The a-typical effects of olanzapine on body weight regulation

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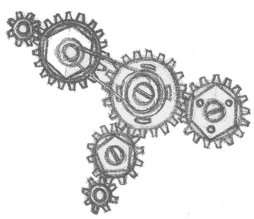

Chapter 6:

Psychogenetics

Roman High/Low Avoidance rat selection lines differ in their response to Olanzapine treatment at the level of body weight regulation and mesolimbic cortical mRNA expression.

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Abstract

Subjects treated with the antipsychotic drug olanzapine (OLZ) often experience weight gain, however, variation in drug responsiveness related to weight gain has been observed. To understand the underlying mechanisms of this variation, we investigated the effects of OLZ treatment in the Roman low and high avoidance (RLA and RHA, respectively) rat lines, which previously have been characterized as a valid model for studying the different aspects of impulsive behavior and its related psychopathologies. The main outcome of this study is that the RHA line was susceptible to OLZ-induced weight gain, while the RLA line did not accumulate extra weight in response to OLZ treatment. When allowed access to running wheels, RHA rats treated with OLZ displayed enhanced weight gain compared to sedentary RHA rats treated with OLZ. We suggest that the increased susceptibility to OLZ treatment in the (active) RHA is related to increased dopaminergic sensitivity. This was supported by the findings that running wheel activity in the RHA reduced circulating prolactin (PRL) and increased levels of dopamine receptor D1 (Drd1) mRNA expression in the prefrontal cortex (PFC). Finally, we observed a "complex interplay among environmental factors, genetic predisposition, and the receptor binding profile of the antipsychotic drug (Kinon et al, 2005)". Above all, we demonstrate that the RHA selection line, which was presumed to be obesity resistant, is more susceptible to OLZ-induced weight gain compared to the RLA. Moreover, our results show that the RHA strain is not only a proposed model to study the underlying mechanisms of psychopathologies, such as schizophrenia, but also could be a preferred model to study the metabolic consequences of antipsychotic drug treatment. All together our data underscores the value of studying individual variation in drug responsiveness via selection lines.

Keyword: Roman Avoidance, Olanzapine, weight gain, activity, prolactin, prefrontal cortex, dopamine.

Introduction

Animal models mimicking the behavioral phenotype of schizophrenia have been used in the attempt to increase the understanding of the possible underlying neurological mechanisms of this psychopathology [1,2]. One of these models is the Roman rat strain, which has been selectively bred for low and high avoidance (RLA and RHA respectively) behavior, mimicking several aspects of impulsive behavior found in schizophrenia. From this point of view it is remarkable that studies focusing on the effects of antipsychotic agents, like Olanzapine (OLZ), on neuronal, physiological, and behavioral mechanisms have only made limited use of such models.

Olanzapine is a widely used second generation antipsychotic (SGA) agent and is developed to exhibit mood stabilizing properties and limiting extrapyramidal side effects (e.g. tardive dyskinesia, akinesia, and akathisia) when compared to first generation antipsychotics [3,4]. These effects result from the a-typical property of SGA's to antagonize both dopamine and serotonin receptors [5,6], whereas first generation antipsychotics (FGA's) are specifically antagonistic on the dopamine D2 receptor (Drd2). Unfortunately the use of SGAs, and in particular OLZ, is associated with negative side effects including severe weight gain [7,8].

Weight gain on OLZ treatment is, however, not consistently observed in all users. In fact, Kinon *et al* (2005) observed that the majority of patients actually did not exhibit weight gain during OLZ treatment [9]. In their study, including 1336 patients, only 15% of patients displayed severe weight gain during the first 6 weeks of treatment (≥7% BW gain). It was also observed that individuals showing severe weight gain responded better to OLZ's potency to reduce the positive symptoms of schizophrenia (e.g. delusions, and hallucinations). Kinon *et al* concluded that "antipsychotic drug induced weight gain may reflect a complex interplay among environmental factors, a patient's psychiatric history and genetic predisposition, and the receptor binding profile of the antipsychotic drug" [9].

This reminded us of a passage in the seminal paper by L.L. Thurstone (1927) in which he provided a definition of personality, later translated by others into coping style: "The way an individual perceives and interacts with the environment is to a large extent determined by the individual's personality. Personality can be defined as a set of behavioral strategies that are embedded within the individual. These behavioral strategies are deployed throughout life to guide the individual's interaction with the environment [10]. This suggests a strict relation between personality and genetic background. Both Kinon and Thurnstone emphasize on the

importance of environmental interaction, but where Kinon *et al* suggest that environmental factors might only influence the individual's responsiveness to treatment, Thurnstone more strictly state that the individual's embedded behavioral strategy is a direct consequence of its environment. Indeed, animal studies have shown that under a given environmental situation (e.g., stress) individuals may display totally different predictable behavioral responses (based on its inbred selection background), that each serve adaptive value in terms of Darwinian fitness.

The Roman High/Low Avoidance rat strain is such an animal model. The selection is based on active avoidance performance in the shuttle-box, but the two selection lines are, additionally, characterized by several behavioral and physiological differences. In general the Roman Low Avoidance (RLA) rat is more anxious and emotionally reactive in comparison with the Roman High Avoidance (RHA) rat [11,12]. The RLA strain activates the HPA-axis leading to increased ACTH and corticosterone levels during stress, whereas the RHA is characterized by a more sympathetically regulated stress response. In addition, in comparison to its RLA counterpart, the RHA rat is more sensitive to develop amphetamine and apomorphine-induced stereotypic behavior; this seems related to a higher striatal dopamine turnover rate [13,14]. Both selection lines, RHA and RLA, also differ in the monoaminergic make-up of the brain, their emotionality and the vulnerability to drug addiction [14-17].

Previous studies from our group revealed that the two Roman strains also differ in the susceptibility to develop diet–induced-obesity (DIO) and related insulin resistance [18]. While RLA's were found to be prone to develop DIO on a palatable high caloric diet leading to significant weight gain, adiposity and insulin resistance, RHA rats are diet-resistant and remain insulin sensitive on a palatable high caloric diet. Finally, we showed that the two Roman rat strains differ in the response to antidiabetic drugs [19]. Rosiglitazone (an insulin sensitizer through GLUT4 activation) was equally effective in improving insulin sensitivity in both selection lines, but treatment with the glucocorticoid receptor antagonist RU486 only improved insulin sensitivity in the RLA without any effect in the RHA. For the same reasons that the RHA and RLA selection lines can be regarded as a unique animal model for the study of personalized medicine [20], they may also serve to study the mechanisms underlying the individual differences in the diabetogenic and weight gain-inducing properties of OLZ. Therefore, in the present study, we decided to administer OLZ or placebo to rats of both the RHA and RLA strain and study the effects on weight gain and associated co-morbidities. To this end, we provided the experimental animals with a highly palatable medium fat diet and monitored daily food intake and changes in body weight. After 14 days of OLZ treatment, glucose and insulin profiles were obtained in

an intravenous glucose tolerance test (IVGTT). Blood samples and brain tissue were collected at the termination of the experiment to investigate related or underlying hormonal and neuronal mechanisms, with special emphasis on central mRNA receptor expression in the cortico-mesolimbic system. The latter was performed to reveal comparisons between the selection lines and the schizophrenic population, in which increased dopaminergic receptor expression has been observed in the prefrontal cortex (PFC) compared to control individuals [21]. Studies were performed in sedentary rats and in rats that had access to running wheels. The latter condition was included because OLZ is known to cause drowsiness and lethargy, which could underlie in part the adverse metabolic side effects of OLZ, and these effects would obviously be inflated (i.e., compared to the control treatment) if rats were given the opportunity to be physically active. Alternatively, we have previously demonstrated that the susceptibility to develop DIO and insulin resistance on a palatable high fat diet were markedly different between Roman High and Low Avoidance rats with voluntary access to a running wheel [22], and these differences might be lost when treated with OLZ. Taken together, the variation in rat strains, diet, and housing conditions may allow us to study the metabolic, hormonal and behavioral effects of OLZ and investigate whether these effects are mainly induced by the genetic background, the environment, or a combination of strain, treatment, and environment.

Material and Methods

2.1 Animals

All procedures were approved by the Animal Experimentation Committee of the University of Groningen, NL.

Roman Low/High Avoidance breeding pairs were obtained from the breeding colony of the Clinical Psychopharmacology Unit (APSI; University of Geneva, Switzerland), and bred locally at the animal facility of the University of Groningen. Breeding pairs were characterized and female offspring was kindly provided by Dr. C.M. Coppens (Dept. of Behavioral Physiology, RuG, NL).

Animals were individually housed with ad lib access to standard chow (3.8 kcal/g, 14% fat, RMB-H 2181, Arie Blok Diervoeding, Woerden, NL) and water. Climate was controlled at 20±2 ˚C, humidity 60±5%. Lights went off at 11:00 AM (CT12) on a 12:12hr light-dark cycle. At experimental day -1, all animals were put on a medium fat diet with lard (4.7 kcal/g; 45% fat, Arie Blok Diervoeding, Woerden, NL).

2.2 Drug and administration protocol

Olanzapine (powder) was kindly provided by Abbott (Fournier Laboratory, France). To obtain an administration of 2 mg/kg, OLZ was diluted in 0.9% NaCl saline, using 1M HCl, the final solution was adjusted to pH 6.5 using 1M NaOH and diluted to a concentration of 1mg/ml OLZ. Animals were administered 2ml/kg OLZ solution or saline twice a day at CT12 and CT18 via a permanent gastric catheter.

2.3 Surgical procedure

All surgical procedures were performed using isoflurane-O2/N2O gas-anesthesia. Animals were equipped with a permanent gastric catheter for intragastric drug administration. 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.

Two jugular vein silicon catheters (sampling cannula: 0.95 mm OD, 0.50 mm ID, infusion cannula: 0.64 mm OD, 0.28 mm ID) were inserted into the right and left jugular vein and kept in place with a ligature. Both catheters were pulled subcutaneously towards the head where they were connected to curved metal sleeves made of 19G surgical needles. The metal sleeves were fixed to the skull with dental cement and screws [48]. To prevent the formation of blot cloths, catheters were filled with a 55% polyvinyl pyrrolidone-heparin (PVP) solution [23]. Both exteriorized jugular vein cannulas and gastric cannula were closed by plastic caps made of a piece of flame-sealed PE100 tubing. Post-surgery analgesia (0.1 mg/kg Finadyne diluted in 0.1 ml/kg saline subcutanously) was administered 15 minutes before animals were taken off anesthesia. Catheters were rinsed twice a week starting 2 days after surgery untill the start of the experiment after which catheters were used at a regular basis.

2.4 Experimental set-up

Body weight, food intake, and water intake were measured daily at CT12. From day -7 till day -1 all animals received 2ml/kg saline intragastrically at CT12 and CT18; from day 0 till day 14 designated animals received 2ml/kg OLZ while control animals continued on 2 ml/kg saline. A single blood sample (1ml) was drawn at day -1 and day 7, and a final blood sample (trunk blood) was collected at day 14. All blood samples were collected in plastic tubes on ice containing per 1ml whole blood 10 µL EDTA; samples were centrifuged for 15 min, 2500 rpm at 4˚C, and plasma was stored at -20 ˚C until further analysis. At day 14, an intravenous glucose tolerance test (IV-GTT) was performed at CT12. Animals were sacrificed at CT16 on day 14. Animals were shortly placed in an isoflurane filled chamber for sedation followed by decapitation. Trunk blood (± 10 mL) was collected and brains were excised and frozen with liquid nitrogen

and stored at -80˚C for further analysis. Consecutively, parametrial, retroperitoneal, visceral, subcutaneous (skin), and brown adipose tissue, liver, kidneys, and adrenals were carefully excised and weighed. Carcass, skin and liver were dried in an oven at 80˚C for 5 days. After drying, tissues were put in a petroleum based Soxlet fat extractor to dissolve the remaining fat and dried for another 24hr. The dried tissues were weighed before and after fat extraction in order to measure the amount of fat content of each tissue.

Experiment 1: Sedentary

The sedentary RLA (N=16; 272 ± 4.7 g) and RHA (N=16; 260 ± 5.4 g) rats were housed individually in clear Plexiglas cages (25x25x30cm) filled with wood chipped bedding and gnawing stick on a plastic bottom. Both RLA and RHA rats were split into a control and drug treated group (n=8/group). In these groups of animals, a telemetry transmitter (model TA10TA-F40, Data Sciences, St. Pail, MN) was inserted in the abdominal cavity during surgery. This transmitter registered core body temperature and home cage activity every 10 minutes throughout the duration of the study. Telemetry signals were received by separate transponder plates (model RA1010, Data Sciences) underneath each cage. Data were collected and analyzed using specialized recording and analysis software (Dataquest IV, Data Sciences;[24]).

Experiment 2: Exercise

The exercise group consisted of 12 RLA rats (257 ± 3.2 g) and 12 RHA rats (255 ±3.8 g), which were split into a control and OLZ treated group (n=6) matched for body weight. These animals were housed individually in Nalgene polycarbonate running wheel cages (50x27x36cm) filled with wood chipped bedding, a gnawing stick, and had free access to a running wheel (diameter 27cm; Mini Mitter, Oregon, USA). Spontanous Running wheel activity was registered continuously during the experiment.

