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Genetic predisposition to obesity affects behavioural traits including food reward and anxiety-like behaviour in rats

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HIGHLIGHTS

- Rat strains that differ in their genetic predisposition to develop obesity also differ in behavioural tests linked to anxiety, exploration, and reward.
- The lean Obese Resistant rats typically displayed the most marked difference from the other strains (Sprague-Dawley, Obese Prone, and Zucker rats).
- Differences in weight within strains did not explain differences in behaviours, suggesting that weight status does not impact on behaviour.

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ABSTRACT

Here we sought to define behavioural traits linked to anxiety, reward, and exploration in different strains of rats commonly used in obesity research. We hypothesized that genetic variance may contribute not only to their metabolic phenotype (that is well documented) but also to the expression of these behavioural traits. Rat strains that differ in their susceptibility to develop an obese phenotype (Sprague-Dawley, Obese Prone, Obese Resistant, and Zucker rats) were exposed to a number of behavioural tests starting at the age of 8 weeks. We found a similar phenotype in the obesity susceptible models, Obese Prone and Zucker rats, with a lower locomotor activity, exploratory activity, and higher level of anxiety-like behaviour in comparison to the leaner Obese Resistant strain. We did not find evidence that rat strains with a genetic predisposition to obesity differed in their ability to experience reward from chocolate (in a condition place preference task). However, Zucker rats show higher motivated behaviour for sucrose compared to Obese Resistant rats when the effort required to obtain palatable food is relatively low.

Together our data demonstrate that rat strains that differ in their genetic predisposition to develop obesity also differ in their performance in behavioural tests linked to anxiety, exploration, and reward and that these differences are independent of body weight. We conclude that genetic variations which determine body weight and the aforementioned behaviours co-exist but that future studies are required to identify whether (and which) common genes are involved.

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1. Introduction

Obesity has increased markedly during the past three decades and involves a complex interplay of a number of behavioural, genetic, and environmental factors [1,2]. Over-eating disorders that cause over-weight and obesity are increasingly viewed as brain disorders in which reward-driven urges for palatable rewarding foods "hijack" decision-making circuits [3–5]. Differences in cognitive function and in the way the reward system responds to food have been associated with variations in body mass index [5]. Consistent with this, genetic studies also point towards a role for the central nervous system in explaining obesity susceptibility [6].

Surprisingly, only few studies have explored behaviours linked to reward, anxiety or cognitive/memory function in strains of rats that differ in their genetic predisposition to develop obesity and

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that are commonly used in obesity research [7]. Indeed, it is unclear whether their obesity-predisposing genotype impacts on the development of these behaviours, as any differences detected could also be influenced by their diverging body weights. In the present study, therefore, we sought to characterize a number of such behaviours in (1) normal Sprague-Dawley rats and also in different rat strains commonly used in obesity research, namely (2) Zucker rats that carry a mutated form of the extracellular domain of the leptin receptor rendering them hyperphagic and with reduced energy expenditure [8–10] and (3) Obese Prone (OP) and Obese Resistant (OR) rats that diverge in body weight when placed on a high fat diet due to a polygenically inherited form of obesity [11]. We hypothesized that baseline genetic differences rather than differences in body weight *per se* may differentially affect behaviours linked to reward, anxiety, and cognitive function in these rat strains.

2. Material and methods

2.1. Animals

Adult male rats (age 8–12 weeks) were used for the behavioural tests: Sprague-Dawley rats (Charles River, Sulzfeld, Germany), Obese Resistant rats (Crl:OP(CD)), Obese Prone rats (Crl:OP(CD)), and Zucker rats (Crl:ZUC-*Lepr*^{fa}) (Charles River, Wilmington, MA, USA). They were housed in a 12-h light/dark cycle (lights on at 6 am) with regular chow (Teklad diet 2016, Harlan Laboratories, Cambridgeshire, UK) and water available *ad libitum* in their home cages. All animal procedures were carried out with ethical permission and in accordance with the University of Gothenburg Institutional Animal Care and Use Committee guidelines.

