; ST: short-term; MT: mid-term; LT: long-term.

**Fig. 3.** Acute effects of olanzapine on BAT temperature. (A-D) Olanzapine had no effect on BAT temperature during the short- and mid-term treatment. (E) Long-term olanzapine decreased BAT temperature in the light phase acutely from 45-180 minutes post-treatment. (F) Long-term olanzapine decreased BAT temperature in the dark phase acutely from 45-180

minutes post-treatment. n=12/group; *$P$<0.05, **$P$<0.01 vs Control. C: control; O: olanzapine; ST: short-term; MT: mid-term; LT: long-term.

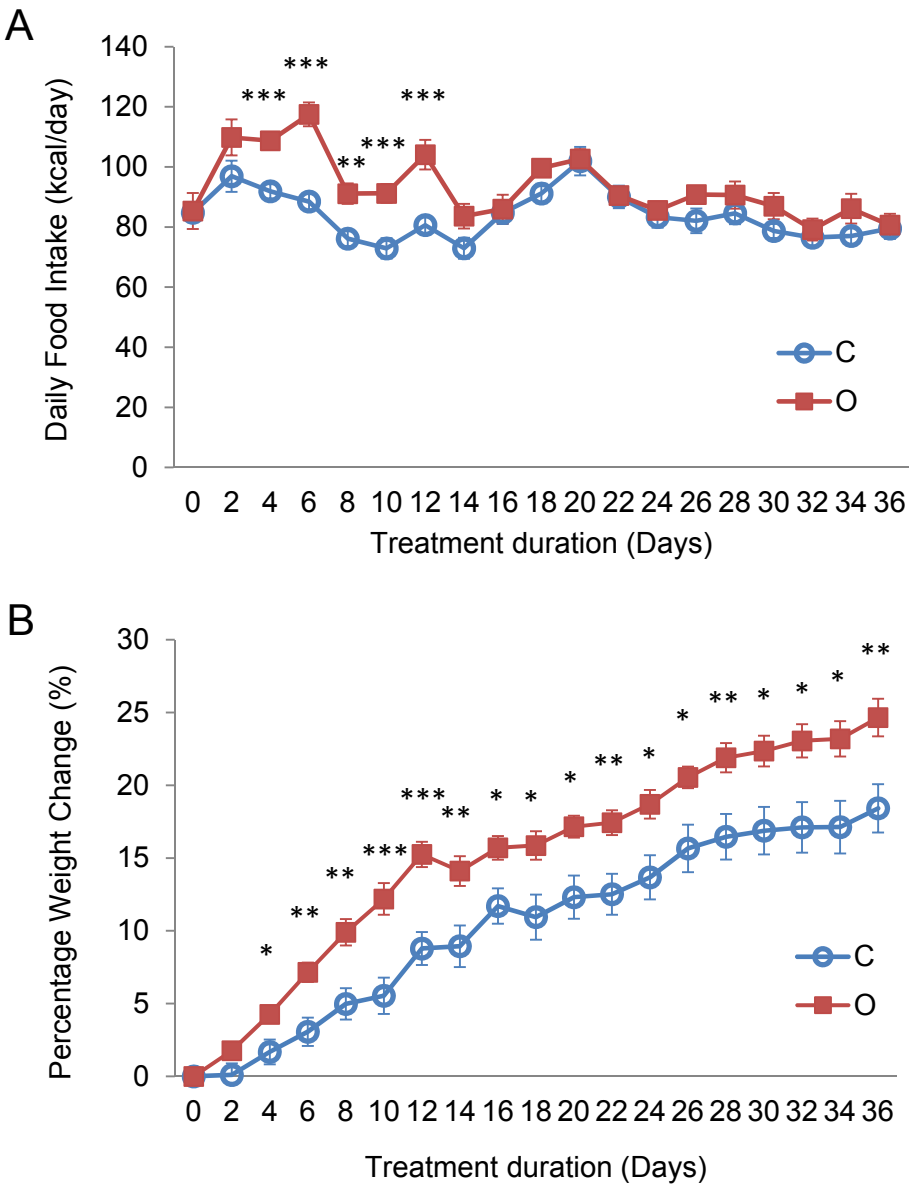
**Fig. 4.** Olanzapine downregulated protein expressions of UCP1 and PGC-1α at BAT in the mid- to long-term. (A) UCP1 expressions at BAT. (B) PGC-1α expressions at BAT. (C) Correlations of BAT temperature and UCP1 expressions at BAT. (D) Correlations of BAT temperature and PGC-1α expressions at BAT. $n$ =6/group. *$P$<0.05 vs control. C: control; O: olanzapine; ST: short-term; MT: mid-term; LT: long-term.

