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## The a-typical effects of olanzapine on body weight regulation

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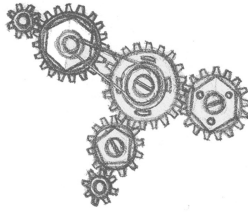
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## Chapter 4:

# Physiology and Behavior

**Olanzapine causes hypothermia, inactivity, a deranged feeding pattern and weight gain in female Wistar rats.**

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## **Abstract**

Olanzapine is an atypical antipsychotic drug antagonizing predominantly 5-HT and dopamine, but also histamine, muscarin, and  $\alpha$ -adrenergic receptors. In humans, Olanzapine induces weight gain and increases the risk of type 2 diabetes. The underlying mechanisms of Olanzapine-induced weight gain are unclear. To study this we administered Olanzapine (5 mg/kg) in female Wistar rats on a medium fat diet for 14 days via a permanent gastric catheter twice a day, just prior to the onset and at the middle of dark phase. Food and water intake, locomotor activity and body temperature were measured. Olanzapine acutely induced hypothermia, markedly decreased locomotor activity and increased body weight during 14 days of treatment. Olanzapine treatment did not result in an alteration of 24 hour food intake, but diurnal patterns of feeding behavior and body temperature were dramatically changed. We conclude that in female Wistar rats Olanzapine has an acute hypothermic effect; that the effect of Olanzapine on feeding behavior is secondary to the effect on activity; and that Olanzapine-induced weight gain is primarily the result of reduction in locomotor activity.

## **Keywords:**

Olanzapine, hypothermia, locomotor activity, body weight, feeding behavior, meal size, rat

## Introduction

Over the recent years, considerable attention has been paid to the metabolic side effects of the a-typical antipsychotic drug (APD) Olanzapine. Olanzapine is primarily designed to be antagonistic on 5-HT<sub>2A/C</sub> and dopamine receptors [1,2] for treatment of schizophrenia and bipolar disorder. However, besides its effect on psychotic illnesses, Olanzapine in humans causes body weight gain and increases the risk of developing type 2 Diabetes [3-5].

The underlying mechanisms are still unclear, as several contradictions with respect to Olanzapine's actions on metabolic pathways have been reported in humans or animals [6-9]. Olanzapine does not only act on serotonin or dopamine receptors, but has antagonistic properties on histaminergic, muscarinic, and  $\alpha$ -adrenergic receptors as well [1,2,10]. Receptors targeted by Olanzapine are located in CNS neuronal networks involved in energy homeostasis [11-15], but can also be found throughout the digestive tract [16], in skeletal muscle [17] and adipose tissue [18]. The wide-spread distribution of putative action site for Olanzapine explains the multitude of mechanisms and various time-dose relationships that have been reported with Olanzapine treatment in humans.

Experimental studies in animals on the mechanisms underlying Olanzapine's metabolic effects also provide confusing data. Female rats appear to increase body weight more profoundly than male rats [19,20] and this increase in body weight, predominantly adipose tissue, may be related to a decreased level of physical activity (the sedative effect of Olanzapine), or by an increase in food intake, although again contradictory results have been found [21-23]. Furthermore, Olanzapine has also been shown to induce hypothermia [24] and to block orexin-A induced hyperthermia [25], but these effects have not been investigated in the face of Olanzapine's effect on body weight gain.

One of the gaps between the pre-clinical rodent studies and human clinical studies is the huge discrepancy between dosing. The main cause is that Olanzapine's half life time in rats is about 2.5 hrs compared to 21-54 hrs in human [26]. Therapeutic doses in human are aimed to reach 65-80% D<sub>2</sub>-receptor occupancy [26-28], to reach such levels in male Sprague-Dawley rats (250-275 grams) Kapur *et al* show that Olanzapine needs to be administered continuously using an osmotic minipump at 7.5 mg/kg/day [26]. However, Van der Zwaal *et al* (2008) showed that Olanzapine is vulnerable to degradation at body temperature therefore long term infusion of Olanzapine using minipumps is not recommended [29]. Rat studies show the most pronounced effects on food intake and body weight gain when

Olanzapine is administered at 1-2 mg/kg twice a day [19,23]. Whereas studies focusing on central receptor activity in specific brain areas [2,30-34] or Olanzapine's effect on thermogenesis [24,25,35] use often a single administration up to 10 mg/kg.