2.5 Intravenous-Glucose Tolerance Test

An IV-GTT was performed at day 14 at the start of the dark phase (CT12). Prior to the start of IV-GTT, animals were fasted for 4 hours. A baseline blood sample was drawn 60 minutes (t=-60) before starting of the intravenous glucose infusion (t=0). A single dose of 2 mg/kg OLZ was intragastrically administered immediately after the baseline blood sample was drawn at t=-60. Blood samples (0.2 ml) were taken at time points -60, 0, 5, 10, 15, 20, 25, 30, 40, 60, and 120 minutes. Glucose (150mg/ml) was infused via the infusion cannula starting at t=0 at a rate of 0.1 ml/min and stopped at t=30min. Blood samples were immediately put on ice during IV-GTT in vials containing 10µl EDTA (0.09 g/ml). Whole blood samples of 50µl diluted in 450µl 2%

heparin solution were stored at -20°C until analysis of glucose concentrations by the ferry-cyanide method [25] in a Technicon auto analyzer. The remaining blood samples (150 µl) were centrifuged (15min, 2500 rpm, 4°C) and plasma was collected and stored at -20°C until insulin determination. Plasma insulin levels were measured in duplicates using a commercial radioimmunoassay kit (Rat Insulin, ^{125}I-Insulin Cat# RI-13K, Linco Reasearch, Nucli Lab, NL).

2.6 Plasma analysis

Circulating leptin, triglycerides (TGC), free fatty acids (FFA), corticosterone (Cort), and prolactin levels were determined in plasma samples collected at day -1 (baseline), day 7 (wk 1), and day 14 (wk 2), using an ^{125}I-Rat Leptin RIA (#RL-83K, Linco Research , Nucli Lab, NL), L-Type Triglyceride M enzymatic kit (WAKO Chemicals GmbH, D), NEFA -HR2 enzymatic kit (WAKO Chemicals GmbH, D), ImmuChem Cort ^{125}I-RIA KIT (MP Biomedicals, Germany GmbH, Eschwegge, D), and PRL ^{125}I-RIA KIT (MP Biomedicals Germany GmbH, Eschwege, D).

2.7 Central gene expression

The Prefrontal Cortex (PFC), Nucleus Accumbens (Nac), and Ventral Tegmental Area (VTA) were isolated from 500 µm thick frozen coronal sections using a Harris uni-core tissue puncher (ID 0.75 mm; Ted Pella, Inc., Redding, CA, USA) based on the coordinates for adult rat brains [26]. mRNA expression on the following genes were measured using RT-PCR: the dopamine 1 receptor (Drd1), the dopamine 2 receptor (Drd2) in VTA, Nac and PFC, the serotonin 1a receptor (Hrt1a) in VTA and PFC, the serotonin 2a receptor (Hrt1a), and the serotonin 2c receptor (Hrt2c) in the PFC, and the dopamine transporter (Slc6a3), and Tyrosine hydroxylase (Th) in the VTA. Total RNA from the tissue punches was obtained using the RNeasy Lipid Tissue Mini Kit with the Qiazol reagent (Qiagen, Valencia, CA). A QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA) was used to generate cDNA for subsequent quantitative real-time PCR. All reactions were carried out in triplicate using 1X Taqman master mix (Applied Biosystems, Foster City, CA), 1X Taqman probes for each gene (Drd1, Drd2, Hrt1a, Hrt2a, Hrt2c, Th, Slc6a3 and Actb) (Life technologies, Grand Island, NY), and 1 µg of cDNA template in a total volume of 20 µL. Real-time reactions were performed with standard PCR conditions (50°C for 2 min; 95°C for 10 min; and 95°C for 15 sec and 60°C for 1 min for 40 cycles) on an Applied Biosystems 7900HT Fast Real-Time PCR System. Each set of triplicates was checked to ensure that the threshold cycle (Ct) values were all within 1 Ct of each other. Negative RT samples were used to control for possible contamination of gDNA. To determine relative expression values, the -ΔΔCt method (Applied Biosystems, Foster City, CA) was used, where triplicate Ct values for each tissue sample were averaged and subtracted from those derived from

the housekeeping gene Beta-actin (Actb). The average Ct difference for the control group was subtracted from those of the test samples, and the resulting -ΔΔCt values were raised to a power of two to determine normalized relative expression.

2.8 Data analysis

All data are expressed as average±sem. Home-cage and running wheel activity data were analyzed as a change in percentage of activity relative to baseline activity per individual. Baseline activity was calculated per individual as the average activity per day from day -7 until day -1; this average was set at 100%. Activity data during treatment are presented as a percentage of this baseline value. Body composition parameters were analyzed as a percentage of total BW measured at sacrifation. Statistical analysis was performed using repeated measures (rm) analysis of variance (ANOVA) between subjects for time dependent analysis. To check for significant differences between groups One-way ANOVA (post hoc Tuckey HSD) was used. Where appropriate, paired samples t-test was performed within each group. To assess strain, environment, treatment, or interaction effects general linear model (GLM) repeated measures or Univariate ANOVA was performed. All statistical analysis were performed in SPSS20 outcomes were regarded significant with a significance level of $P<0.05$.

Results

Experiment 1: Sedentary Environment

1.1 Body weight gain.

High fat diet induced weight gain (see figure 1A and B) was only affected by strain, with higher ΔBW in the RLA rats compared to the RHA rats ($P<0.001$, $F_{14,378}=7.766$, GLM rmANOVA), independent of treatment. When analyzing the strains separately, however, OLZ increased ΔBW exclusively in the RHA ($P<0.001$, $F_{14,182}=7.695$, GLM rmANOVA). Sources of significance are indicated in the figure legend.

1.2 Food intake.

Cumulative food intake (in kcal/day) calculated over 14 days of treatment is illustrated in fig 2A (Averages: RHA-Sed-Con = 938±30kcal, RHA-Sed-OLZ = 955±55kcal, RLA-Sed-Con = 1158±66, RLA-Sed-OLZ = 1166±44). A main effect of strain on cumulative food intake was observed ($P<0.01$, $F_{1,26}=12.929$), with higher levels found in the RLA selection line, and this was confirmed by repeated measures analysis (fig. 2C) revealing higher daily food intake in the RLA ($P<0.05$, $F_{13,388}=1.770$, GLM rmANOVA) compared to the RHA line. At day -1, when animals were put on a

palatable high fat diet, caloric intake of the new diet was higher in the RLA compared to RHA ($P<0.05$, $F_{1,26}=4.530$, Univariate ANOVA).

1.3 Home cage activity.

Diurnal home cage activity was affected most profoundly by treatment ($F_{23,621}=42.638$, $P<0.001$, GLM rm-ANOVA), with OLZ clearly reducing it compared to control treatment. While a main of strain was found on home cage activity ($F_{23,621}=1.908$, $P<0.01$, GLM rm-ANOVA), it interacted strongly with treatment ($F_{23,621}=4.161$, $P<0.001$, GLM rm-ANOVA), with the difference between the OLZ- and

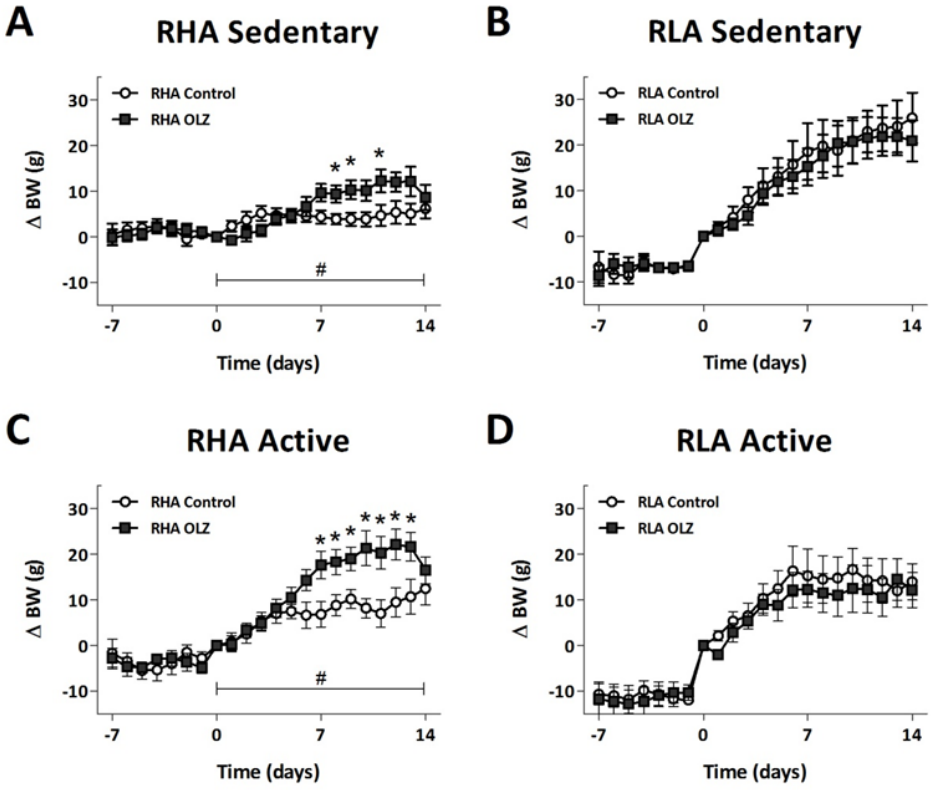


Fig. 1: Body weight (ΔBW) A) in the RHA strain in the sedentary environment, B) in the RLA strain in the sedentary environment, C) In the RHA strain in the active environment, and D) in the RLA strain in the active environment. A) ΔBW gain was increased in the RHA-OLZ treated group compared to control at day 8, 9, and 11 (*P<0.05). B) ΔBW gain of the RLA in the sedentary environment was not different in the OLZ and control treated groups. C) ΔBW gain of the RHA in the active environment was increased during OLZ treatment compared to control at days 7, 8, 9, 10, 11, 12, and 13 (*P<0.05). D) Body weight gain of the RLA in the active environment was not different in the OLZ and control treated groups.

control-treated RHA rats being significantly larger than in the RLA rats. As illustrated in fig. 3A/B, total diurnal home cage activity was higher in RHA controls (=109.5±3.2 AU) than RLA controls (=71.85±3.75 AU; P<0.001) and in both strains OLZ treatment reduced total home cage activity (RHA-Sed-OLZ=48.2±2.7 AU; RLA-Sed-OLZ=50.15±1.9 AU; P<0.001, $F_{3.30}$=81.141, post hoc HSD) only during the dark phase to comparably low levels. Analyzing sources of significance revealed that OLZ in the RHA strain only reduced dark phase home cage activity (RHA-Sed-Con=77.7±4.7 AU,

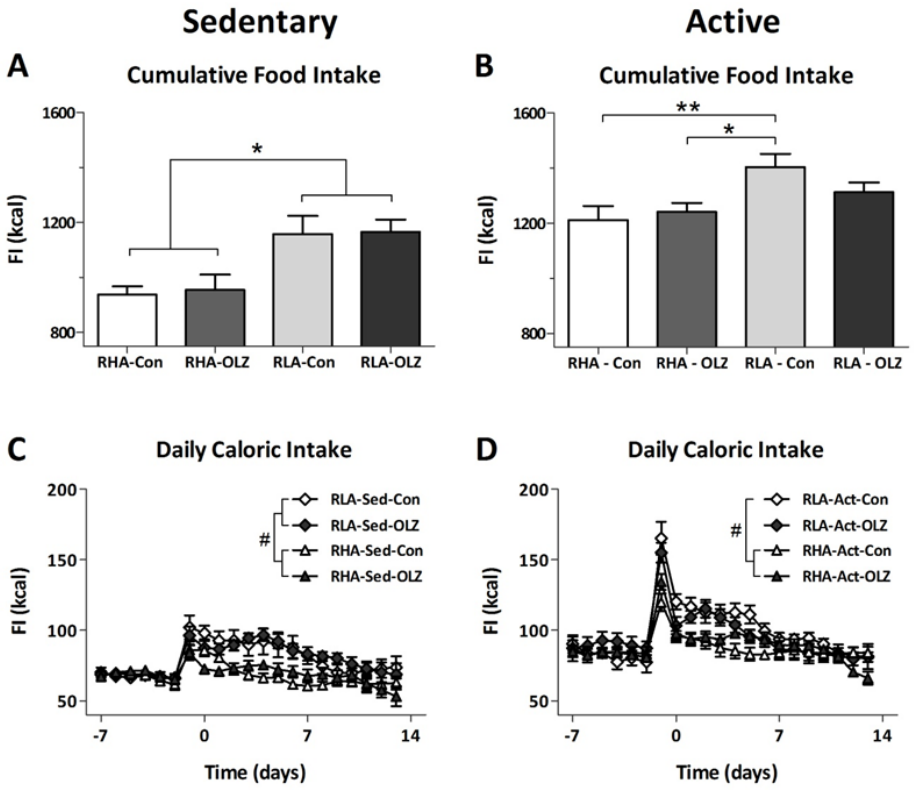


Fig. 2: Cumulative food intake over the 14-day treatment period (in kcal) A) of RHA and RLA strains in the sedentary environment, B) of RHA and RLA strains in the active environment. Daily patterns of food intake (in kcal) of C) sedentary (Sed) housed RHA and RLA strains, and D) actively (Act) housed RHA and RLA strains. A) Fourteen days of cumulative food intake (kcal) was lower in the RHA groups compared to both RLA groups independent of treatment (*P<0.05). B) Fourteen days of cumulative food intake in the active environment was a higher in the RLA control group compared to both RHA groups (*P<0.05, **P<0.01), no treatment effect has been observed. C) Daily food intake in the sedentary environment was higher in the RLA strain during treatment compared to the RHA strain ($^{\#}$P<0.05, rm-ANOVA). D) Daily food intake in the active environment over the duration of treatment was higher in the RLA control group compared to both RHA groups on days ($^{\#}$P<0.05, rm-ANOVA).