**Fig. 5.** Olanzapine downregulated mRNA expressions of TH at the hypothalamus and brainstem in the mid- to long-term. (A) TH mRNA expressions at hypothalamus. (B) TH mRNA expressions at brainstem. (C, D) Correlations of BAT temperature and TH mRNA expressions at hypothalamus and brainstem. $n$ =6/group. *$P$<0.05 vs control. C: control; O: olanzapine; ST: short-term; MT: mid-term; LT: long-term.

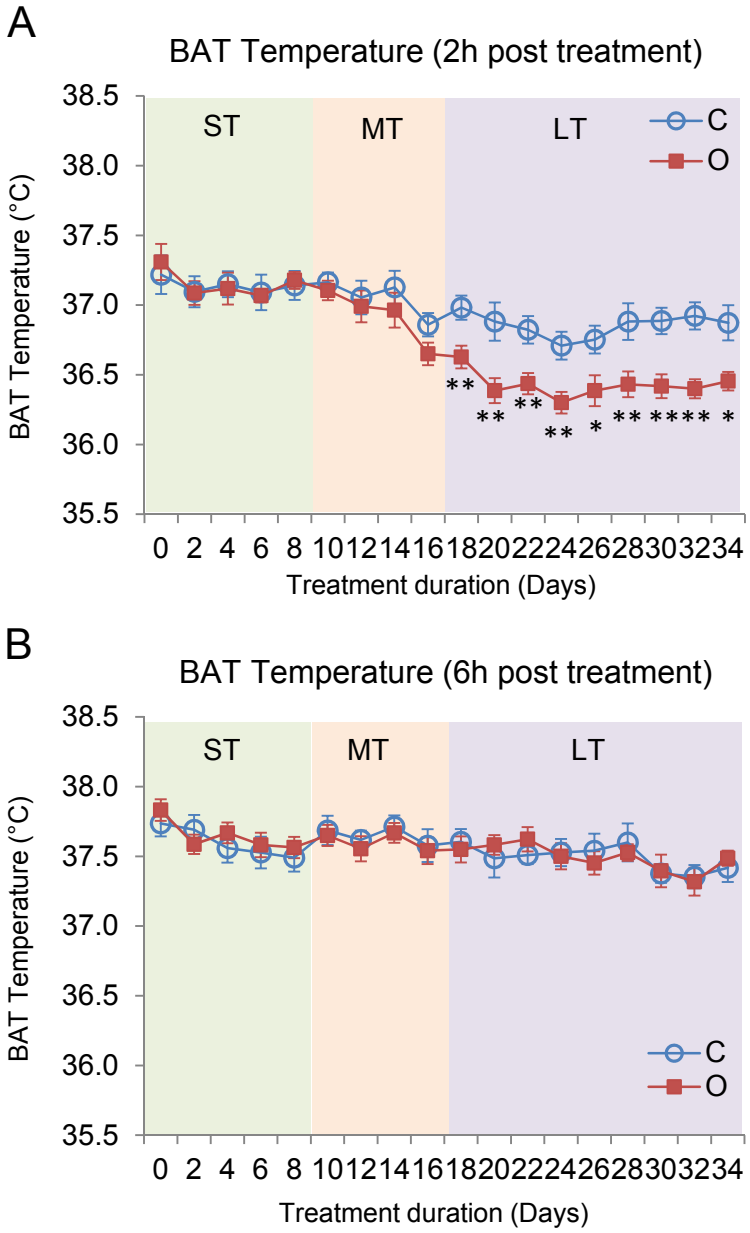
**Fig. 6.** Olanzapine decreased percentage of multilocular brown adipocytes in BAT. (A) Representative picture of interscapular BAT after HE-staining from short-term, mid-term, and long-term control and olanzapine groups, showing unilocular white adipocytes (black arrows), paucilocular adipocytes (red arrows) and multilocular brown adipocytes (white arrows). Scale bar: 20 μm. (B) Percentage of multilocular brown adipocytes in interscapular BAT in rats treated with control or olanzapine for 8 (short-term), 16 (mid-term), or 36 (long-term) days. Mid- and long-term olanzapine treatment significantly decreased the percentage of multilocular brown adipocyptes in interscapular BAT. $n$=6 rats per group. *$P$<0.05, **$P$<0.01vs control. C: control; O: olanzapine; ST: short-term; MT: mid-term; LT: long-term.