In this study, we re-evaluated a number of potential mechanisms, i.e. food intake patterning, locomotor activity, body temperature, by which Olanzapine may induce weight gain in female rats subjected to a high-fat diet. To amplify Olanzapine's effect on locomotor activity and thermogenesis we decided to administer a dose of 5 mg/kg b.i.d. of Olanzapine. The main outcome of this study paradigm is that weight gain by Olanzapine treatment is predominantly caused by reduced locomotor activity combined with dramatic derangements of several circadian behavioral and physiological patterns.

## **Materials and Methods**

### **2.1 Animals**

All procedures involving animal care and experimental procedures have been approved by the Animal Experimentation Committee of the University of Groningen. Female Wistar rats ( $232 \pm 3.5$  g; on arrival), obtained from Harlan (Horst, NL) were individually housed in clear Plexiglass cages (25\*25\*30 cm) on a plastic floor with wood chip bedding. Female rats were chosen based on the results of Cooper et al [19,20]. Room temperature was controlled at  $22 \pm 2$  °C, under a 12:12 hr light-dark cycle (lights off at 11:00AM). Animals had one week to adapt to the new environment before undergoing surgery, the baseline measurements started one week after surgery when all animals had surpassed their pre-surgical body weight. Prior to drug treatment, animals had ad libitum access to standard chow (3.8 kcal/g) and water. From day 0 (the start of drug treatment) a medium fat diet with lard (4.7 kcal/g; 45% fat, Arie Blok Diets, Woerden, NL) was given to all animals to stimulate weight gain.

### **2.2 Drugs**

Olanzapine (as powder) was kindly provided by Solvay Pharmaceuticals (Fournier Laboratory, France). To obtain a daily administration of 10 mg/kg Olanzapine was diluted to 1.5 mg/ml in 0.9% NaCl saline. Olanzapine was first dissolved in saline using 1M HCl and adjusted to pH 6.5 using 1M NaOH. Animals were administered Olanzapine or saline twice a day, prior to the dark phase and 6 hr after lights went off.

### **2.3 Surgical Procedure**

Animals were equipped with a permanent gastric catheter for stress-free intragastric

drug administration and a telemetry transmitter (model TA10TA-F40, Data Sciences, St. Paul, MN) in the abdominal cavity for continuous temperature and activity registration [36]. Surgical procedures were performed by using isoflurane-O<sub>2</sub>/N<sub>2</sub>O gas-anesthesia. A silicon catheter (1.40-mm OD, 0.80-mm ID) was inserted through the gastric wall at the level of the corpus, extending 0.5 cm into the gastric lumen. The catheter was drawn subcutaneous towards the head where it was fixed to the skull with dental cement [37]. Animals were given 0.1 ml Finadine s.c. for analgesia and 0.25 ml penicillin s.c. to prevent infection.

## **2.4 Circadian Registration**

Core body temperature and activity were recorded throughout the duration of the experiment using radiotelemetry. Telemetry signals were received by separate responder plates (model RA1010, Data Sciences) underneath each cage. Data was collected every 5 minutes by a PC and analyzed by using specialized recording and analyses software (Dataquest IV, Data Sciences)[36].

## **2.5 Experimental set-up**

Data was collected starting at 7 days before the start of drug administration (day 0). Olanzapine was administered intragastric twice a day at CT 11½ (CT 12 = lights off at 11 AM) and at CT 18. From day 0 all animals were switched from the standard lab chow diet to the medium fat diet. Body weight, food intake and water intake was measured every day at CT11½ for 14 days. At day 17 the animals were individually housed in a specialized cage for continuous registration of food intake and locomotor activity for 3 days (TSE Systems GmbH, Bad Homburg, Germany) to monitor circadian feeding patterns, meal sizes and meal numbers. Circadian food intake patterns were calculated as an average of the last two consecutive days, the first day was used for adaptation. These plexiglass cages (40\*23\*15cm) consist of a sensitive weight balanced food station (stainless steel food container for standard size food pellets); water bottles were weight once a day prior to dark phase. Olanzapine administration was continued all the time twice a day at the above mentioned time points. At the end of the experiment rats had ad lib access to two bottles with either a 10% (g/vol) sucrose solution or a water bottle for 24 hours in a two bottle preference test. The bottles were presented at CT 12 and intake was measured at time point 6 and 24 hrs after presentation.

## **2.6 Data Analyses**

Activity data was analyzed as a percentual change of activity relative to baseline per individual. Baseline was calculated as the average activity per day during one week prior to day 0 per individual, this average was set as 100 percent. All activity data

were then expressed as percentage of this baseline value.