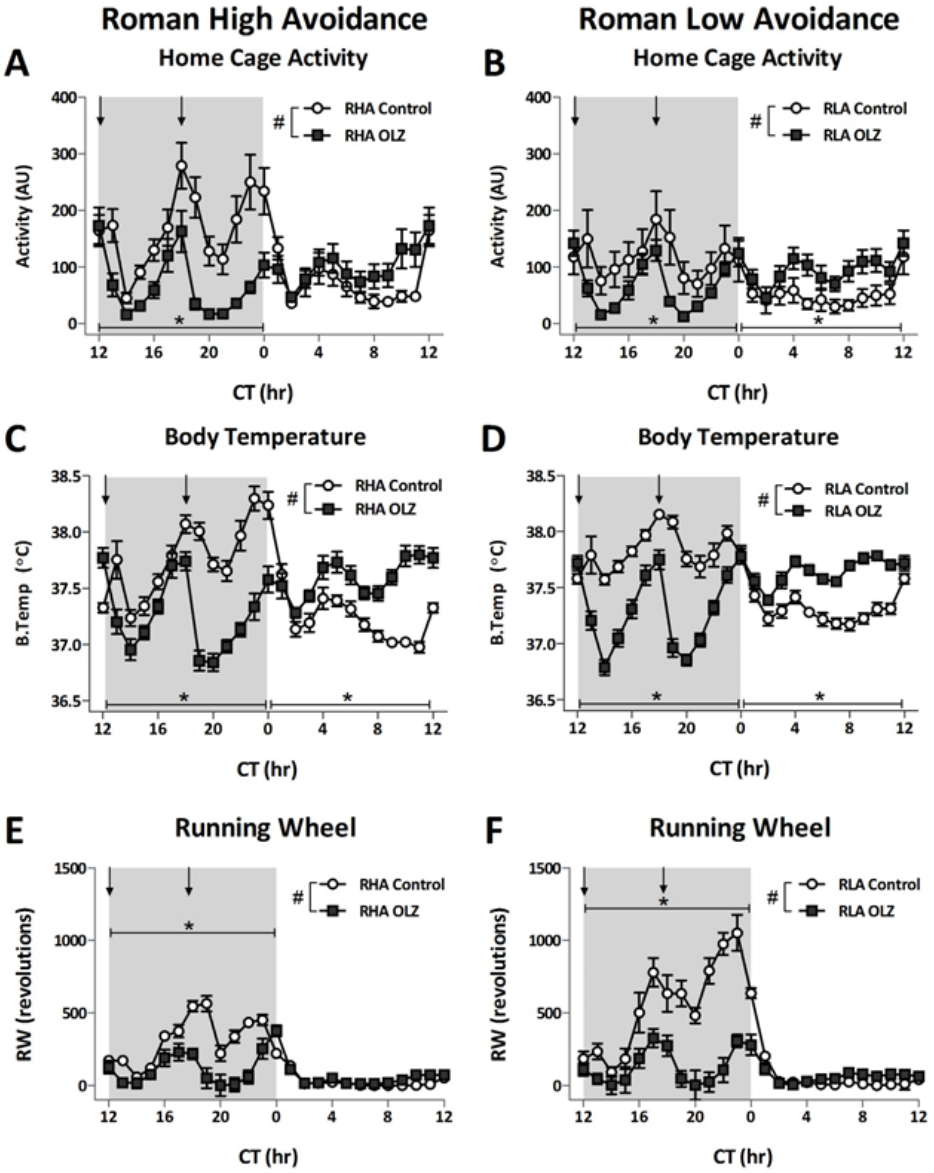


Fig. 3: Circadian home cage activity (A, B), temperature patterns (C, D), and running wheel behavior (E,F) of RHA and RLA selection lines. A) RHA diurnal home cage activity was reduced by OLZ treatment ($^{\#}P<0.01$), especially during the dark phase ($^{*}P<0.01$) immediately after drug administration (arrows). B) RLA diurnal home cage activity was reduced by OLZ ($^{\#}P<0.01$). Like the RHA, OLZ reduced home cage activity during the dark phase ($^{*}P<0.01$), but unlike the RHA, light phase home cage activity was increased in the RLA OLZ-treated group ($^{*}P<0.01$). C) RHA diurnal body temperature showed a drop by OLZ treatment (arrows) and changed diurnal temperature patterns ($^{\#}P<0.01$). During the dark phase OLZ reduced body temperature ($^{*}P<0.01$), whereas body temperature was increased in the OLZ group during the light phase ($^{*}P<0.01$). D) RLA diurnal body temperature also revealed an OLZ-

RHA-Sed-OLZ=18.3±1.5 AU; P<0.001, $F_{1,14}$=201.578), and did not affect light phase activity (RHA-Sed-Con=31.74±1.5 AU, RHA-Sed-OLZ=29.9±1.7 AU). In the RLA strain OLZ decreased home cage activity during the dark (RLA-Sed-Con=49.7±2.3 AU, RLA-Sed-OLZ=20.5±1.2 AU; P<0.001, $F_{1,15}$=124.431), but increased home cage activity during the light phase (RLA-Sed-Con=22.12±1.7 AU, RLA-Sed-OLZ=29.6±1.9 AU; P<0.05, $F_{1,15}$=8.866). During the light phase the RLA-Sed-Con group displayed less home cage activity compared to all other groups (P<0.05, $F_{3,30}$=5.842).

1.4 Thermoregulation.

As is illustrated in fig.3C/D, OLZ reduced body temperature immediately after the two administrations in the dark phase in both selection lines, whereas an increase of body temperature was observed during the light phase. Regarding the diurnal body temperature patterns, a very strong main effect of treatment was found (P<0.001, $F_{23,598}$=80.520, GLM rm-ANOVA), that also interacted with strain ($F_{23,598}$=6.222, P<0.001, GLM rm-ANOVA). While strain itself was a main effect too (P<0.01, $F_{23,598}$=2.075, GLM rm-ANOVA), these results may be explained by the difference in diurnal body temperature patterns between both control groups (P<0.001, $F_{23,276}$=5.767, rm-ANOVA), but not between the OLZ treated groups. Moreover, retrieving the sources of significance revealed a hypothermic response (relative to control treatment) following the first OLZ injection in the RLA strain (P<0.05, $F_{23,20}$=5.652), but not in the RHA. No differences were found between the hypothermic responses following the second OLZ administration in the RHA and RLA rats, nor during the rebound of body temperature in the light phase, where OLZ treated rats had actually increased body temperatures versus control treated rats. Compared to diurnal home cage activity all groups displayed a positive correlation between activity and body temperature per hour (RHA-Sed-Con: R^2=0.848, P<0.001; RHA-Sed-OLZ: R^2=0.828, P<0.001; RLA-Sed-Con: R^2=0.869, P<0.001; RLA-Sed-OLZ: R^2=0.865, P<0.001; Pearson 2-tailed).

induced hypothermic response immediately after administration (arrows) and also changes in diurnal temperature patterns were found ($^{\#}$P<0.01). During the dark phase OLZ reduced body temperature (*P<0.01), whereas body temperature was increased in the OLZ group during the light phase (*P<0.01). Collectively in all groups body temperature was highly correlated to home cage activity (Pearson 2-tailed). E) RHA running wheel activity was decreased by OLZ ($^{\#}$P<0.01), predominantly during the dark phase (*P<0.01). Unlike home cage activity no increase of running wheel activity was observed during the light phase. F) Total RLA running wheel activity was higher compared to control RHA (P<0.01). Like the RHA, OLZ reduced running wheel activity ($^{\#}$P<0.01,), especially during the dark phase (*P<0.01). *CT=circadian time; lights off at CT0; arrows indicate drug/saline administration at CT12 and CT18. Open circles indicate control treated groups, dark filled squares indicate OLZ treated groups.*

1.5 Glucose and insulin responses.

No main effects or interactions hereof were found on circulating glucose levels or the AUC_{Gluc} during the IVGTT (fig. 4A), nor in the basal glucose levels preceding the IVGTT. In addition, within strain analysis did not reveal a difference in these parameters either (see Table 1 for details).

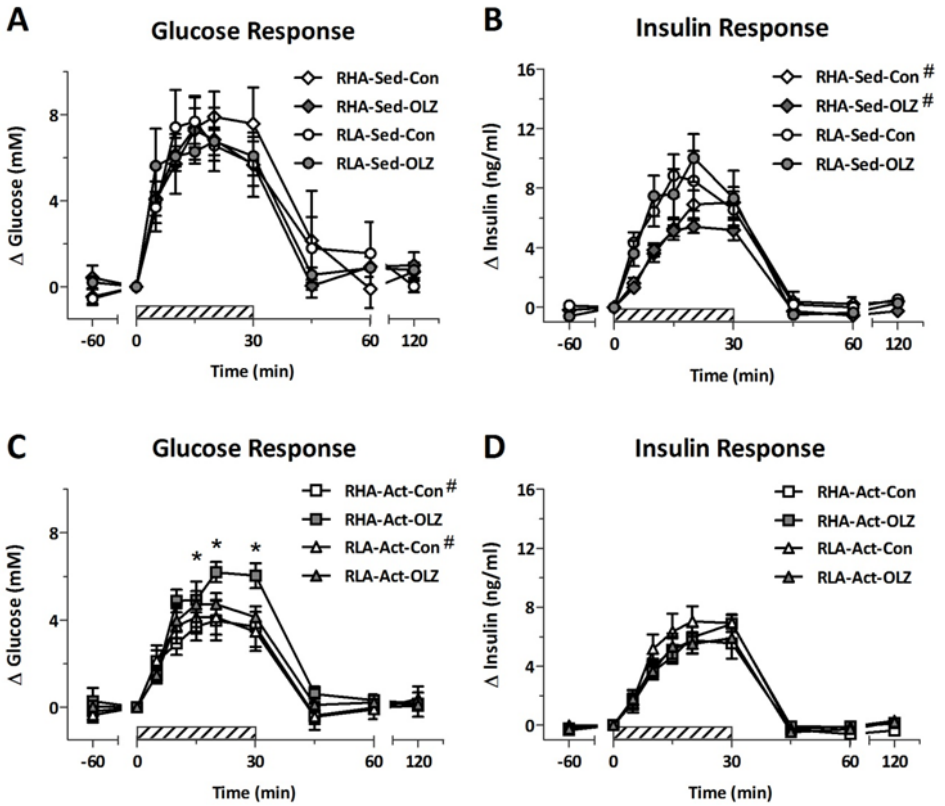


Fig. 4: Delta Glucose (A, C) and Insulin (C, D) responses during intravenous glucose tolerance test in a sedentary (A, B) and active (C, D) condition. A) Glucose responses in the sedentary environment were not different among groups. B) Insulin responses in the sedentary environment showed a strain effect, an increased insulin response was observed in both RLA groups compared to both RHA groups ($^{\#}P<0.05$). C) Glucose responses in the active environment were affected by treatment with increased circulating glucose levels in the OLZ groups compared to the Control groups ($^{\#}P<0.05$). Within strain analysis showed increased circulating glucose levels in the RHA-Act-OLZ group compared to the RHA control group at time points 15, 20, and 30 minutes (*P<0.05). D) Insulin responses in the active environment were not affected by strain, treatment, or strain*treatment interaction.

Analysis of basal insulin levels (fig 4b, table 1) revealed a strain effect ($F_{1,25}$=16.983, P<0.001), with generally higher levels in the RLA strain than in the RHA strain (P<0.01, $F_{3,25}$=7.511). In addition, circulating insulin levels during IVGTT were also affected by strain (P<0.001, $F_{8,200}$=3.828, GLM rm-ANOVA), with generally higher levels found in the RLA compared to the RHA selection strain. A main effect of strain on AUC_{Ins} reached significance ($F_{1,25}$=4.088, P=0.054). No effect of treatment or interaction with strain was observed.