**Fig. 7.** Olanzapine decreased locomotor activity in rats. Locomotor activity in the open field test was traced using the Ethovision software. (A) Examples of locomotor activity from rats treated with olanzapine or control. (B) Distance moved and (C) velocity in the open field test. $n$ =11-12/group *p<0.05, **p<0.01, ***p<0.001 vs control. C: control; O: olanzapine; ST: short-term; MT: mid-term; LT: long-term.

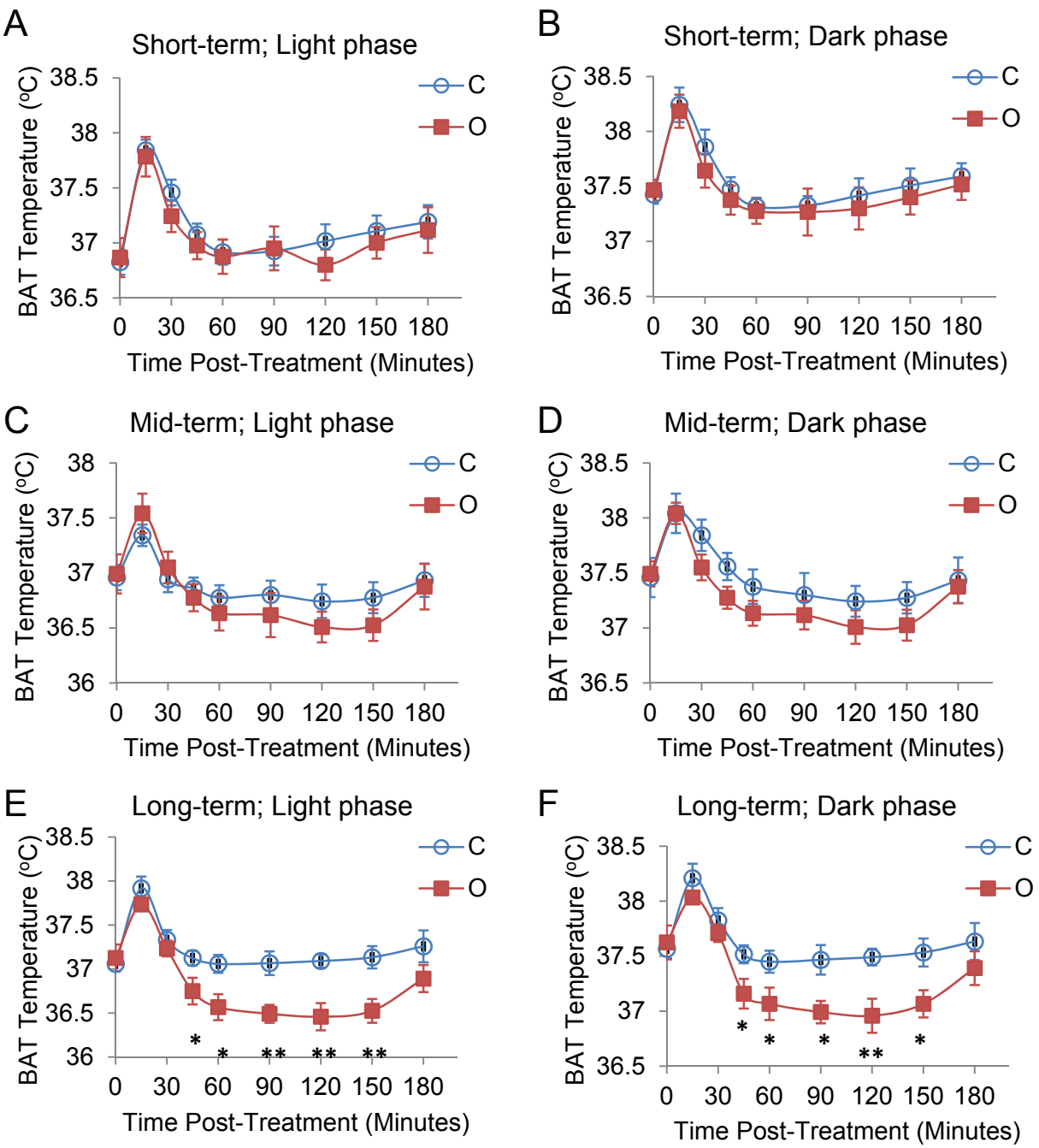
# Figure 1


A
Daily Food Intake (kcal/day)
140
120
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Treatment duration (Days)
C
O
B
Percentage Weight Change (%)
30
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Treatment duration (Days)
C
O