All data are expressed as averages  $\pm$  SEM. Statistical analyses were performed using repeated measures (rm)ANOVA between-subjects for time dependent analyses. A multivariate (m)ANOVA between subjects test was used to calculate significance at different time points during time related treatment. Activity and body temperature data were analyzed from baseline average to day 14 of treatment. Because average daily activity and body temperature data consist of a too large amount of data points, rmANOVA was performed for four consecutive 6 hour time frames. All data expressed without a time dependent factor are analyzed using one-way ANOVA test. All statistical analyses were performed in SPSS16, outcomes were regarded significantly different when  $P < 0.05$ .

## Results

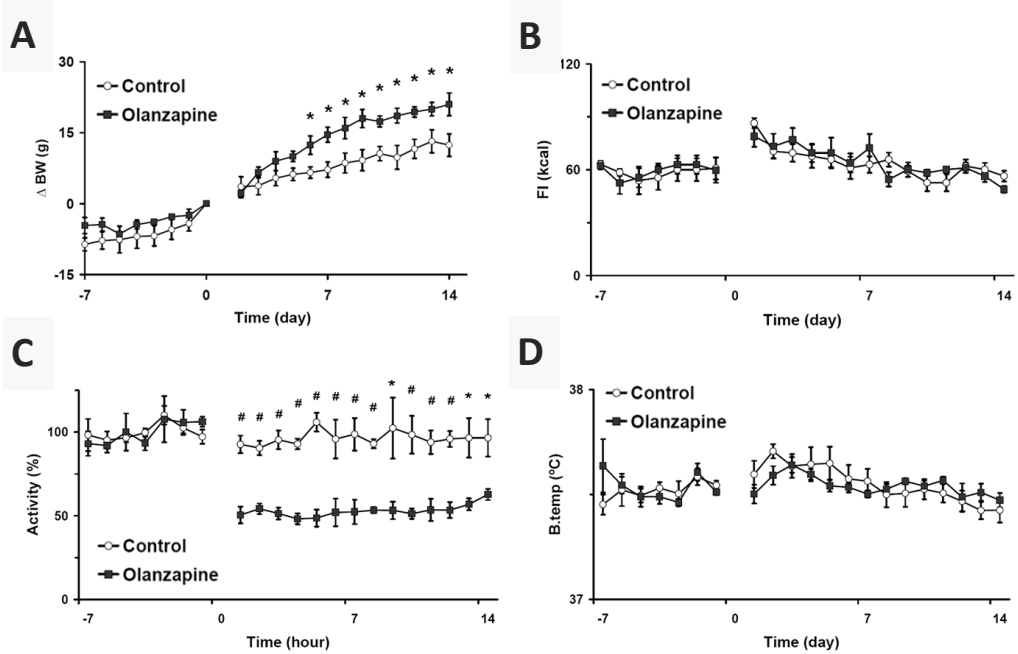
### 3.1 Chronic treatment

Figures 1 present 24 hr food intake, activity, body temperature and the changes in body weight in the Olanzapine treated animals and controls before and during treatment. Olanzapine treatment (5 mg/kg b.i.d.) stimulated body weight gain (fig. 1a) which was significant after 6 days (mANOVA:  $P < 0.05$ ) and persisted during the rest of treatment (rmANOVA:  $F_{14,112} = 3.893$ ,  $P < 0.01$ ). The increase of body weight was not accompanied by changes in 24 hr food intake (fig. 1b) or average 24 hr body temperature (fig. 1d). Olanzapine decreased the activity of the animals during 14 days of treatment (fig. 1c), this was significant when compared with both baseline activity and the values in the control rats (rmANOVA:  $F_{15,90} = 2.914$ ,  $P < 0.01$ ; mANOVA:  $P < 0.05$  and  $P < 0.01$ ).