2.2. Experimental procedure

Behavioural testing commenced when rats of the different strains were 8 weeks old. Each test is described in full below. Two cohorts of rats, comprising all strains (N = 12 per strain), were compared in each cohort (Fig. 1). Cohort 1 was first tested in the elevated plus maze before commencing training for the lever pressing for sucrose paradigm, that began 4 days later. Cohort 2 was first exposed to the Open Field test and then, 2 days later, training for the conditioned place preference experiments began. Finally, in cohort 2, we performed the novel object recognition test, according to the schedule in Fig. 1. All experiments started in the morning and continued during the entire day, using a balanced design between morning and afternoon for the different experimental groups. The baseline average body weight of the different strains (in each case stated for the first followed by the second cohort) were: Sprague Dawley (SD): 309 ± 3.5 g and 332 ± 4.7 g; Obese Resistant (OR): 249 ± 3.1 g and 208 ± 2.6 g; Obese Prone (OP): 291 ± 8.1 g and 218 ± 5.2 g; Zucker rats (*fa/fa*): 334 ± 6.6 g and 265 ± 5.8 g. The body weight on the day of each behavioural experiment was also recorded.

2.3. Elevated Plus Maze (EPM)

The EPM apparatus (Med Associates Inc., St Albans, Vermont, USA) consisted of two open arms (50 × 10 cm^2) made of black PVC (polyvinyl chloride), crossed by two closed arms (50 × 10 cm^2) with protective walls (40 cm high), and a central platform (10 × 10 cm) placed elevated 70 cm above the ground. Under dim light (around 100 lx over the open arms and 60 lx over the closed arms) the rat was placed in the central platform facing to one open arm and the session lasted 5 min whereas the rat was allowed to freely move in the whole apparatus. The EPM apparatus was cleaned between each trial with 5% ethyl alcohol. The rat behaviour was recorded by an automated system and the following parameters were determined: the number of entries into the open and closed arms (an entry was counted when the four paws were placed on the respective arm), time spent in the open and closed arms, and the number of explorations (when the upper body crossed the boundary of the open or closed arm).

2.4. Open field

This test was performed to study locomotor activity, exploration, and anxiety-like behaviour (Bailey and Crawley, 2009). In addition, the selective D2,3-receptor agonist quinpirole was injected to study whether different rat strains show an altered dopamine-linked locomotor activity. After 15 min of habituation in the Open Field arena (90 × 90 cm) with protective Plexiglas walls (30 cm; Med Associates Inc., St Albans, Vermont, USA) corresponding to 24 h before the start of the Open Field test, each animal received an i.p. injection of vehicle (0.9% saline) or quinpirole (0.5 mg/kg) in a crossover design with at least 78 h in between: they were exposed to the Open Field for 1 h and the locomotor activity was recorded by an automated system using infrared beams in X-, Y- and Z-plane. The Open Field arena was washed with 5% ethyl alcohol between each session. The following activity parameters were measured: distance travelled, ambulatory counts, ambulatory time, vertical counts, and vertical time. In addition, the activities in the peripheral and central part (45 × 45) of the arena were analyzed to study anxiety-like behaviour. In addition to the behavioural changes observed after dopaminergic activation with quinpirole, the Open Field results of vehicle-injected rats were used to evaluate the strain characteristics.

2.5. Conditioned place preference (CPP)

This test was undertaken to study reward behaviour for palatable food, as described previously [12]. Briefly, a CPP apparatus (Med Associates, MED-CPP2-RS, ST Albans, VT, USA) comprising of two connected chambers (30 × 21 × 21 cm) differing in visual (white and black) and tactile (hard plastic with tactile qualities and smooth transparent plastic) cues were used, illuminated by dim light (40–45 lx) and behaviour was recorded automatically.

The CPP procedure consists of three phases: 1st phase habituation/pre-test, 2nd phase conditioning, and the 3rd phase CPP-test. During habituation the door was open between the two chambers allowing the rats to explore freely both compartments for 15 min. The second day of habituation was used as a pre-test (initial preference), in which the time spent in each compartment was recorded. The following conditioning session consisted of 20 sessions/animal (20 min each) and was conducted in 10 consecutive days in a crossover design. During the conditioning session, the least preferred compartment was paired with a rewarding/palatable food (chocolate pellets; Ms, Marabou, Kraft Foods, Upplands Väsby, Sweden) and the preferred chamber with less-rewarding food (normal chow diet). One day following the last conditioning session, the CPP test was performed during which the animals had access to both chambers without food and the time spent in each compartment was measured during 15 min. If the rat previously experienced reward from the palatable food, it will spend more time in the palatable food-paired chamber, even when the food is no longer available. All procedures were conducted in satiated animals and between each session the chambers were cleaned with 5% ethyl alcohol.