1.6 Body composition (for actual data see table 2).

a. Adiposity

Adiposity (calculated as a percentage of total body weight) in the sedentary housed animals was not affected by treatment. In contrast, a strain effect on adiposity was observed with higher levels in the RLA compared to the RHA groups of total adiposity (P<0.001, $F_{1,26}$=28.695), parametrial (P<0.001, $F_{1,26}$=27.974), retroperitoneal (P<0.001, $F_{1,26}$=30.874), visceral (P<0.05, $F_{1,26}$=7.738), subcutaneous (P<0.001, $F_{1,26}$=28.980), and intermuscular adiposity ($F_{1,26}$=8.151, P<0.01).

b. Organ weights

Analysis of liver weights did not reveal a strain effect (P=0.054), but a strain*treatment interaction was found (P<0.01, $F_{1,26}$=7.884). Post hoc analysis revealed lower liver weights in RHA-Sed-OLZ compared to RLA-Sed-OLZ rats (P<0.01, $F_{3,26}$=4.789). Kidney or BAT weights were comparable in all sedentary housed groups. In contrast, a strain effect was observed on adrenal weight ($F_{1,26}$=8.822, P<0.01), with increased adrenal weights in the RHA compared to RLA selection line. Post hoc analysis revealed higher adrenal weights in RHA-Sed-Con compared to RLA-Sed-Con rats (P<0.05, $F_{3,26}$=4.107).

Table 1: Baseline and area under the curves of glucose and insulin responses in the sedentary environment.

	RHA Sedentary		RLA Sedentary	
	Control	*OLZ*	*Control*	*OLZ*
Baseline				
Glucose (mM)	6.10±0.40	5.95±0.23	6.19±0.47	6.34±0.16
Insulin (ng/ml)	0.89±0.05[a]	0.83±0.11[a]	1.26±0.19	1.74±0.18[b]
Area under the Curve				
Glucose	274±75	215±58	253±66	231±23
Insulin	200±26	149±11	247±42	250±45

[a]P<0.05, oneway-ANOVA. RHA show a strain effect of lower baseline insulin levels compared to RLA.
[b]P<0.01, oneway-ANOVA post hoc LSD. RLA-Sed-OLZ shows higher baseline insulin levels compared to both RHA groups.

Table 2: Body composition within the sedentary environment

		RHA Sedentary		RLA Sedentary	
		Control	*OLZ*	*Control*	*OLZ*
Parametrial (%)		1.34±0.20*^{A}	1.46±0.10*^{b}	2.07±0.14	2.09±0.12
Retroperitoneal (%)		1.76±0.21*^{A}	2.05±0.24*^{b}	2.94±0.24	2.93±0.19
Visceral (%)		2.27±0.13*	2.26±0.07*	2.77±0.17	2.67±0.15
Abdominal depots (%)		5.37±0.36*^{A}	5.77±0.30*^{B}	7.78±0.51	7.69±0.43
Subcutaneous (%)		2.83±0.19*^{A}	3.92±0.44*^{b}	6.77±0.79	6.96±0.76
Intermuscular (%)		2.29±0.17*^{a}	2.86±0.29*	3.56±0.40	3.06±0.18
Total Adiposity (%)		10.71±0.60*^{A}	12.75±0.98*^{b}	18.28±1.63	17.92±1.21
Liver	g	9.53±0.35	8.91±0.17	9.52±0.30	10.26±0.22$^{\#C}$
Kidneys	g	2.10±0.09	2.09±0.05	2.13±0.06	2.18±0.07
Adrenal	g	0.093±0.006*^{a}	0.081±0.005*	0.068±0.005	0.075±0.004
BAT	g	0.605±0.077	0.630±0.054	0.505±0.044	0.630±0.056

Adiposity: *Strain effect: RHA lower compared to RLA; P<0.05, univariate-ANOVA. ^{A}RHA-Sed-Con<RLA-Sed-Con/OLZ; P<0.01, oneway-ANOVA post hoc. ^{a}RHA-Sed-Con<RLA-Sed-Con/OLZ; P<0.05, oneway-ANOVA post hoc. ^{B}RHA-Sed-OLZ<RLA-Sed-Con/OLZ; P<0.01, oneway-ANOVA post hoc. ^{b}RHA-Sed-OLZ<RLA-Sed-Con/OLZ; P<0.05, oneway-ANOVA post hoc. Liver: #Strain*treatment interaction: RLA-Sed-OLZ<other groups; P<0.01, univariate-ANOVA. ^{C}RLA-Sed-OLZ > RHA-Sed-OLZ; P<0.01, oneway-ANOVA post hoc. Adrenals: *Strain effect: RHA-Sed<RLA-Sed; P<0.01, univariate-ANOVA. ^{a}RHA-Sed-Con>RLA-Sed-Con; P<0.05, oneway-ANOVA post hoc.

Experiment 2: Active environment

2.1 Body weight gain.

High fat diet induced weight gain, as shown in fig 1C/D, was affected by a strain*treatment interaction ($F_{14,280}$=1.902, P<0.05, GLM rmANOVA), with higher ΔBW by OLZ treatment only in the RHA group. When analyzing each strain separately, the above mentioned effect of OLZ to increase ΔBW was confirmed in the OLZ group ($F_{14,140}$=6.152, P<0.0001, GLM rmANOVA), with sources of significance found on day 7 (P<0.05, $F_{1,10}$=6.376), day 8 (P<0.05, $F_{1,10}$=6.353), day 9 (P<0.05, $F_{1,10}$=6.381), day 10 (P<0.05, $F_{1,10}$=7.926), day 11 (P<0.05, $F_{1,10}$=7.353), day 12 (P<0.05, $F_{1,10}$=7.013), and day 13 of treatment (P<0.05, $F_{1,10}$=5.582, oneway-ANOVA).

Combined analysis of ΔBW in the sedentary and active groups revealed a main effect of strain(P<0.01, $F_{14,658}$=2.210), treatment (P<0.001, $F_{14,658}$=3.688), and a strain*environment interaction (P<0.001, $F_{14,658}$=5.346, GLM rmANOVA), with higher ΔBW in RLA rats in the sedentary environment than in the active environment, but this was not found in the RHA rats. Analyzing each strain separately revealed an effect of environment (P<0.001, $F_{13,322}$=6.153) and treatment (P<0.001, $F_{13,322}$=8.631, GLM rmANOVA) in the RHA strain, with ΔBW being increased by OLZ treatment, and

being surprisingly reduced in the active environment compared to the sedentary environment. In the RLA strain, however, only a main effect of environment was observed ($F_{14,336}$=1.999, P<0.05, GLM rmANOVA), confirming the abovementioned interaction effect, and a general unresponsiveness of the RLA to alter ΔBW by OLZ.

2.2 Food intake.

Cumulative food intake (fig. 2B) was affected by strain ($F_{1,21}$=11.482, P<0.01) and by a strain*treatment interaction ($F_{1,21}$=4.499, P<0.05), with generally higher cumulative intake in the RLA selection line, and with OLZ lowering cumulative intake only in the RLA.

Figure 2D illustrates the daily caloric intake during baseline and 14 days of treatment of animals in the active environment. Repeated measures analysis revealed a strain effect ($F_{13,273}$=3.288, P<0.001, GLM rmANOVA), and a strain*treatment interaction ($F_{13,273}$=2.235, P<0.01, GLM rmANOVA), confirming the aforementioned main effects on cumulative intake. Analyzing each strain separately indeed confirmed the main effect of treatment in the RLA strain (P<0.001, $F_{13,143}$=3.837, rmANOVA), but not in the RHA.

At day -1 animals received a palatable medium fat diet, intake at day -1 showed a strain effect (P<0.001, $F_{1,24}$=16.866), meaning higher caloric intake of the unfamiliar diet in the RLA selection line.

Combining sedentary and active groups in one analysis revealed that total 14 days cumulative food intake was affected by strain ($F_{1,54}$=24.946, P<0.001) and by environment (P<0.0001, $F_{1,54}$=46.734), meaning that food intake was increased in the RLA compared to the RHA selection line, and that food intake was increased in the active environment compared to the sedentary environment, without interactions between the two.

2.3 Running wheel activity.

Average diurnal running wheel activity, as illustrated in fig.3E/F, was affected by treatment (P<0.001, $F_{24,480}$=14.656, GLM rm-ANOVA), by strain (P<0.001, $F_{24,480}$=3.698, GLM rm-ANOVA), and by an interaction of strain*treatment (P<0.001, $F_{24,480}$ =3.279, GLM rm-ANOVA). In general, running wheel activity was higher in the RLA than in the RHA strain, but these differences were lost during the treatment with OLZ (explaining the interaction). When analyzing the strains separately, OLZ affected diurnal running wheel activity in both the RHA (P<0.001, $F_{24,240}$=8.161, rm-ANOVA) and RLA (P<0.001, $F_{24,240}$=9.202, rm-ANOVA).

Analyzing total daily running wheel activity revealed a strong main effect of treatment (P<0.001, $F_{1,24}$=20.057), with generally lower levels in the OLZ treated groups. Furthermore, a main effects of strain was found (P<0.05, $F_{1,24}$=6.338), that also interacted with treatment (P<0.05, $F_{1,24}$=4.416). These results corroborate above

mentioned findings. Within strain analysis revealed that OLZ decreased average total running wheel activity per day in both the RHA (RHA-Act-Con=3964±659 rev/day, and RHA-Act-OLZ=2117±386 rev/day; P<0.05, $F_{1,11}$=5.859) and the RLA strain (RLA-Act-Con=7555±1418 rev/day, and RLA-Act-OLZ=2440±476 rev/day; P<0.01, $F_{1,11}$=14.263); post hoc analysis revealed increased daily running wheel activity in the RLA-Act-Con group compared to all other groups (P<0.001, $F_{3,23}$=10.271). In addition, in both strains only average dark phase running wheel activity was decreased by OLZ treatment: RHA-Act-OLZ (=1491±320 rev) compared to RHA-Act-Con (=3529±648 rev; P<0.05, $F_{1,11}$=7.952), and the RLA-Act-OLZ (=1637±404 rev) compared to the RLA-Act-Con (=6992±1137 rev; ; P<0.01, $F_{1,11}$=19.689, oneway-ANOVA) .

When comparing home cage activity (sedentary environment) and running wheel activity (active environment) it is remarkable that the RHA strain displayed increased home cage activity, whereas the RLA was more active in the running wheel.

2.4 Glucose and insulin responses.

Analysis of the glucose responses during the IVGTT in the active environment (fig.4C, and table 3) only revealed a treatment effect (P<0.01, $F_{8,160}$=2.996, GLM rm-ANOVA), with increased glucose levels in the OLZ treated groups relative to the control treated ones. When analyzing the strains separately, a treatment effects was observed on the glucose response during the IVGTT only in the RHA (P<0.001, $F_{8,80}$=4.495, rm-ANOVA), with increased levels in OLZ treated versus control treated rats. In the RHA, increased circulating glucose levels were observed at time points: 10min (P<0.05, $F_{1,11}$=9.274), 20min (P<0.05, $F_{1,11}$=6.083), and 30min (P<0.05, $F_{1,11}$=6.134). Area under the curve analysis (Table 3) consistently revealed an effect of treatment in the RHA strain, with a higher AUC_{Gluc}, in the OLZ group relative to the controls (P<0.05, $F_{1,11}$=7.006). Basal circulating glucose levels were affected by treatment (P<0.05, $F_{1,20}$=4.973), with lower glucose levels in the OLZ treated groups compared to controls, irrespective of selection line.

The insulin response during an IVGTT (fig.4D) was neither affected by strain or treatment. In addition, no differences in AUC_{Ins} between groups were observed.

Table 3: Baseline and area under the curves of glucose and insulin responses in the active environment.

	RHA Active		RLA Active	
	Control	*OLZ*	*Control*	*OLZ*
Baseline				
Glucose (mM)	6.35±0.17	5.70±0.37[a]	5.97±0.24	5.61±0.26[a]
Insulin (ng/ml)	0.77±0.10[b]	0.58±0.13[b]	0.81±0.16	1.15±0.16
Area under the Curve				
Glucose	114±19	171±17[c]	117±34	138±19
Insulin	157±21	174±15	201±27	160±17

[a]P<0.05, oneway-ANOVA. Baseline glucose levels show a treatment effect of lower baseline glucose levels in the OLZ treated groups.
[b]P<0.05, oneway-ANOVA. Baseline insulin levels show a strain effect of lower baseline insulin levels in the RHA groups.
[c]P<0.05, oneway-ANOVA. Within strain analyses show increased AUC_{Gluc} in the RHA-Act-OLZ compared to RHA-Act-Con.

Nevertheless, basal insulin levels were affected by strain ($P<0.05$, $F_{1,20}=5.177$), with lower basal insulin levels in the RHA group; post hoc analysis revealed lower basal circulating insulin levels in the RHA-Act-OLZ compared to RLA-Act-OLZ ($P<0.05$, $F_{3,23}=3.127$).