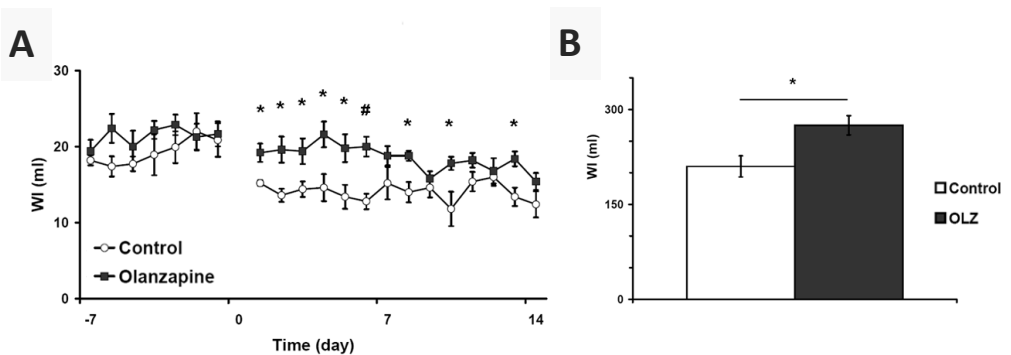
Figure 2 presents both the 24 water intake and the cumulative intake over day 0-14 during Olanzapine or saline treatment. The switch from the standard lab chow to the medium fat diet led to a reduction in water intake in the controls but not in Olanzapine treated animals (fig. 2). Both the water intake per day ( $F_{1,8} = 8.304$ ,  $*P < 0.05$ ; one-way ANOVA) and 14 day cumulative water intake (rmANOVA:  $F_{15,120} = 2.703$ ,  $P < 0.01$ ) were significantly increased in the Olanzapine-treated animals in comparison to the control animals.

### 3.2 Telemetry Data

The average activity and body temperature over both the 12 hr dark and 12 hr light phase were calculate to investigate the diurnal rhythms in activity, food intake and body temperature. Dark and light phase activity are expressed as percentage of



**Figure 1:** a) Effects of intragastric administration of Olanzapine (n=5) or saline (n=5) on body weight gain in grams, expressed as changes from day 0, the start of drug administration. b) The effect of Olanzapine (n=5) and saline (n=5) on 24 hour food intake, expressed as kcal. Day -7 to -1 show standard chow intake, day 1 to 14 show medium fat diet intake. c) The effect of Olanzapine (n=4) and saline (n=4) on 24 hour activity expressed as a percentage of baseline activity. d) The effect of Olanzapine (n=5) and saline (n=5) on average 24 hour body temperature. In all graphs, Olanzapine is presented as filled squares and saline controls as open circles. Significance is presented as \*  $P < 0.05$ , and #  $P < 0.01$ .

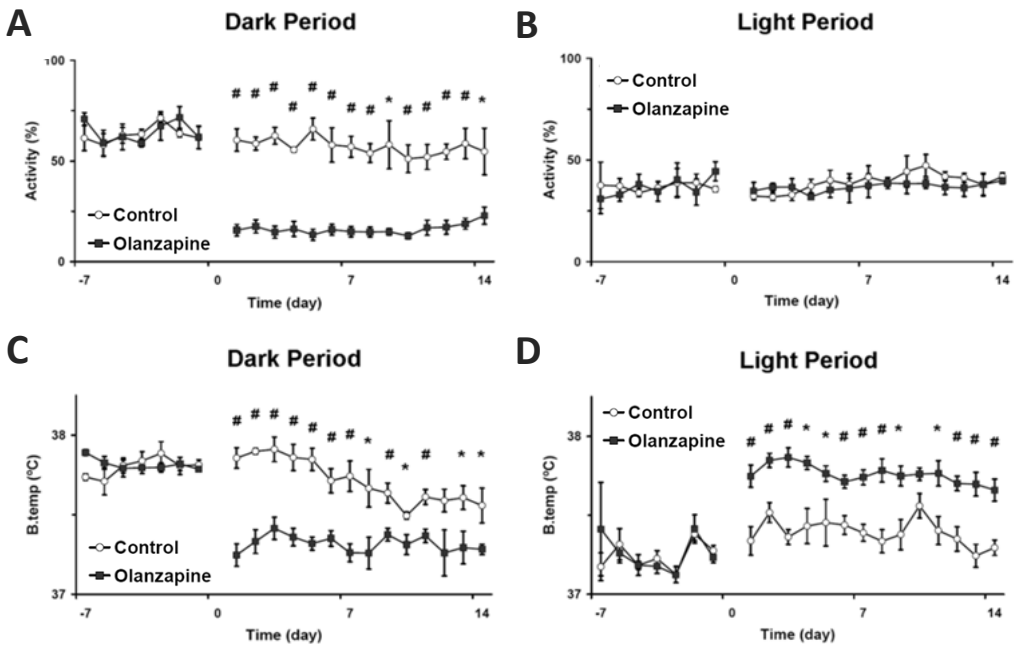


**Figure 2:** a) The effect of Olanzapine (n=5) and saline (n=5) treatment on 24 hour water intake (WI), expressed as ml. b) Cumulative water intake after 14 days of Olanzapine or saline treatment. Olanzapine is presented as filled squares, control as open circles. Significance is presented as \*  $P < 0.05$ , and #  $P < 0.01$ .