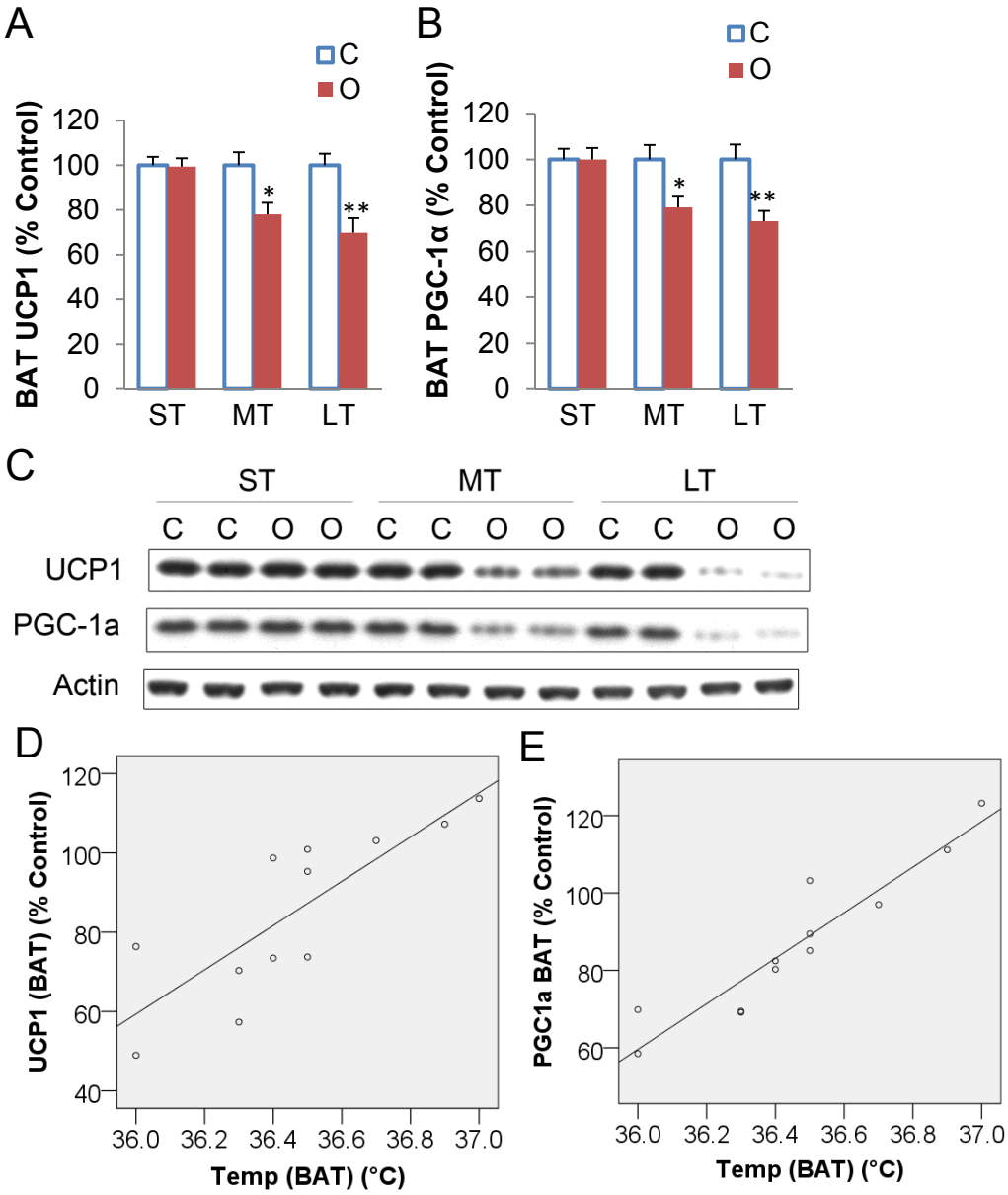
# Figure 2


A
BAT Temperature (2h post treatment)
BAT Temperature (°C)
38.5
38.0
37.5
37.0
36.5
36.0
35.5
ST
MT
LT
C
O
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Treatment duration (Days)
B
BAT Temperature (6h post treatment)
BAT Temperature (°C)
38.5
38.0
37.5
37.0
36.5
36.0
35.5
ST
MT
LT
C
O
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Treatment duration (Days)