Repeated measures analysis of the IVGTT induced glucose responses including both sedentary and active groups revealed an environment effect ($P<0.001$, $F_{8,360}=4.753$, GLM rm-ANOVA), and an environment*treatment interaction ($P<0.05$, $F_{8,360}=2.410$, GLM rm-ANOVA). These results are explained to indicate that the glucose responses were generally lower in the active rats, but that treatment contributed significantly to this effect. As mentioned already above in the analysis of only the active groups, OLZ increased the IVGTT-induced glucose responses in the RHA rats, which apparently underlied the interaction effect in the combined analysis too. When analyzing the selection lines separately, the effect of environment, with lower glucose responses in the active environment, was only found in the RLA ($P<0.05$, $F_{8,192}=2.886$, GLM rm-ANOVA). In the RHA strain, however, an environment effect ($P<0.05$, $F_{8,168}=2.239$, GLM rm-ANOVA), and an environment*treatment interaction ($P<0.05$, $F_{8,168}=2.216$, GLM rm-ANOVA) on glucose responses was found, which consistently points to OLZ treatment as a limiting factor to lower the glucose response in the active RHA rats. Analysis of the glucose areas under the curve back-up above-mentioned results, again showing the environment effect ($P<0.01$, $F_{1,53}=9.909$), with lower AUC_{Gluc} in the active environment compared to the sedentary environment, and analysis in the RHA strain alone revealed a lower AUC_{Gluc} of RHA-Act-Con compared to RHA-Sed-Con ($P<0.05$, $F_{3,24}=1.690$), but not when comparing the RHA-Act-OLZ with the RHA-Sed-OLZ group. In the RLA, an environment effect was observed ($P<0.05$, $F_{1,28}=6.850$), and additionally a lower AUC_{Gluc} in the RLA-Act-Con compared to the RLA-Sed-Con ($P<0.05$, $F_{3,24}=2.365$). In contrast, no effects or interactions were found between groups in basal circulating glucose levels.

Analysis of the insulin responses of both sedentary and active groups revealed a strain effect ($P<0.001$, $F_{8,360}=3.595$, GLM rm-ANOVA), with lower insulin responses in the RHA compared to RLA irrespective of environment or treatment. Within the RHA strain, no differences between groups were found in the insulin responses, which presumably rendered the RHA strain prone to glucose intolerance in the case of OLZ treatment. In the RLA strain, an environment effect ($P<0.05$, $F_{8,192}=11.015$, GLM rm-ANOVA) was found, with lower insulin responses in the active environment compared to the sedentary environment, essentially tracking the lower glucose responses in the active condition. Between-subjects analysis of the AUC of the insulin response did not reveal a strain, environment, or treatment effect on AUC_{Ins}. Overall basal circulating

insulin levels were affected by strain ($P<0.001$, $F_{1,52}=20.261$), with lower baseline insulin levels in the RHA compared to RLA selection line, and an environment effect ($P<0.01$, $F_{1,52}=11.493$), where lower circulating insulin levels in the active versus sedentary rats were found. Within strain analysis did not reveal any effect of treatment or environment in the RHA. In the RLA strain, however, an environment effect ($P<0.01$, $F_{1,28}=8.439$) was observed, with increased basal circulating insulin levels in the sedentary environment compared to the active environment, which is in line with the repeated measures analysis.

2.5 Body composition (see table 4).

a. Adiposity.

A main effect of strain was found on several parameters related to adiposity, with the RLA in relation to the RHA strain having increased total body adiposity ($P<0.05$, $F_{1,19}=5.732$), subcutaneous adiposity ($P<0.05$, $F_{1,19}=8.140$), and intermuscular adiposity ($P<0.05$, $F_{1,19}=6.628$), but not abdominal adiposity. When analyzing the strains separately, an effect of treatment was observed in the RHA, with parametrial adiposity ($P<0.05$, $F_{1,10}=5.422$) and subcutaneous adiposity ($P<0.05$, $F_{1,10}=5.422$) being increased in the OLZ treated versus control treated rats, and a near significant effect in the same direction of treatment was found on total body adiposity ($P=0.051$, $F_{1,10}=5.051$).

When including sedentary and active groups in one analysis, strain*environment interactions were found on total adiposity ($P<0.05$, $F_{1,54}=6.939$), parametrial adiposity ($P<0.01$, $F_{1,46}=11.934$), retroperitoneal adiposity ($P<0.05$, $F_{1,46}=5.156$), subcutaneous adiposity ($P<0.001$, $F_{1,46}=7.596$), and intermuscular fat content ($P<0.05$, $F_{1,46}=5.097$), that were all increased in the RLA sedentary groups compared to the RLA active versus RLA sedentary rats, and these effects were significantly less strong (hence the interaction) in the RHA strain.

When analyzing the strains separately, a strong effect of environment was observed ($P<0.001$, $F_{1,24}=18.309$), with increased adiposity in the RLA sedentary housed groups compared to the RLA active housed groups. Within the RHA selection line, an effect of environment was found too ($P<0.05$, $F_{1,22}=5.261$), with also higher total body adiposity level in the sedentary condition compared to the active condition. In addition, an effect of treatment was found, with higher total body adiposity in the OLZ treated RHA rats compared to respective controls ($P<0.01$, $F_{1,22}=10.031$).

b. Organ weights.

Within the active environment, liver weights were affected by strain ($P<0.05$, $F_{1,19}=4.942$), with higher liver weights in the RLA compared to the RHA selection line.

Strain also interacted with treatment ($P<0.05$, $F_{1,46}=5.944$), indicating that OLZ treatment contributed significantly to the lower liver weights in the RHA strain. Analyzing all groups of the active and sedentary environment together did not reveal an effect of any significant effects. Analysis of kidney weights of rats in the active environment revealed a treatment effect ($P<0.05$, $F_{1,23}=7.139$), with lower kidney weights in the OLZ treated groups. In the joint analysis of sedentary and active groups, an environment*treatment interaction was found ($P<0.05$, $F_{1,46}=4.833$), with lower kidney weights in the OLZ treated groups and with the active housing condition significantly contributing to this effect. Within the active environment, a strain effect was found on adrenal weights ($P<0.001$, $F_{1,19}=22.403$), with increased adrenal weight in the RHA groups compared to the RLA groups. In addition, a strain effect was observed ($P<0.001$, $F_{1,45}=22.403$), with higher adrenal weight in the RHA, and the strain*treatment interaction ($P<0.05$, $F_{1,45}=5.718$) revealed that the OLZ treatment actually significantly reduced this difference. Brown adipose tissue weight in the active environment was affected by strain ($p<0.01$, $F_{1,45}=9.323$), with increased BAT weight in the active RHA groups compared to the active RLA groups. In the joint analysis of including both environments, a main effect of environment ($P<0.001$, $F_{1,45}=23.994$) was observed, with increased BAT weights in the sedentary housed rats compared to the active housed ones.

Table 4: Body composition within the active environment.

		RHA Active		RLA Active	
		Control	*OLZ*	*Control*	*OLZ*
Parametrial (%)		0.95±0.10	1.39±0.07[a]	1.26±0.15	1.20±0.11
Retroperitoneal (%)		1.79±0.15	2.29±0.12	2.56±0.38	2.13±0.28
Visceral (%)		1.53±0.09	1.75±0.14	1.80±0.07	1.82±0.15
Abdominal depots (%)		4.27±0.30	5.43±0.20	5.62±0.45	5.15±0.43
Subcutaneous (%)		2.60±0.17*[b]	3.74±0.19*[a]	4.61±0.55	4.43±0.58
Intermuscular (%)		1.83±0.10*[b]	2.19±0.08*	2.54±0.23	2.43±0.14
Total Adiposity (%)		8.70±0.51*[b]	11.36±0.38*	12.77±1.22	12.01±1.08
Liver	g	9.85±0.26*	9.26±0.32*	9.77±0.18	10.13±0.54
Kidneys	g	2.25±0.06	2.07±0.049	2.13±0.03	1.93±0.10
Adrenal	g	0.091±0.006*	0.082±0.003*	0.069±0.004	0.069±0.004
BAT	g	0.514±0.041*	0.495±0.024*	0.338±0.024	0.353±0.028

Adiposity: *Strain effect: RHA-Act<RLA-Act; P<0.05, univariate-ANOVA. [a]Within strain RHA-Act-OLZ>RHA-Act-Con; P<0.05, oneway-ANOVA. [b]RHA-Act-Con<RLA-Act-Con/OLZ; P<0.05, oneway-ANOVA post hoc. Liver: *Strain effect: RHA-Act<RLA-Act; P<0.05, univariate-ANOVA. Adrenals: *Strain effect: RHA-Act>-RLA-Act; P<0.001, univariate-ANOVA. Brown adipose tissue (BAT): *Strain effect: RHA-Act>RLA-Act; P<0.01, Uunivariate-ANOVA.

Circulating compounds.

During baseline (day -1) and after sacrifice at day 14, blood samples were taken and circulating leptin, corticosterone, triglycerides, free fatty acids, and prolactin were measured (as illustrated in fig. 5).

a. Circulating leptin levels:

GLM rm-ANOVA revealed main effects of strain ($P<0.01$, $F_{1,46}=10.315$) and environment ($P<0.01$, $F_{1,46}=9.478$) on circulating levels of leptin, irrespective of treatment (fig. 5A/B). Circulating leptin levels were increased in the RLA strain compared to the RHA strain, and lower circulating leptin levels were observed in the active environment compared to the sedentary environment. OLZ did not affect circulating leptin levels at both days measured.

Univariate-analysis of baseline leptin levels revealed a strain effect ($P<0.01$, $F_{1,56}=16.849$) and a strain*environment interaction ($P<0.05$, $F_{1,56}=5.088$), the latter related to increased leptin levels in the sedentary housed RLA rats. Analyzing each strain separately revealed an environment effect in the RHA strain ($P<0.01$, $F_{1,28}=11.232$), which was absent in the RLA selection line. Analyzing each environment separately, only in the sedentary environment a strain effect was observed ($P<0.01$, $F_{1,31}=25.267$), with higher circulating leptin levels in the RLA compared to RHA strain.

A main effect of strain ($P<0.001$, $F_{1,54}=16.017$) and environment ($P<0.05$, $F_{1,54}=5.712$, univariate-ANOVA) on circulating leptin levels at day 14 of treatment were found, revealing increased leptin levels in the RLA compared to the RHA and increased levels of leptin in the sedentary housed groups compared to the active housed groups..

b. Circulating corticosterone levels.

A main effect of treatment ($P<0.05$, $F_{1,47}=5.077$, GLM rm-ANOVA) was found on circulating Cort levels (fig. 5C/D) irrespective of strain and environment, revealing lower circulating Cort levels in the OLZ treated groups compared to the control groups. Additionally, a strain*environment*treatment ($P<0.05$, $F_{1,47}=5.077$, GLM rm-ANOVA) effect was observed related to the increase of circulating Cort levels in the RLA-Sed-Con group. A similar main effect of treatment was found when both selection lines were analyzed separately.

Univariate-analysis of baseline Cort levels revealed an environment effect irrespective of strain ($P<0.001$, $F_{1,56}=13.481$), with increased baseline Cort levels in the active environment. Analyzing each selection line separately, an environment effect was observed only in the RLA strain ($P<0.01$, $F_{1,27}=8.848$), with higher Cort levels in the active environment at baseline. At day 14 of treatment a

strain*environment*treatment interaction ($P<0.01$, $F_{1,54}=4.826$) was observed related to the increased Cort levels specifically in the RLA-Act-Cont group compared to all other groups.

c. Circulating Triglyceride levels.

No main effects of strain, environment, or treatment were observed over the duration of the study on circulating TGC levels (fig. 5E/F) determined by GLM rm-ANOVA.

Univariate-analysis at baseline, however, revealed an environment effect ($P<0.01$, $F_{1,56}=18.609$), with lower TGC levels at baseline in the active compared to sedentary environment, irrespective of strain.

d. Circulating free fatty acid levels.

Over the duration of treatment no main effects of strain, environment, or treatment were observed on circulating FFA levels (fig. 5G/H) determined by GLM rm-ANOVA.

e. Circulating prolactin levels.

GLM rm-ANOVA analysis revealed a main effect of environment ($P<0.01$, $F_{1,47}=5.371$) and treatment ($P<0.01$, $F_{1,47}=5.350$) on circulating prolactin levels (fig. 5I/J). Analyzing each strain separately revealed a treatment effect in the RHA ($P<0.01$, $F_{2,46}=8.501$), with increased prolactin levels in the OLZ treated RHA rats irrespective of environment, whereas an environment effect was observed in the RLA ($P<0.01$, $F_{2,48}=5.252$), with higher prolactin levels in the sedentary environment.

Univariate-analysis at baseline revealed a main effect of strain ($P<0.05$, $F_{1,56}=5.023$), with lower circulating prolactin levels in the RHA group. Analyzing each strain separately did not reveal a main effect of environment within each strain. Analysis within each environment revealed a strain effect in the active environment ($P<0.05$, $F_{1,56}=7.728$), with lower prolactin levels in the active RHA group, which was absent in the sedentary environment. Within strain analysis also revealed a treatment effect at day 14, with higher prolactin levels in the OLZ treated RHA groups ($P<0.01$, $F_{1,26}=10.035$) irrespective of environment. In contrast, in the RLA strain, an environment effect ($P<0.01$, $F_{1,27}=9.502$) was observed, with higher levels of prolactin in the sedentary environment compared to the active environment, irrespective treatment.