2.6. Novel object exploration

Exploratory behaviour and novel object exploration were assessed, as described previously [13]. Briefly, the apparatus com-

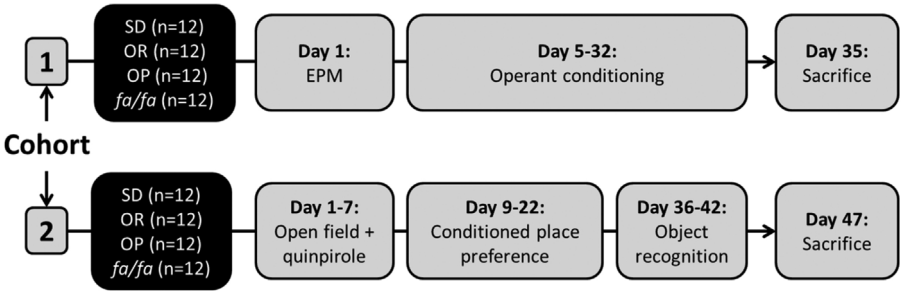

Fig. 1. Schematic diagram of the study design. Two cohorts, comprising different strains of rats, were exposed to different behaviour tests starting at the age of 8 weeks. SD (Sprague-Dawley), OR (Obese Resistant), OP (Obese Prone), *fa/fa* (Zucker), EPM (Elevated Plus Maze).

prised a wooden, square chamber (1 × 1 x 0.5 m) that was placed in a sound-shielded room to minimize any environmental disturbances. The sessions were performed with two types of objects made of neutral (e.g. odorless) material and that differed in shape: a ceramic cup as sample object and a plastic bottle as novel object. A video camera was placed above the chambers to record the exploratory behaviour.

On the experimental day, each rat was brought to the testing room and habituated to the empty chambers for 30 min. Following habituation, two identical copies of the sample object were placed in the chamber and during a 5 min sample session the rats had time to explore the two equal objects. The rats were placed in the chamber between the two objects facing away from the object. After a delay period of 10 min in their home cage one sample object was replaced by a new (novel) object and the object exploration was recorded for 1 min (choice session). In order to avoid a place preference, the object positions were counterbalanced between rats. Object exploration was defined as direct contact of the nose with the object and/or being within a 2 cm radius of an object and was determined by visual analysis of the video.

2.7. Operant conditioning procedure

To test food-motivated behaviour we used the progressive ratio (PR) operant conditioning test. Food-induced operant conditioning training and testing were performed in rat conditioning chambers (30.5 × 24.1 × 21.0 cm; Med-Associates, Georgia, VT, USA) containing a metal grid floor, two retractable levers with light bulbs above, a food pellet dispenser that can deliver 45 mg of sucrose pellets (Test Diet, Richmond, IN, USA), and behaviour was recorded automatically. The training included four stages: first three sessions on a fixed ratio (FR) starting with FR1 (single press on the active lever = delivery of one sucrose pellet) followed by FR3 (3 presses/pellet) and finally FR5 (5 presses/pellet), where a minimum of 50 presses per session was required to obtain the next FR session. Once trained, we could then introduce a progressive ratio (PR) schedule in which the cost of a reward (i.e. number of lever presses) was progressively increased for each subsequent reward. During the FR and the first three PR sessions (PR1), all rats were restricted to a mild food restriction paradigm resulting in a gradual loss of about 10% of their initial body weight over a period of one week. PR sessions (PR2) were continued until a stable baseline (15% for three consecutive sessions) was reached and the mean value of the last 3 PR-sessions was calculated as a trait of food-motivated behaviour.