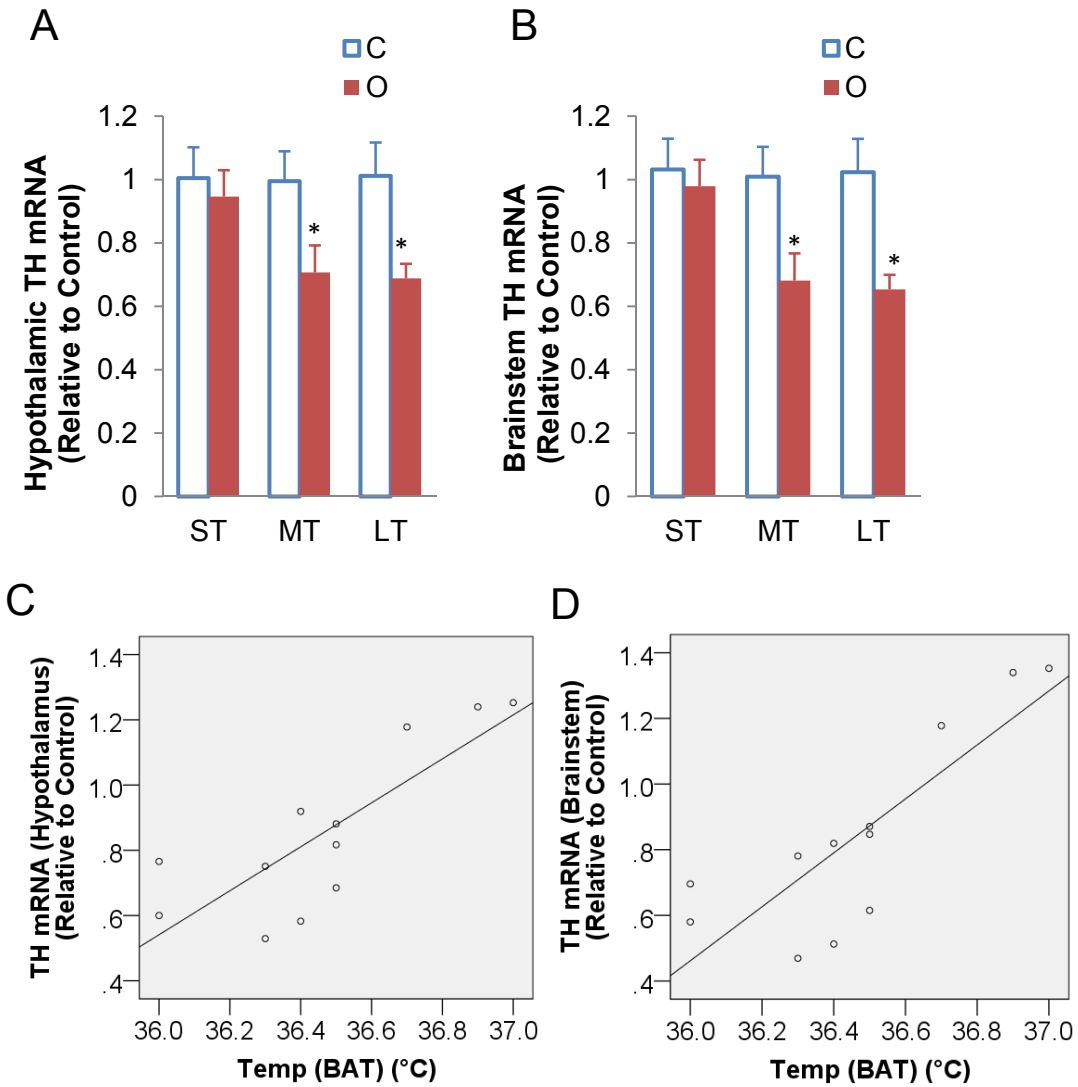
# Figure 3


A
Short-term; Light phase
B
Short-term; Dark phase
C
Mid-term; Light phase
D
Mid-term; Dark phase
E
Long-term; Light phase
F
Long-term; Dark phase
BAT Temperature (°C)
Time Post-Treatment (Minutes)
C
O
0 30 60 90 120 150 180
*
**