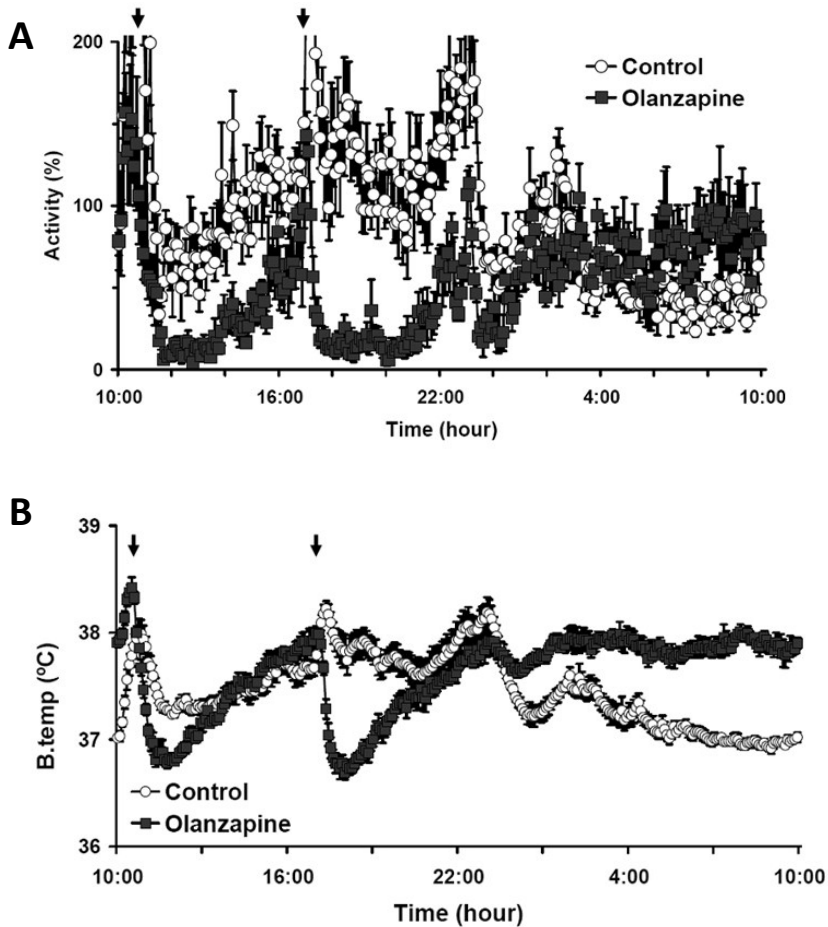
average 24 hr activity in the baseline measurements in the week before day 0. Figure 3a-b shows that Olanzapine significantly reduced the activity in the dark period (fig. 3a, rmANOVA:  $F_{15,90}=5.558$ ,  $P<0.01$ ), while light phase activity was unaffected (fig. 3b). Figure 3c-d shows that Olanzapine treatment led to a significant reduction in body temperature in the dark phase (fig. 3c, rmANOVA:  $F_{15,120}=8.630$ ,  $P<0.01$ ), and a significant increase in the light phase (fig. 3d, rmANOVA:  $F_{15,120}=4.231$ ,  $P<0.01$ ).

Figure 4 provides more detailed information (every 5 minutes) on body temperature and activity. Drug administration (indicated by arrows) caused an acute significant drop of both body temperature (rmANOVA CT 12-18:  $F_{71,568}=12.144$ ,  $P<0.01$ ; and CT 18-0:  $F_{71,426}=2.13$ ,  $P<0.01$ ) and locomotor activity (CT 12-18; rmANOVA:  $F_{71,426}=1.778$ ,  $P<0.01$ ; and CT 18-0:  $F_{71,426}=2.13$ ,  $P<0.01$ ), which persisted throughout the dark period. In the light period Olanzapine treatment caused an increase in body temperature (figure 4b, rmANOVA CT 0-6:  $F_{71,568}=9.184$ ,  $P<0.01$ ,



**Figure 3 a and b:** The effect of Olanzapine and saline on locomotor activity in the dark (3a) and light (3b) period. Activity is expressed as a percentage of total 24 hour activity during the baseline period. **Figure 3c and d:** The effect of Olanzapine and saline on body temperature in the dark (3c) and light (3d) period. In all graphs, Olanzapine is presented as filled squares, controls as open circles. Significance is presented as \*  $P<0.05$ , and #  $P<0.01$ .