2.8. Gene expression analysis

To explore whether different strains of rats diverge in the expression of dopamine-related genes, we performed expression studies in the nucleus accumbens (NAc), a key mesolimbic area involved in food reward behaviour. The NAc was rapidly removed, frozen in liquid nitrogen and stored for later use in −80 °C. Total RNA was extracted with the RNeasy Mini Lipid Kit (Qiagen, Hilden, Germany) according to the guideline of the manufacturer. First strand cDNA synthesis was prepared with 500 ng total RNA and the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, CA, USA). Gene expression profiling was performed with an ABI Prism 7900HT sequence detection system (Applied Biosystems), in which the mean value of *TATA box binding protein* (*Tbp*) and *beta-2 microglobulin* (*B2m*) were used as endogenous controls. Data were normalized according to the protocol of [14].

2.9. Statistical analysis

Statistical analysis was performed using the Student's *t*-test for single comparisons and one-way ANOVA for differences between more than two groups. All parameters for one-way ANOVA were initially tested with Leveneís statistics for homogeneity of variances. At equal variances, data were analyzed by one-way or two-way analysis of variance (ANOVA) followed by *post hoc* Bonferroni test and by Games Howell *post hoc* test at unequal variances. The Pearson correlation test was used to determine the relationship between body weight and behavioural variables within each strain. All statistical analyses were conducted using the SPSS software. A p-value <0.05 was considered significant and values are expressed as means ± SEM, unless stated otherwise.

3. Results

3.1. Increased locomotor activity in obesity-resistant rats

To evaluate the different strain characteristics in respect to classical behavioural traits, the data were divided into several sub-phenotypes resembling activity, anxiety, exploration, and food-reward. We first tested whether genetic strain differences are linked to alterations in locomotor activity, a variable that is integral to most of the behavioural tests. Parameters measured to assess locomotor activity were: (i) The total number of entries in the Elevated Plus Maze (EPM) and, in the Open Field, (ii) the distance travelled, (iii) the average velocity, and (iv) the crossings between the inner and outer zone in the Open Field (Fig. 2). When rats were tested in the EPM for 5 min, Obese Resistant (OR) rats displayed a significantly higher number of total entries in comparison to Sprague Dawley (SD), Obese Prone (OP), and Zucker (*fa/fa*) rats, whereas the latter strain (*fa/fa*) showed the lowest locomotor activity among all strains reflected by the lowest number of total arm entries (Fig. 2A). Similar (non-significant) trends were observed for the different groups for total distance travelled during 60 min in the Open Field (Fig. 2B). In this paradigm, further indicators that OR rats were more active included an increase in the maximum number of zone entries (Fig. 2C) and the highest average velocity (Fig. 2D), parameters that were rather similar for the other groups.