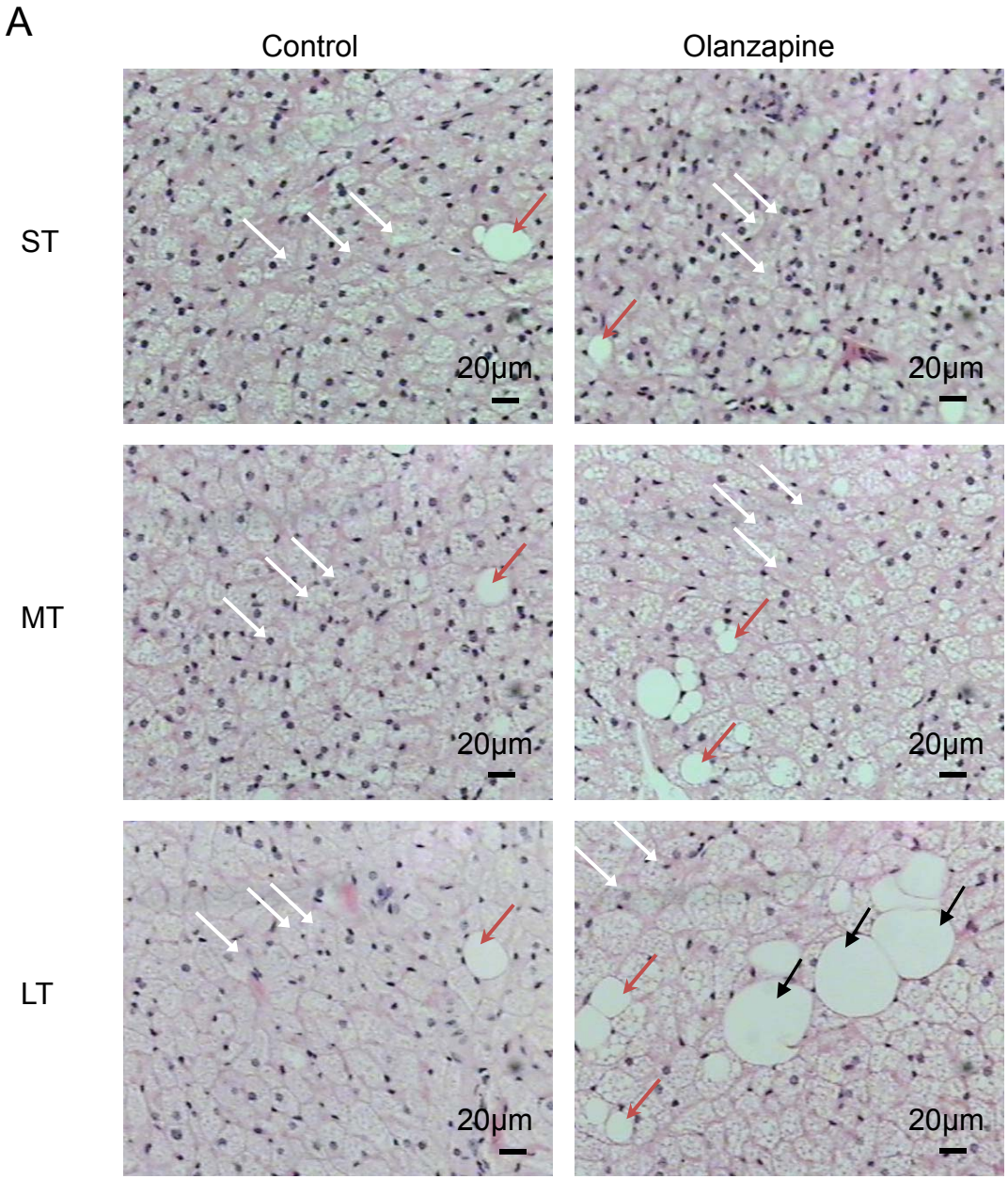
# Figure 4

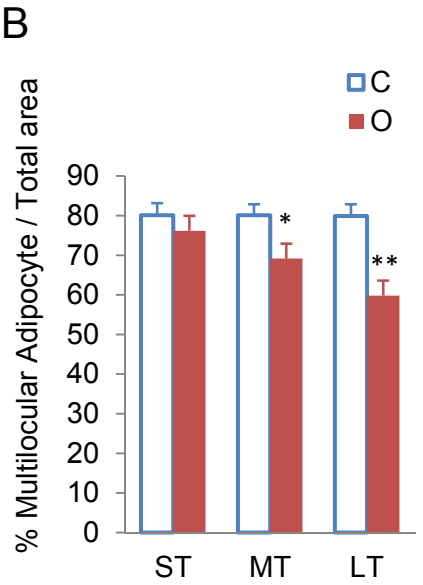

A
C
O
BAT UCP1 (% Control)
120
100
80
60
40
20
0
*
**
ST
MT
LT
B
C
O
BAT PGC-1α (% Control)
120
100
80
60
40
20
0
*
**
ST
MT
LT
C
ST
MT
LT
C C O O C C O O C C O O
UCP1
PGC-1a
Actin
D
UCP1 (BAT) (% Control)
120
100
80
60
40
36.0 36.2 36.4 36.6 36.8 37.0
Temp (BAT) (°C)
E
PGC1a BAT (% Control)
120
100
80
60
36.0 36.2 36.4 36.6 36.8 37.0
Temp (BAT) (°C)