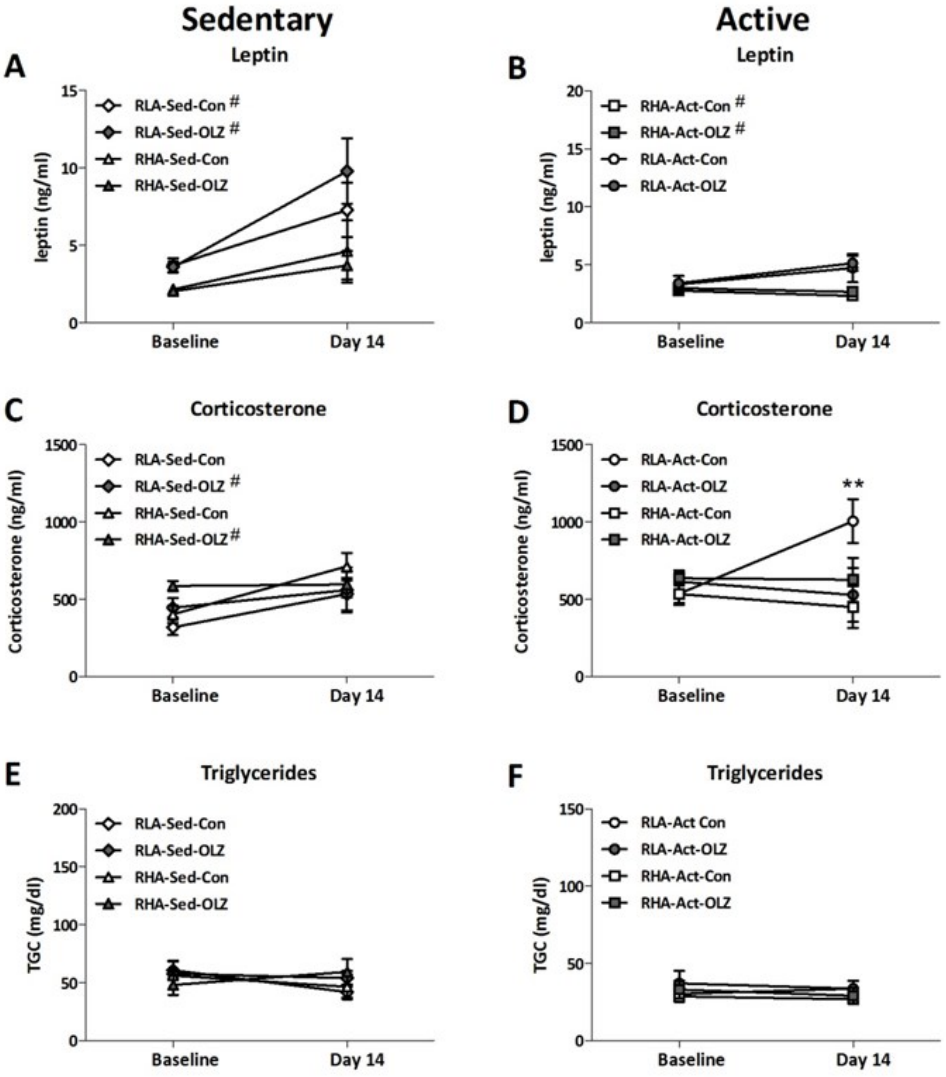
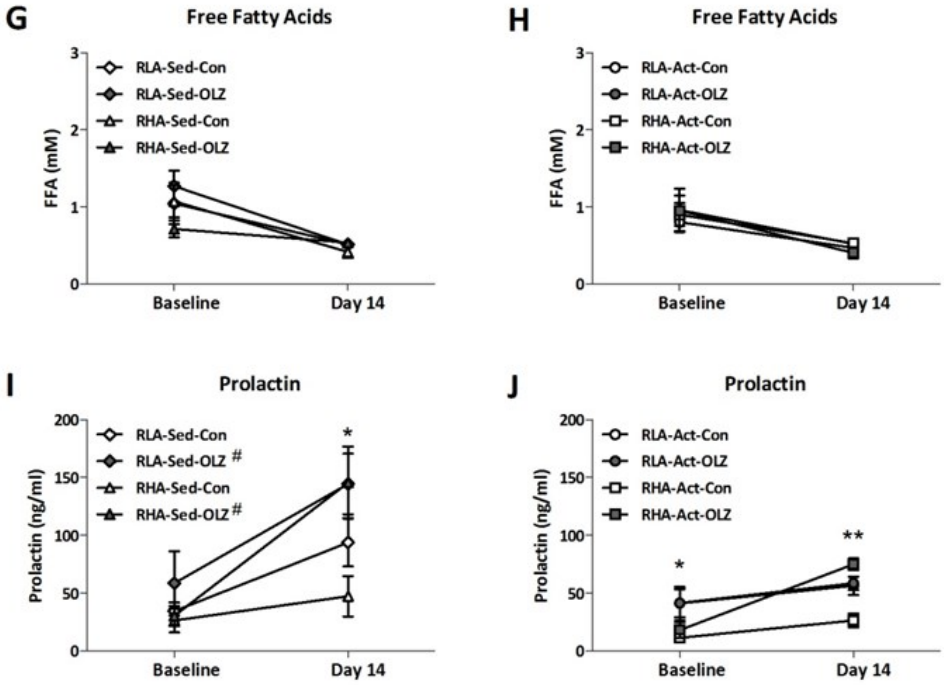


Fig. 5: Circulating compounds at baseline and day 14 of treatment. A,B) Analysis of leptin levels revealed a strain effect, with increased leptin levels during treatment in the RLA ($^{\#}P<0.01$, GLM rm-ANOVA). In addition, an environment effect was observed, with increased leptin levels during treatment in the sedentary environment ($P<0.01$, GLM rm-ANOVA), independent of strain. C,D) A main effect of treatment on circulating Cort levels was observed,lower Cort levels in the OLZ treated groups ($P<0.05$, GLM rm-ANOVA). In the RLA, an environment effect of lower Cort levels at baseline in the sedentary environment was found ($^{\#}P<0.01$, Univariate-ANOVA). At day 14 of treatment, a strain*environment* treatment interaction was observed indicative of increased Cort levels in the active control treated RLA (**$P<0.01$, Univariate-ANOVA). E,F) No main effects of strain, environment, or treatment on circulating TGC levels were observed during treatment. Baseline TGC levels were lower in the active environment compared to the sedentary environment ($P<0.01$, Univariate-ANOVA), independent of strain.



G,H) A decrease of FFA levels was observed in all groups from baseline to day 14, without any effects of strain, environment, or treatment I,J) Main effects were observed of environment, with decreased prolactin levels in the active environment ($P<0.01$), and of treatment ($P<0.01$), with increased prolactin levels in the OLZ groups . Analysis of each strain separately revealed an environment effect in the RLA ($P<0.01$), and a treatment effect in the RHA ($P<0.01$, GLM rm-ANOVA). Baseline prolactin levels were lower in the RHA compared to RLA ($P<0.05$, Univariate-ANOVA). Also within the active environment prolactin levels were lower in the RHA compared to the RLA (*$P<0.01$). At day 14 a treatment effect was observed in the RHA, with increased prolactin levels in the OLZ treated groups (**$P<0.01$, Univariate-ANOVA). Within the sedentary groups a treatment effect was observed, with increased prolactin levels in the OLZ treated groups (*$P<0.05$, Univariate-ANOVA).

Central mRNA expression

Expression levels of mRNA levels in *Ventral Tegmental Area*, *Nucleus Accumbens*, and *Prefrontal Cortex* are indicated as relative expressions of mRNA compared to the RHA -sedentary housed control group (RHA-Sed-Con); for details see table 5. Using a univariate analysis, main effects are discussed first followed by univariate analysis within each strain and/or environment. It was chosen not to analyse each treatment group separately, because the comparison between OLZ vs control treatment was considered to be the main interest of the study (i.e., and thus were not separated).

Ventral Tegmental Area.:

Dopamine receptor 1.

A strong main effect of treatment was found ($P<0.001$, $F_{1,55}=41.380$), with decreased VTA Drd1 expression levels by OLZ relative to control treatment. When analyzing effects within each separate strain or environment, the effect of treatment remained, indicating the robustness of OLZ-induced reductions of VTA Drd1 mRNA expression irrespective of strain or environment.

Dopamine receptor 2.

A main effect of treatment was found ($P<0.001$, $F_{1,55}=9.455$), with increased VTA Drd2 mRNA expression due to OLZ. In addition, a main effect of environment was found ($P<0.001$, $F_{1,55}=9.872$), with higher VTA Drd2 mRNA expression levels in the active environment compared to the sedentary environment. When analyzing each strain separately, a treatment effect was found only in the RHA rats ($P<0.01$, $F_{1,25}=7.853$), demonstrating that the RHA line contributed most to the effect of OLZ to reduce VTA Drd2 mRNA expression (i.e., in the RLA line treatment almost reached significance). Separate analysis of environment did not reveal effects of treatment or strain.

Serotonin receptor $5HT_{1A}$.

A main effect of treatment was found ($P<0.001$, $F_{1,55}=12.738$,), with decreased VTA $5HT_{1A}$ mRNA expression by OLZ treatment. When analyzing each strain separately, comparable treatment effects were observed in the two strains (RHA: $P<0.05$, $F_{1,23}=5.105$; RLA ($P<0.05$, $F_{1,25}=7.804$). In the RHA strain, however, treatment interacted with environment too ($P<0.05$, $F_{1,23}=5.580$), which is explained by the fact that the active environment contributed strongest to OLZ-induced reductions in VTA $5HT_{1A}$ mRNA expression the RHA rats. This was confirmed when each environment was analyzed separately.

Tyrosine Hydroxylase.

A main effect of environment was found ($P<0.05$, $F_{1,55}=5.893$), with lower VTA TH mRNA expression levels in the active environment compared to the sedentary environment. When analyzing each environment separately this revealed a treatment effect only in the active environment ($P<0.05$, $F_{1,21}=5.365$), with lower VTA TH mRNA expression levels by OLZ relative to control. Analyzing each strain separately did not reveal significant outcomes.

Dopamine Active Transporter.

A strong main effect of treatment was found ($P<0.001$, $F_{1,55}=27.061$), with lower VTA DAT mRNA expression levels observed in the OLZ-treated rats relative to control-treated rats, which remained only in the RLA rats ($P<0.01$, $F_{1,25}=52.843$) when analyzing each strain separately. A more subtle main effect of environment was found ($P<0.05$, $F_{1,55}=4.937$), with lower VTA DAT mRNA expression levels in the active versus sedentary rats, which only remained in the RHA rats ($P<0.05$, $F_{1,23}=4.719$) when analyzing each strain separately. The above-mentioned effect of treatment remained when analyzing active ($P<0.001$, $F_{1,21}=26.073$) and sedentary ($P<0.001$, $F_{1,25}=39.221$) environments separately, indicating that OLZ lowered VTA DAT mRNA expression levels irrespective of environment. Collectively, these data suggest that RHA rats respond more to environment and RLA rats more to OLZ to lower VTA DAT mRNA expression levels.

Summary of VTA expression levels.

OLZ caused profound reductions in Drd1, DAT, and $5HT_{1A}$ mRNA expression levels and increased Drd2 mRNA expression levels. Running wheel availability did not affect Drd1 and $5HT_{1A}$ expression levels but increased Drd2, and lowered TH, and DAT expression levels. Strain was not a main factor in altering expression levels. However, when analyzing the strains separately the RHA rats responded somewhat stronger to OLZ (either or not in relation to environment) than the RLA rats with alterations in Drd2 and $5HT_{1A}$ expression levels. RLA responded stronger to OLZ than RHA rats with alterations in DAT.

Nucleus Accumbens:

Dopamine receptor 1.

A strong main effect of environment was found ($P<0.001$, $F_{1,55}=14.034$), as well as interactions between environment, treatment and strain ($P<0.001$, $F_{1,55}=14.962$) on NAc Drd 1 mRNA expression levels. The interactions, which in fact improved the model slightly, are explained by the fact that generally higher expression levels of NAc Drd 1 mRNA were found in the active rats than in the sedentary rats, but with

control-treated rats contributing mainly to this effect in the RHA strain and OLZ-treated rats mainly contributing to this effect in the RLA strain. These interactions were confirmed by analyzing each environment and strain separately. Also a subtle main effect of strain alone was found ($P<0.05$, $F_{1,55}=6.831$), with slightly higher expression levels in RHA than RLA rats.

Dopamine receptor 2.

A main effect of strain was found ($P<0.01$, $F_{1,54}=9.215$, Univariate-ANOVA), with higher NAc Drd2 mRNA expression levels in the RHA rats compared to RLA rats. Strain also interacted with environment and treatment ($P<0.01$, $F_{1,54}=8.803$, Univariate ANOVA), but the inclusion of these interactions did not improve significance of the model. When analyzing the strains separately, an effect of environment was found ($P<0.05$, $F_{1,25}=5.495$) in the RLA rats, with lower NAc Drd2 mRNA expression in the active ones than in the sedentary ones. In the RHA rats, however, environment interacted with treatment ($P<0.01$, $F_{1,20}=8.170$), with higher NAc Drd2 mRNA expression levels found in the active ones, with the control-treated rats contributing to this effect. Conversely, when analyzing each environment separately, an effect of strain ($P<0.01$, $F_{1,20}=10.471$) was found in the active environment, with lower expression levels in the RLA rats compared to the RHA rats. In the sedentary environment, an interaction effect between strain and treatment ($P<0.05$, $F_{1,27}=4.679$) was observed, with increased NAc Drd2 mRNA expression in the RHA rats treated with OLZ.