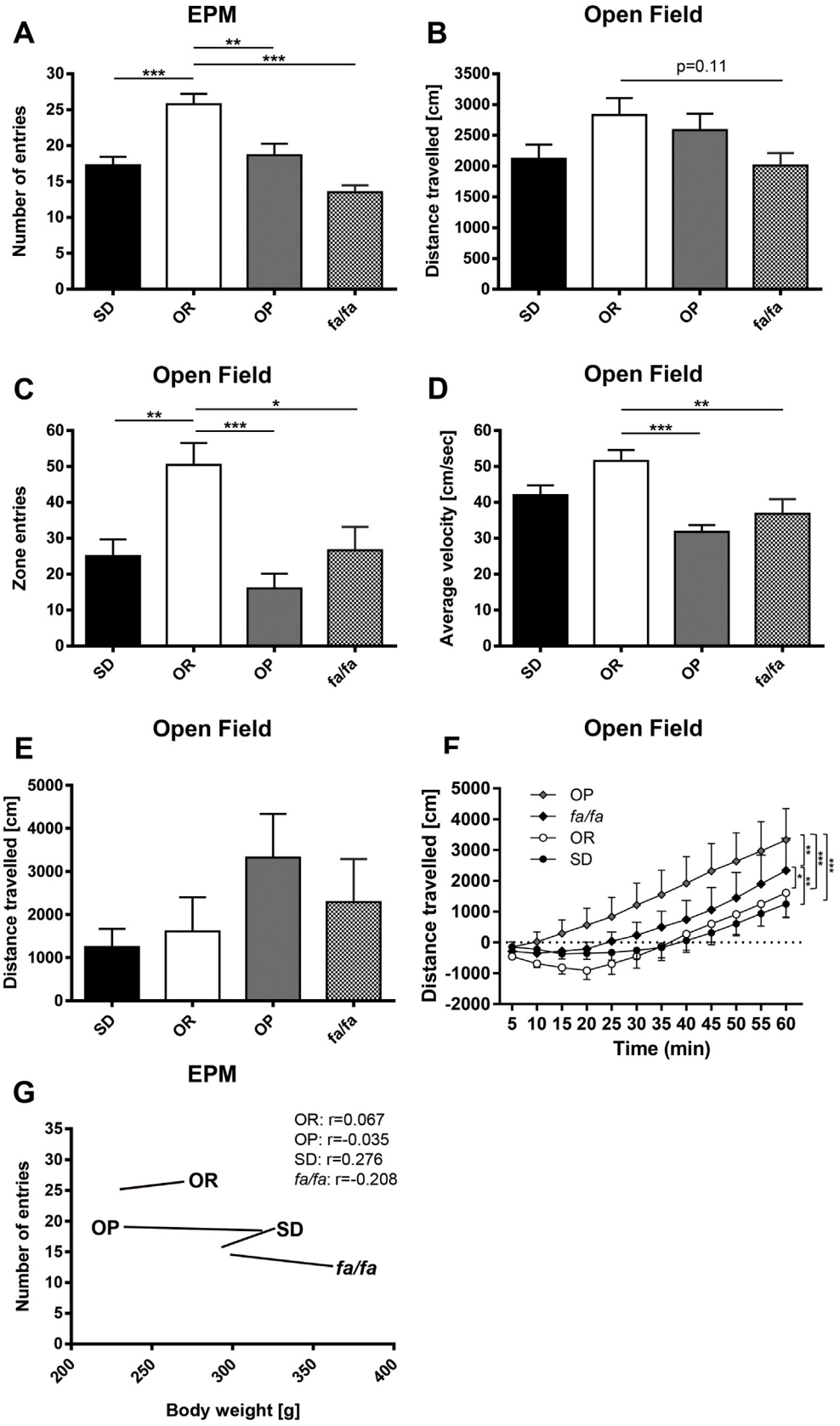

Fig. 2. Parameters linked to locomotor activity in different strains of rats. (A) Total number of entries (open plus closed arm entries during 5 min) in the elevated plus maze (EPM) paradigm. In an Open Field arena the activity of the rats was recorded for 60 min and analyzed as (B) distance travelled, (C) number of zone entries, and (D) average velocity. (E) Change in distance travelled 60 min after peripheral injection of the D2 receptor agonist quinpirole in an Open Field arena and (F) 60 min time course after quinpirole injection. (G) Association between number of entries and body weight within each strain and corresponding Pearson correlation coefficient. Data represent mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by *post hoc* Bonferroni test (EPM: number of entries: $F_{(3,44)} = 15.0$, $p < 0.0001$; Open Field: distance travelled: $F_{(3,44)} = 2.5$, $p = 0.07$; zone entries; $F_{(3,44)} = 7.4$, $p < 0.0005$; average velocity: $F_{(3,44)} = 7.7$, $p < 0.0001$) or (F) two-way ANOVA followed by *post hoc* Bonferroni test OP-SD: $F_{(1,264)} = 34.4$, $p < 0.0001$; OP-OR: $F_{(1,264)} = 30.9$, $p < 0.0001$; OP-*fa/fa*: $F_{(1,264)} = 10.4$, $p < 0.005$; *fa/fa*-OR: $F_{(1,264)} = 6.6$; $p < 0.05$; *fa/fa*-SD: $F_{(1,264)} = 6.9$, $p < 0.01$); $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. N = 12 per group.

We also explored whether different strains differ in their locomotor activity after dopaminergic activation with the D2 receptor agonist, quinpirole. Peripheral injection of quinpirole increased the activity to a similar extent in all strains, indicated by the distance travelled after D2 receptor stimulation (Fig. 2E). Nevertheless, when we analyzed the time course of the response after quinpirole injection we observed that the OP strain had a significantly higher sensitivity to the D2 receptor agonist in comparison to all other strains with SD rats showing the lowest response (Fig. 2F).