# Figure 5

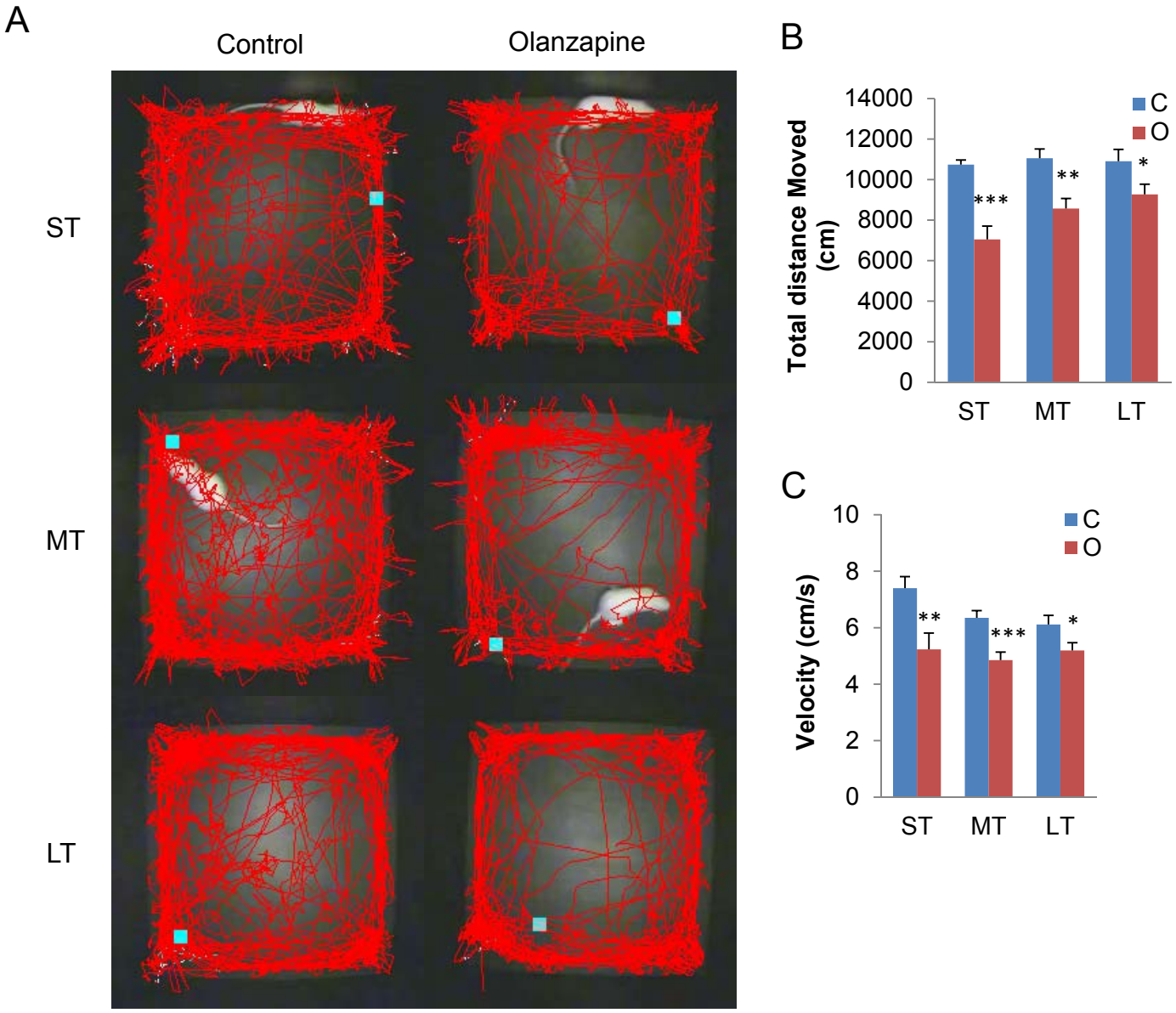

A
C
O
Hypothalamic TH mRNA (Relative to Control)
1.2
1
0.8
0.6
0.4
0.2
0
*
*
ST
MT
LT
B
C
O
Brainstem TH mRNA (Relative to Control)
1.2
1
0.8
0.6
0.4
0.2
0
*
*
ST
MT
LT
C
TH mRNA (Hypothalamus) (Relative to Control)
1.4
1.2
1.0
.8
.6
.4
36.0
36.2
36.4
36.6
36.8
37.0
Temp (BAT) (°C)
D
TH mRNA (Brainstem) (Relative to Control)
1.4
1.2
1.0
.8
.6
.4
36.0
36.2
36.4
36.6
36.8
37.0
Temp (BAT) (°C)

# Figure 6

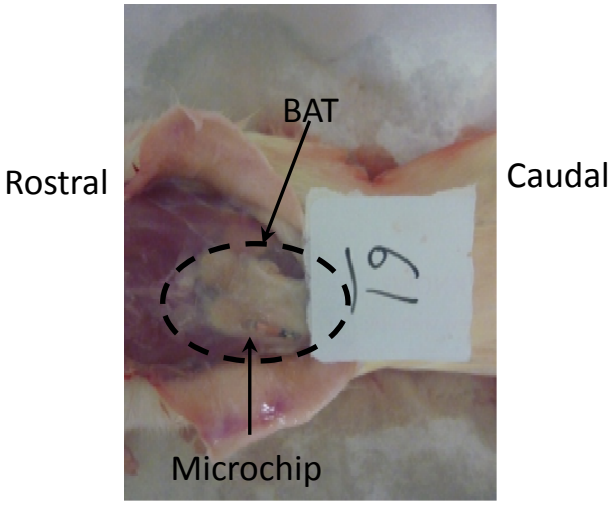

A
Control
Olanzapine
ST
MT
LT
20μm
20μm
20μm
20μm
20μm
20μm


B
C
O
% Multilocular Adipocyte / Total area