Summary of nucleus accumbens expression levels.

Environment (Drd1) and strain (Drd1, Drd2) were important factors in the NAcc to alter mRNA expression levels (opposite to the VTA where treatment was a strong main factor to alter Drd1 and Drd2 mRNA expression levels). Physical activity increased Drd1 mRNA expression particularly in the control-treated RHA rats, which mostly underlied the main effect of strain. Also Drd2 mRNA expression levels were clearly higher in RHA rats than RLA rats, with subtle effects of treatment and environment as interacting factors.

Prefrontal Cortex.:

Dopamine receptor 1.

A main effect of strain was found ($P<0.001$, $F_{1,55}=19.738$), with higher PFC Drd1 mRNA expression levels in the RHA rats compared to RLA rats. In addition, a main effect of treatment was found ($P<0.05$, $F_{1,46}=4.217$), which improved considerably in interaction with strain ($P<0.01$, $F_{1,46}=8.865$). The latter was due to the fact that OLZ

caused reductions in PFC Drd1 mRNA expression levels compared to control treatment in the RLA, but not RHA rats. This was confirmed when analyzing the RLA strain separately ($F_{1,25}$=5.106, P<0.01), indeed with reduced PFC Drd1 mRNA expression levels by OLZ treatment. In the RHA strain, however, an effect of environment was found (P<0.05, $F_{1,23}$=7.256), with increased PFC Drd1 mRNA expression levels in the active environment compared to the sedentary environment. These results were largely confirmed when analyzing the environments separately.

Dopamine receptor 2.

A main effect of treatment was effect was found (P<0.05, $F_{1,55}$=4.252), with lower PFC Drd2 mRNA expression levels by OLZ treatment. No effects were observed when analyzing the results separately within each strain or environment.

Serotonin receptor 5HT$_{1A}$.

A main effect of strain was found (P<0.01, $F_{1,46}$=8.022), with higher PFC 5HT$_{1A}$ mRNA expression levels in the RHA rats than RLA rats. In addition, an interaction effect between environment and treatment was found (P<0.05, $F_{1,46}$=6.081), with expression levels being reduced in the OLZ treated rats compared to the levels found in controls in sedentary but not active ones. This effect was confirmed in the RHA (P<0.05, $F_{1,23}$=6.873), but not in RLA rats when analyzing the strains separately. When analyzing each environment separately, expression levels of rats in the active environment were affected by strain (P<0.01, $F_{1,21}$=9.245), with generally higher levels found in the RHA rats than in the RLA rats, confirming the mian effect. In the sedentary environment, however, an effect of treatment was found (P<0.05, $F_{1,27}$=7.368), with OLZ reducing expression levels compared to levels observed in the controls-treated rats, largely confirming the interaction.

Serotonin receptor 5HT$_{2A}$.

A strong main effect of strain was found (P<0.001, $F_{1,46}$=23.624), with higher PFC 5HT$_{2A}$ mRNA expression levels in the RHA strain compared to the RLA strain. In addition, a main effect of treatment was observed ($F_{1,46}$=7.343, P<0.01), with lowered expression levels by OLZ. When analyzing the strains separately, an effect of treatment was observed with expression levels again being reduced by OLZ (P<0.05, $F_{1,23}$=4.772), but this effect was only observed in the RHA selection line. When analyzing the environments separately, in the active environment an effect of strain was observed (P<0.001, $F_{1,21}$=14.250), with higher expression levels found in the RHA rats compared to the RLA rats. In the sedentary environment, also a strain effect was observed (P<0.01, $F_{1,27}$=8.093), again with higher expression level in the RHA rats than in the RLA rats. In addition, a treatment effect was observed in the sedentary

rats ($P<0.01$, $F_{1,27}=10.839$), with expression levels being reduced by OLZ treatment. The data collectively indicate that the sedentary RHA rats mostly contributed to the main effect of OLZ to lower PFC $5HT_{2A}$ mRNA expression

Serotonin receptor $5HT_{2C}$.

A main effect of strain was observed ($P<0.01$, $F_{1,46}=13.693$), with higher PFC $5HT_{2C}$ mRNA expression levels in the RHA rats than in the RLA rats. In addition, an interaction effect between strain, environment, and treatment was observed ($P<0.05$, $F_{1,46}=5.222$, Univariate-ANOVA), but this interaction was weaker and did not improve the significance of the main effect. When each selection line was analyzed separately, however, no effects of environment nor treatment was observed, which indeed confirms the fact that differences between strains are most striking. When analyzing the environments separately, again a strain effect was observed in the active environment ($P<0.05$, $F_{1,21}=6.494$), with lower PFC $5HT_{2C}$ mRNA expression levels in the RLA rats compared to the RHA rats. In the sedentary environment, a strain effect was found too, alone ($P<0.001$, $F_{1,27}=6.936$), as well as in interaction with treatment ($P<0.01$, $F_{1,27}=11.639$), with generally lower expression levels observed in the RLA rats than in the RHA rats, but with the control-treatment most contributing to this effect.

Summary of Prefrontal Cortex expression levels.

Strain was the most important factor here with higher expression levels of Drd1, $5HT_{1A}$, $5HT_{2a}$, and $5HT_{2c}$ in RHA rats than in RLA rats. Treatment was also an important factor, mostly in interaction with strain, in which OLZ reduced expression levels of Drd1 (mostly in RLA), Drd2 (irrespective of strain), $5HT_{1A}$ (in sedentary RHA), and $5HT_{2a}$ (mostly RHA).

Conclusions and discussion

The main outcome of this study is the divergence in susceptibility to OLZ-induced weight gain between the RHA and RLA lines. The RHA line was susceptible to OLZ-induced weight gain, while the RLA line did not accumulate extra weight in response to OLZ treatment. It confirms our hypothesis that the RHA rat, i.e. the selection line that may serve as an antecedent model for psychopathologic disorders [27,28], is also more susceptible to OLZ-induced weight gain. The model also has face validity with the human data of Kinon *et al* (2005), who showed increased clinical efficacy of OLZ treatment in subjects developing severe body weight gain [9].

The increased susceptibility to OLZ-induced weight gain in the RHA strain is in line with our initial hypothesis that the active environment, which reduced weight gain in

the RLA, strongly inflated weight gain in the OLZ treated RHA selection line. This divergence was based on the expectation that OLZ treated rats would run less than the control treated ones. The fact that the latter did not happen in the RLA could be due to 1) a different orchestration of behavior and metabolism in the RLA strain, and/or 2) a difference in drug responsiveness in the RLA strain compared to the RHA strain. In line with the findings of Boersma et al (2012 [22]) is our finding that the RLA strain maintained a stable body weight in the presence of a running wheel, and this did not change when the RLA strain was treated with OLZ. Our study additionally demonstrated that even a small amount of running wheel activity, which was maintained while being sedated by OLZ treatment, was sufficient in the RLA strain to improve glucose intolerance and/or insulin resistance, compared to sedentary living RLA rats. However, due to the fact that OLZ did not affect caloric intake in the sedentary RLA it is impossible to ascribe the reduced food intake in the active RLA as a direct consequence of OLZ treatment. Above all, it shows that the RLA strain is not able to control body weight regulation under influence of a palatable diet in the absence of physical activity. In contrast to running wheel activity, home cage activity was higher in the control treated sedentary housed RHA rats compared to RLA, which underscores the activity-related behavioral differences between both selection lines. We also found that home cage activity was strongly correlated with body temperature in both strains. The relatively weak OLZ-induced hypothermic response observed during the first half of the dark phase in the RHA was in line with the relatively low home cage activity and body temperature of the control RHA during that same period. Accordingly increased home cage activity during the second half of the dark phase was reflected by increased body temperature in the control RHA. Although speculative, the seemingly enhanced hypothermic response to OLZ in the RLA is a consequence of the difference in home cage activity patterns between the selection lines instead of an increased susceptibility to OLZ-induced hypothermia in the RLA. This is supported by the comparable hypothermic responses in both lines during the second half of the dark phase.

Associated with the augmented weight gain in the RHA strain due to OLZ treatment - which appeared to be enhanced by the active environment - is the finding that these RHA rats also become relatively glucose intolerant. These findings are surprising when compared to previous findings from our group showing that the RHA selection line is obesity resistant and maintains glucose tolerance independent of running wheel activity [18,22]. This finding is however important in relation to the observed hyperglycemia in OLZ treated patients [29].

The underlying mechanisms for the increased susceptibility to OLZ-induced weight

gain in the RHA strain may, at least in part, be explained by the changes in circulating hormones and central mRNA expression. First, we found that RHA rats have decreased levels of circulating prolactin, specifically in the active environment. However, the RHA rats also have the highest OLZ-induced increase in prolactin at day 14 of treatment. Prolactin is secreted from the anterior pituitary gland and its secretion is inhibited by dopamine [30]. One may argue that the lower basal prolactin levels can be considered as a sign of increased dopaminergic activity [31]. Antagonism of Drd2 receptors by OLZ at the level of the anterior-pituitary may then explain the observed elevated increase of prolactin. Furthermore, prolactin increases

Table 5: Central mRNA expression in the Ventral Tegmental Area (VTA), Nucleus Accumbens (NAc), and Prefrontal Cortex (PFC).

		Roman High Avoidance				Roman Low Avoidance			
		Sedentary		Active		Sedentary		Active	
Region	*mRNA*	*Control*	*OLZ*	*Control*	*OLZ*	*Control*	*OLZ*	*Control*	*OLZ*
VTA	*Drd1*	1.00±0.17	0.36±0.15^{T}	0.73±0.26	0.07±0.01^{T}	1.10±0.20	0.40±0.18^{T}	1.01±0.23	0.07±0.01^{T}
	Drd2	1.00±0.18	2.11±0.32^{T}	2.56±1.26^{E}	3.44±0.40^{TE}	1.02±0.16	2.28±0.52^{T}	2.01±0.97^{E}	4.64±0.90^{TE}
	5HT$_{1A}$	1.00±0.55	0.75±0.49	3.88±1.44	0.16±0.01^{t}	3.00±1.24	0.86±0.43	2.62±0.94	0.17±0.01^{t}
	TH	1.00±0.36	0.83±0.49	0.23±0.06^{E}	0.11±0.01^{E}	0.66±0.32	0.12±0.02	0.17±0.05^{E}	0.12±0.01^{E}
	DAT	1.00±0.22	0.51±0.20^{T}	0.70±0.24^{E}	0.10±0.01^{ET}	0.97±0.14	0.40±0.19^{T}	0.97±0.20^{E}	0.12±0.02^{ET}
NAc	*Drd1*	1.00±0.24	1.68±0.09^{t}	2.51±0.15^{e}	1.63±0.26^{e*t}	1.24±0.10	1.24±0.05	1.26±0.30	1.72±0.23
	Drd2	1.00±0.23	1.49±0.05^{e*t}	1.70±0.27	1.21±0.10^{e*t}	1.20±0.12	1.15±0.05	0.78±0.14^{e}	0.99±0.18^{e}
PFC	*Drd1*	1.00±0.33	0.21±0.07^{T}	2.08±0.73^{e}	1.57±0.51^{Te}	0.38±0.13^{S}	0.23±0.07ST	0.30±0.14^{S}	0.10±0.05ST
	Drd2	1.00±0.10	0.83±0.14	1.22±0.29	1.16±0.24	0.81±0.11	0.94±0.30	1.53±0.40	0.57±0.16^{t}
	5HT1A	1.00±0.13	0.31±0.10^{e*t}	0.65±0.36	1.20±0.31	0.64±0.35^{S}	0.17±0.07^{S}	0.28±0.09^{S}	0.21±0.09^{S}
	5HT2A	1.00±0.15	0.39±0.06^{t}	0.97±0.25	0.78±0.22^{t}	0.45±0.11^{S}	0.40±0.11^{S}	0.32±0.04^{S}	0.21±0.03^{S}
	5HT2C	1.00±0.18st	0.34±0.03	0.75±0.24	0.72±0.24	0.27±0.04^{S}	0.42±0.12^{S}	0.37±0.04^{S}	0.25±0.06^{S}