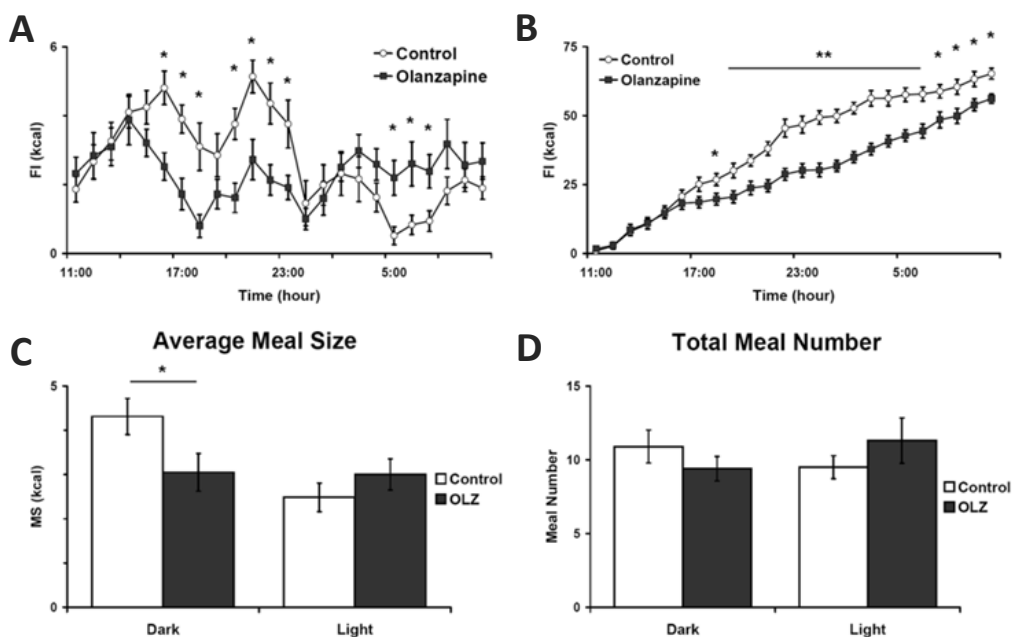
and CT 6-12:  $F_{71,568}=11.664$ ,  $P<0.01$ ) with no significant changes in activity (fig. 4a). Figure 4 also shows a diurnal rhythm in the control group, by which activity and body temperature are higher during the dark phase (11:00hr-23:00hr) compared to the light phase. This daily rhythm is disturbed by the administration of Olanzapine.



**Figure 4:** a) The effect of Olanzapine and saline administration on locomotor activity expressed as average of 14 days of treatment. Activity is expressed as the percentage of average baseline activity per 5 minutes per 24 hour. b) The effect of Olanzapine and saline administration on body temperature (degrees Celsius) expressed as the average body temperature over 14 days of treatment. Arrows indicate the time of drug administration. Lights turned off at 11:00 hour. Olanzapine is presented as filled squares, controls as open circles.

### 3.3 Food Registration Data

At day 17 and 18, feeding behavior was continuously monitored during two consecutive days. Figure 5a gives the average intake over these two days is per hour. There were marked differences in feeding patterns between both groups (rmANOVA:  $F_{23,414}=3.864$ ,  $P<0.01$ ). Fig. 5b shows the cumulative food intake over 24 hours and reveals that there is a decrease of food intake during the second half of dark phase, while food intake during the light period is increased when compared to control values. The 24 hour cumulative data (fig. 5b) shows that Olanzapine reduced food intake during the dark (Controls:  $45.45 \pm 3.22$  kcal; Olanzapine:  $28.72 \pm 2.14$  kcal; One-way ANOVA:  $F_{1,18}=18.739$ ,  $P<0.01$ ). During the light period Olanzapine increased food intake relative to control treatment (Controls:  $19.74 \pm 2.80$  kcal; Olanzapine:  $27.45 \pm 1.69$  kcal. ANOVA:  $F_{1,18}=5.546$ ,  $P<0.05$ ). During the dark period meal size (fig. 5c) was decreased by Olanzapine (One-way ANOVA:  $F_{1,18}=4.606$ ,  $P<0.05$ ), without significant effects on meal number (fig.5 d).