90
80
70
60
50
40
30
20
10
0
*
**
ST
MT
LT

# Figure 7

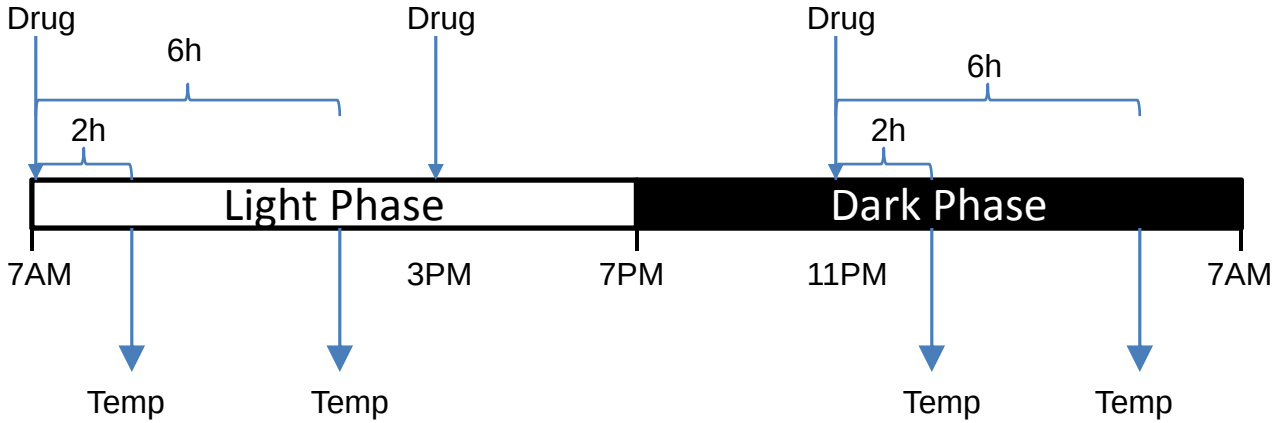

A
Control
Olanzapine
ST
MT
LT
B
Total distance Moved (cm)
14000
12000
10000
8000
6000
4000
2000
0
C
O
***
**
*
ST
MT
LT
C
Velocity (cm/s)
10
8
6
4
2
0
C
O
**
***
*
ST
MT
LT

# Supplementary Figure S1




Example of the temperature detection microchip being placed right into the interscapular brown adipose tissue (BAT). This was double-checked for each rat after euthanasia.

# Supplementary Figure S2




Time of drug administration and brown adipose tissue temperature measurements during the day.