Main effects: Ventral tegmental area (VTA); *Drd1:* ^{T}Treatment effect: OLZ reduces VTA Drd1 expression in both strains; P<0.001, Univariate-ANOVA. ***Drd2:*** ^{T}Treatment effect: OLZ increases VTA Drd2 expression in both strains, P<0.001, Univariate-ANOVA. ^{E}Environment effect: VTA Drd2 expression is increased in the active environment; P<0.001, Univariate-ANOVA. ***5HT$_{1A}$:*** ^{t}Treatment effect within the active environment: OLZ reduces VTA 5HT$_{1A}$ expression; P<0.001, Univariate-ANOVA. ***TH:*** ^{E}Environment effect: TH expression is lower in the active environment; P<0.001, univariate-ANOVA. ***DAT:*** ^{E}Environment effect: VTA DAT expression is lower in the active environment; P<0.05, univariate-ANOVA. ^{T}Treatment effect: OLZ reduces VTA DAT expression; P<0.001, Univariate-ANOVA. **Nucleus Accumbens (NAc); *Drd1:*** Within the RHA: ^{e}Environment effect: NAc Drd1 expression is increased in the active environment; P<0.01, univariate-ANOVA. ^{t}Treatment effect: OLZ increases NAc Drd1 expression in the sedentary group; P<0.05, oneway-ANOVA post hoc). ^{e*t}Enivronment*treatment interaction: OLZ reduces NAc Drd1 expression in the active environment; P<0.01, univariate-ANOVA. ***Drd2:*** ^{e}Environment effect within the RLA: NAc Drd2 expression is decreased in the active environment; P<0.05, univariate-ANOVA). ^{e*t}Environment*treatment interaction within the RHA: OLZ increases Nac Drd2 expression in the sedentary environment, but increases Drd2 expression in the active environment; P<0.01, univariate-ANOVA). **Prefrontal Cortex (PFC); *Drd1:*** ^{S}Strain effect: RHA show higher PFC Drd1 expression; P<0.001, univariate-ANOVA. ^{T}Treatment effect: OLZ decreases PFC Drd1 expression; P<0.05, univariate-ANOVA. ^{e}Environment effect within RHA: PFC Drd1 expression is higher in the active environement; P<0.05, univariate-ANOVA). ***Drd2:*** ^{t}Treatment effect: OLZ reduces PFC Drd2 expression in RLA-Act-OLZ compared to RLA-Act-Con; P<0.05, oneway-ANOVA post hoc. ***5HT$_{1A}$:*** ^{S}Strain effect: PFC 5HT$_{1A}$ is higher in the RHA compared to RLA; P<0.01, univariate-ANOVA. ^{e*t}Environment*treatment interaction in the RHA: RHA-Sed-OLZ shows reduced PFC 5HT$_{1A}$ expression; P<0.05, univariate-ANOVA. ***5HT$_{2A}$:*** ^{S}Strain effect: PFC 5HT$_{2A}$ is lower in the RLA strain; P<0.001, univariate-ANOVA. ^{t}Treatment effet in RHA: OLZ reduces PFC 5HT$_{2A}$ expression; P<0.01, univariate-ANOVA. ***5HT$_{2C}$:*** ^{S}Strain effect: PFC 5HT$_{2C}$ is lower in the RLA strain; P<0.01, univariate-ANOVA. stStrain*treatment interaction in the sedentary environment shows higher PFC 5HT$_{2C}$ expression in RHA-Sed-Con compared to sedentary groups; P<0.01, univariate ANOVA. *All data is expressed as relative expression to RHA-Sed-Con (avg±sem).*

food intake in female rats [32,33]. The observed lower prolactin levels in the RHA confirms the work of Steimer *et al* (1997), who found blunted prolactin levels in the RHA selection line after an open field test and a correlation between low prolactin levels and increased locomotion in the open field [11]. Likewise, we also observed increased home cage locomotor activity in the RHA line combined with decreased prolactin levels in the active environment. This may be a sign of enhanced dopaminergic activity [31] in the RHAs, which may have contributed to the more pronounced effect on weight gain observed in the OLZ-treated RHAs in the active environment.

Interestingly, Segal and co-workers found in two separate studies (2004, 2008) differences in prolactin serum levels among different subtypes of schizophrenia patients [34,35]. They observed the lowest prolactin levels in the paranoid type (a positive symptom of schizophrenia), intermediate for the schizoaffective and the highest for the disorganized type patients. Segal *et al* suggested that low serum prolactin levels were a consequence of hyperdopaminergic activity, most prominent in the paranoid type schizophrenia [34]. The studies performed by Segal and colleagues revealed that within the patient population a variation exists in dopaminergic functioning, which possibly is at the basis of the individual's disease phenotype. As an animal model, the RHA selection line's behavioral phenotype is associated with increased dopaminergic activity, such as impulsivity, vulnerability to drugs of abuse, and amphetamine-induced stereotypic behavior [28,36,37]. In addition, low circulating prolactin levels may well be another physiological trait of the RHA, which is also related to the positive symptoms of schizophrenia.

Central mRNA expression analysis revealed higher levels of Drd1, $5HT_{1A}$, $5HT_{2a}$, and $5HT_{2c}$ mRNA expression –suggestive of increased receptor density- in the PFC of the RHA selection line compared to the RLA. These findings are interesting, because especially increased Drd1 expression in the PFC has also been observed in drug-naïve schizophrenic patients [21]. Moreover, Abi-Dargham *et al* (2002) found a correlation between increased Drd1 receptor expression in the PFC and working memory deficits in the schizophrenic subjects. Likewise, the RHA selection line also performs less in working memory tasks compared to the RLA, which according to Willig and colleagues (1991) is related to increased levels of dopamine in the PFC of the RHA [38].

Except for PFC Drd2 mRNA expression, OLZ treatment reduced PFC receptor mRNA expression in the sedentary RHA. Although speculative, the down-regulation of Drd1 in the PFC by OLZ might be causal to the observation that OLZ improves performance in learning tasks in schizophrenic patients [39]. In contrast, running wheel activity

increased PFC Drd1 mRNA expression only in the RHA and the active environment protected the RHA for the receptor mRNA decreasing effects of OLZ. The increase of Drd1 mRNA expression in the PFC and NAc in the active environment might be comparable to the findings of Fernández-Teruel and colleagues (1997), who demonstrated that environmental enrichment increased relative ethanol intake and amphetamine-induced stereotypic behavior in the RHA, which may be related to altered sensitivity of the mesolimbic dopaminergic system [40]. In addition, Kellendonk *et al* (2006) observed decreased working memory performance in transgenic mice over-expressing Drd2 specifically in the striatum. They demonstrated that Drd1 density in the PFC was also increased in the transgenic Drd2 over-expressing mice, which according to the authors was related to the deficit in working memory [41]. This data corresponds to our observation of somewhat increased NAc Drd2 mRNA expression and increased PFC Drd1 mRNA in the active RHA.

VTA mRNA expression levels were not different between the two selection lines. In both lines OLZ reduced VTA Drd1, TH, and DAT mRNA expression, whereas an increase of Drd2 mRNA expression was observed. In the active environment, OLZ additionally reduced $5HT_{1A}$ mRNA expression. The increase of VTA Drd2 mRNA and reduction of DAT mRNA expression may be considered as an adaptive response to OLZ-induced increase in extracellular dopamine concentrations [42]. Both environment (Drd1) and line (Drd1, Drd2) were important factors to increase mRNA expressions levels in the NAc of the RHA, whereas the RLA seemed to be resistant to changes in NAc Drd1 and Drd2 mRNA expression levels due to treatment or environment. The latter is in contrast to the effects observed in the VTA in which OLZ treatment was effective in altering mRNA expression levels in both lines.

The divergence of OLZ's effects on different brain areas was also examined by Xi-Ming *et al* (1998), who previously demonstrated that a subcutaneous (s.c.) injection of OLZ (3 and 10mg/kg) induced an increase of dopamine and norepinephrine (NE) in the PFC and the NAc [43]. However, they found that the OLZ-induced increase of dopamine release was relatively larger in the PFC compared to the subcortical areas. Li *et al* (1998) mentioned in detail that especially this divergence of dopamine release in the PFC compared to subcortical regions is the reason why OLZ is effective in reducing the negative symptoms and antagonizes the positive symptoms of schizophrenia [43]. Because this divergence at the level of receptor expression is higher in the RHAs compared to the RLA, it may be argued that OLZ is more effective in the RHA. As mentioned above, Kinon *et al* (2005) reported that OLZ-induced severe body weight gain was associated with an improvement of especially the positive symptoms of schizophrenia [9]. In this line of thought body weight gain susceptibility

may serve as a marker for drug responsiveness as well as central receptor expression.

From the opposite perspective, lower NAc Drd1 receptor expression is related to increased susceptibility to diet induced obesity (DIO). In this sense it is remarkable that especially the RLA increased running wheel activity, which protected against DIO, but lacked changes in NAc and PFC Drd1 mRNA expression compared to RHA in the active environment. Alsiö *et al* (2010) have previously reported lower NAc Drd1 mRNA expression in obesity prone (OP) compared to obesity resistant (OR) Sprague-Dawley rats on restricted access to a high fat/high sugar palatable diet. Because in their study caloric intake was matched to regular chow intake they concluded that not the calories, but the intake of fat and sugar affected NAc Drd1 expression and this was related to increased palatable food craving, especially in the OP [44]. This possibly relates to the results we found in the active environment, in which palatable diet was *ad lib* available, but due to running wheel activity caloric intake was still regulated and animals did not overeat. Similar to Alsiö *et al* (2010), we also found increased NAc Drd1 mRNA expression specifically in the diet resistant active RHA control compared to active RLA control. Furthermore, Geiger *et al* (2009) also demonstrated a link between dietary obesity and deficits in dopaminergic neurotransmission, concluding that obese animals increased eating palatable food to compensate for depressed dopamine release [45]. The latter especially relates to the increased palatable diet intake in the RLA compared to the RHA at day -1 prior to drug treatment, which has also been observed by Boersma *et al* [46]. Based on Alsiö and co-workers (2010) who demonstrated that especially the dietary composition influenced dopaminergic neurotransmission, it may be possible, although speculative, that OLZ has an increased lipogenic effect in combination with a high fat/high sugar diet in individuals normally resistant to diet induced obesity, like we observed in the RHA line.

Altogether, the two selection lines markedly differ in the central mRNA expression levels in the PFC. The higher Drd1 mRNA expression levels observed in the PFC of the RHA line confirms the similarity between the RHA and the schizophrenic patient population. The increased PFC Drd1 mRNA levels also seem to suggest that running wheel activity may have a negative effect on working memory performance in the RHA. As mentioned, control RLA animals displayed higher running wheel activity compared to RHA animals, whereas RHA control animals showed higher home cage activity levels in the sedentary environment compared to control RLA rats. The higher home cage activity in the RHA is possibly related to increased impulsive behavior [28]. The differences in activity-behavior between both selection line may well be a consequence of underlying differences in dopaminergic activity [12,15].

To the best of our knowledge this is the first study reporting the effects of OLZ in an animal model selected on the basis of different coping styles. The Roman selection line was initially developed as a model to study reactive (RLA) and proactive (RHA) coping styles. More recent studies suggested that the RLA strain may also serve as a model for diet induced obesity (DIO) and accompanied insulin resistance [46], and that the RHA strain may be used as model to study compulsivity, impulsivity [28,47], addiction [48,49], and resistance to DIO and insulin resistance [46]. Because impulsivity and proneness to addiction are also related to schizophrenia, the RHA strain has been suggested to be a fit model to study the underlying mechanisms of psychogenetic disorders, like schizophrenia [28]. Due to the fact that the RLA strain is more prone to DIO and the RHA is comparable to the patient population, we concluded that the Roman lines should be an interesting model to study OLZ-induced weight gain and associated metabolic consequences. Furthermore, the Roman strain has been reported to be highly similar to the rat model predominantly linked to schizophrenia; the APO-SUS and APO-UNSUS [1,50]. The APO-SUS (apomorphine-susceptible) and APO-UNSUS (apomorphine-unsusceptible) is also a Wistar based inbred strain and its selection is based on increased apomorphine induced stereotypic gnawing behavior. The APO-SUS is, like the RHA strain marked by a relatively high two-way active avoidance performance [50,51], and shows lower baseline ACTH levels compared to its counterpart [52,53]. Based on its phenotype of high compulsivity, drug addictiveness, and impulsivity -similar to the RHA- the APO-SUS is categorized as a model for schizophrenia. In addition, the APO-UNSUS line shows to be less sensitive to dopaminergic drugs [50,51], and therefore shows similarities to the RLA strain . However, there are also differences in which the RLA rats better resemble the APO-SUS, e.g. compared to their counterparts they both show an increased corticosterone response upon exposure to a novel environment [50,52,53]. This illustrates that the basis of selection, active avoidance or apomorphine susceptibility, does not select for the same genetic background and results in slightly different behavioral and physiological output.

Furthermore, Piras *et al* (2010) showed that selective serotonin reuptake inhibitors (SSRIs) are anxiolytic in the RLA line and have no or little effect in the RHA [54]. Contrary to our study, the susceptibility to SSRI treatment shows an opposite effect compared to antipsychotic treatment in the Roman High/Low Avoidance strain. Nonetheless, OLZ is not only a dopamine receptor antagonist, but also affects the serotonin receptors 5-$HT_{1A,2A/C,3}$. We found that within the RLA 5-HT mRNA expression levels in the PFC were lower compared to the RHA, but this does not have to be the case in other brain areas. Future studies might want to focus on 5-HT receptor expression dynamics in brain areas such as the amygdala, involved in

depression-like behaviors, and possibly find opposing effects of OLZ in other brain areas than the NAc and PFC, when RLA and RHA are compared. Such studies might have an additional value to study the effectiveness of OLZ in treating the negative compared to the positive symptoms in schizophrenia.

Finally, similar to the citation postulated by Kinon *et al* (2005) we indeed observed a complex interplay among environmental factors, genetic predisposition, and the receptor binding profile of the antipsychotic drug. Above all, we have found that the RHA selection line, which was considered to be obesity resistant, is more susceptible to OLZ-induced weight gain compared to the RLA line. All together our data underscores the value of studying individual variation in drug responsiveness via selection lines.

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