**Figure 5:** a) and b) The effect of Olanzapine and saline treatment on circadian food intake (kcal) per hour (5a) or 24 hour cumulative food intake (kcal). Lights turned off at 11:00 hour and turn on at 23:00 hour. Significance is presented as \* $P<0.05$ . Figure 5 c) and d) The effect of Olanzapine and saline on average meal size (5a) and meal number (5d) in the dark and light period, expressed as an average of two consecutive days. Significance is presented as \* $P<0.05$ . All Olanzapine data is presented as filled squares/bars, controls as open circles/bars.

### 3.4 First day of treatment

The data of day 0, the day that all animals switched to the new diet and received their first injection of saline or Olanzapine, are presented in Table 1. The switch from lab chow to medium fat diet led to a significant increase in food intake in the control group (chow =  $58.35 \pm 3.55$  kcal, medium fat diet =  $100.58 \pm 3.82$  kcal; One-way ANOVA:  $F_{1,8}=64.150$ ,  $P<0.01$ ), but not in the Olanzapine group (chow =  $59.39 \pm 4.57$  kcal, medium fat diet =  $75.20 \pm 5.56$  kcal; One-way ANOVA:  $F_{1,8}=4.826$ ,  $P=0.06$ ). Water intake was lower compared to baseline levels, with no difference between groups. Locomotor activity and body temperature were decreased by Olanzapine when compared to control. This amount of activity within the Olanzapine group during the light phase was significantly lower ( $F_{1,6}=10.569$ ,  $P<0.05$ ; One-way ANOVA) the first day of treatment compared to the average light phase activity of 14 day Olanzapine treatment. 24 Hour body temperature is significantly decreased ( $F_{1,8}=83.175$ ,  $P<0.05$ ; One-way ANOVA) the first day of Olanzapine treatment compared to the average of 14 day treatment.

**Table 1**

Responses at first day of treatment. The effect of Olanzapine or Control treatment on 24 h food and water intake, average 24 h activity, and average 24 h body temperature at the first day of treatment (day 0).

	Control	Olanzapine	$F_{1,8(*1.6)}$	P-value
Food intake (kcal)	$100.58 \pm 3.82$	$75.20 \pm 5.56$	14.155	<0.01
Water intake (ml)	$13.20 \pm 0.92$	$15.40 \pm 1.29$	1.936	
Activity (%)	$105.11 \pm 10.22$	$42.26 \pm 4.72$	*31.191	<0.01
Body temperature (°C)	$37.59 \pm 0.03$	$37.23 \pm 0.03$	115.852	<0.001

### 3.5 Sucrose Preference

At the end of experiment a two bottle preference test for 10% (g/vol) sucrose solution was performed. Table 2 shows that sucrose intake was significantly higher in the controls (6hr =  $17.52 \pm 2.51$  ml; 24hr =  $53.52 \pm 9.18$  ml) when compared to Olanzapine (6hr =  $4.24 \pm 1.61$  ml; 24hr =  $21.76 \pm 4.22$  ml) treated animals, both after 6 ( $F_{1,8}= 19.851$ ,  $P<0.01$ , One-way ANOVA) and 24 hrs ( $F_{1,8}= 9.879$ ,  $P<0.05$ , One-way ANOVA).

**Table 2**

Sucrose preference. The effect of Olanzapine and Control treatment on 6 and 24 h sucrose intake during a two-bottle preference test.

		Control	Olanzapine	$F_{1,8}$	P-value
Sucrose intake (ml)	6 h	$17.52 \pm 2.51$	$4.24 \pm 1.61$	19.851	<0.01
	24 h	$53.52 \pm 9.18$	$21.76 \pm 4.22$	9.879	<0.05

## Conclusions and Discussion

In this study in female Wistar rats we found that chronic Olanzapine treatment led to a significant weight gain without changes in 24 hr food intake. Olanzapine dramatically reduced locomotor activity, caused an acute hypothermia and completely deranged the circadian patterns in food intake.

Since there were no changes in 24 hr food intake, one may argue that the Olanzapine-induced weight gain might be secondary to the marked reduction in locomotor activity, previously described as the sedative effect of Olanzapine [38]. Figure 4 shows in detail the acute reduction of locomotor activity after Olanzapine administration, which persisted throughout the duration of the experiment. Comparable results of reduction in activity by Olanzapine have been reported before in both rats [35,39] and humans [40,40-42].

Olanzapine did not affect 24 hr food intake in this study, but we observed marked differences between dark and light period feeding patterns by Olanzapine. Food intake was markedly reduced in the dark phase and was increased in the light phase. It is tenable to assume that the reduction in dark phase food intake is mainly caused by the sedative effect of Olanzapine. The increased food intake in the normally inactive light phase could then be considered as a compensatory homeostatic response to compensate controlling total 24 hr food intake. These marked derangements in day/night food intake support previous observations by Lee and Clifton (2002) who found that Olanzapine failed to affect 22 hour food intake but also noticed that the size of the first meal as well as the latency to eat was reduced after Olanzapine administration [21]. In the present study we found that Olanzapine during the dark phase reduced meal size, with a small but not significant reduction in meal frequency.

The effects of Olanzapine on body temperature were remarkably similar to the effects on feeding: there was no effect on average 24 hr body temperature but there were marked derangements of the dark and light phase patterns. As shown in fig.4 in detail, Olanzapine induced a dramatic hypothermic response immediately after administration. The dynamics of this response make it unlikely that this reduction in body temperature was secondary to the sedative and hypophagic effects of Olanzapine (i.e., leading to reduced activity- and diet-induced thermogenesis). Instead, data from literature suggest that the hypothermia may be explained by a direct inhibitory effect of Olanzapine on sympathetic outflow and brown adipose tissue (BAT) thermogenesis [24]. Indeed, Olanzapine decreases uncoupling protein 1 (UCP1) expression in BAT and increases Fos expression in orexin-A positive neurons in

perifornical region (PeF) of the lateral hypothalamic area (LHA) projecting to the BAT [35]. Likewise, Olanzapine inhibits the increase in sympathetic activity after icv orexin-A administration [25]. Taken together, these data suggest that the Olanzapine-induced hypothermia has, at least in part, a centrally regulated origin, directly related to the orexin-A system at the level of the lateral hypothalamus (LHA).

Body temperature was significantly increased during the light period (figures 3 and 4). This increase was probably the result of an increased diet-induced thermogenesis caused by the elevated food intake in the light period in the Olanzapine-treated animals.

Olanzapine treatment reduced the intake of sucrose in a two-bottle-preference test, suggesting a reduced motivation for palatable foods (table 2). Likewise, Olanzapine prevented the normal increase in food intake (as seen in the control animals) on day 0, the first day that the animals were confronted with the palatable medium fat diet (table 1). These findings might be explained by Olanzapine's antagonist action on the dopaminergic system, because D2-receptor antagonism has been shown to reduce sucrose preference [43]. Since Olanzapine has been shown to increase swimming activity in a forced swim test [44], one may conclude that Olanzapine does not inhibit activity *per se*, but may predominantly inhibit the motivation to be active.

In this study we used a relative high dose of Olanzapine (5 mg/kg b.i.d). Studies performed by Cooper *et al* [19] and Davoodi *et al* [23] revealed that lower dose (2-4 mg/kg/day) might lead to a higher increase in body weight, partly the result of increased food intake. Oerther *et al* showed that Olanzapine induced hypothermia is dose dependent [24], which probably accounts also for the reduction of activity when compared to our data. As seen in figure 4 control animals increase their activity and body temperature at the time of administration, after which there is a significant drop in temperature and activity. Preliminary studies in our lab revealed that lower doses of Olanzapine will not result in a significant drop of activity and body temperature compared to control treated animals. In addition, we also noticed that there are remarkable differences in the effect of olanzapine on metabolism in, male and female rats, we are currently investigating this. During this study we have not measured if Olanzapine may decrease both basal metabolic rate and locomotor activity. Future studies should reveal if Olanzapine affects both these parameters of energy expenditure.

Based on the data above, we conclude that, in female Wistar rats, Olanzapine-induced weight gain is primarily the result of a major reduction in locomotor activity without changes in 24 hr daily food intake. These finding are in line with data from

human literature in which the sedative effect of Olanzapine is well-documented [40-42]. Evidence in humans for an effect of Olanzapine on food intake is less consistent [42,45-47]. We also found that Olanzapine has a dramatic acute effect on body temperature. Data in literature suggests that this Olanzapine-induced hypothermia is mainly the result of decreased BAT sympathetic activity, involving the orexin-A system at the level of the PeF in the LHA. Future studies are needed to unravel the possible effects of Olanzapine on meal size, meal frequency and feeding patterns.

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