Already at baseline, at the age of 8 weeks, the body weight of the different rat strains started to diverge (Fig. S1). To evaluate whether the observed strain differences in locomotor activity are due to differences in body weight we analyzed the correlation of the measured traits with the corresponding body weight within each strain. The Pearson correlation revealed that there is no association between the behavioural variables studied and the body weight (Table 1 and exemplarily shown for number of entries in Fig. 1G), indicating that the differences observed between the strains could not be attributed to the body weight. The only exception was the parameter "number of zone entries" in the Open Field, for which there was a significant positive correlation with body weight for the OR strain (Table 1).

In summary, we demonstrated that strains of rats that differ in their body weight phenotype also differ in their locomotor activity with OR rats showing the highest level of activity and *fa/fa* rats with the lowest activity level. Importantly, the differences in locomotor activity between the strains were not determined by differences in body weight, highlighting the contribution of genetic modifiers on behavioural traits such as activity.

3.2. Anxiety-related behaviour differs between different strains of rat

To further characterize the behavioural characteristics of the different strains we analyzed the EPM and Open Field data in respect to anxiety-related parameters. These include the percentage of entries into the open arms and time spent in the open and closed arms in the EPM as well as the time spent in the inner area of the Open Field. In the EPM, OR rats entered the open arms significantly more than the *fa/fa* rats, which have the least percentage of entries into the open arms of all four strains (Fig. 3A). Tentatively, this could indicate that the OR rats express the least anxiety-like behaviour and *fa/fa* rats the most. This is further supported by data for time spent in the open arms during the EPM test, for which the *fa/fa* rats spent significantly less time in comparison to SD, OR, and OP rats (Fig. 3B) and consequently more time in the closed arms of the EPM (Fig. 3C). An additional parameter that can be used to assess anxiety-like behaviour is the time spent in the central unprotected part of an Open Field arena. OR rats spent more time in the central zone than any other strain whereas the OP rats spent most of the time in the periphery of the Open Field arena (Fig. 3D).

To further investigate whether the strain differences in body weight also affects anxiety-like behaviour, we calculated the correlation coefficient for the different traits within each strain. The Pearson correlation indicates that the decreased anxiety measured in OR rats in comparison to the other strains is not linked to the fact that they have the lowest body weight of the strains explored here (Table 1). In conclusion, we demonstrate that different strains of rats differ in anxiety-related traits, even when differences in body weight were taken into consideration.

3.3. Exploratory behaviour between different strains of rat

To study exploration, the number of explorations (when the upper body crossed the boundary of the open or closed arm) in the EPM was quantified together with the time exploring a new object in the object recognition test. The exploratory behaviour of the OR strain in the EPM was significantly different to SD, OP, and *fa/fa* rats (Fig. 4A). In addition, exploration was also investigated for a novel object and in line with the EPM data the OR rats explored the first object more than any other group (Fig. 4B). Interestingly, when we replace one object with a new one the OR tended to have the lowest preference for the second object compared to the other strains tested (Fig. 4C). As for the other behavioural parameters exploration did not correlate with the body weight and therefore the observed differences appear to be due to genetic differences.

3.4. Food-motivated behaviour differs between genetically lean and obese rats

To assess the different strain characteristics in respect to food-motivated behaviour, the lever-pressing for sucrose progressive ratio task and the CPP were used. In the first test, rats were trained to press a lever for sucrose (45 mg pellet) and once trained the different groups were exposed to a progressive ratio schedule in which the number of presses required to obtain a single pellet increased progressively. The OR rats pressed the active lever for a sucrose reward significantly more often than *fa/fa* and SD rats (Fig. 5A) and consequently earned more sucrose pellets during PR testing (Fig. 5B), demonstrating that the latter strains were less willing to work hard to obtain sucrose. This effect cannot be explained by a higher basal activity of the OR rats because they do not show more inactive lever presses than the other groups (not shown). Including the time of the break point revealed that the obese susceptible strains reached their final level significantly earlier than the OR rats (which pressed the active lever almost the whole test session; Fig. 5B). To further characterize the willingness of the strains to lever press for a reward we also analyzed the responding during the acquisition period. Interestingly, responding on the active lever was significantly higher in *fa/fa* rats in comparison to the OR strain during the first training (FR1) when the work requirements were relatively low (Fig. 5C). Additionally the *fa/fa* needed less time to pass the acquisition criteria for the FR1 session whereas almost 5 sessions were required for the OR to reach the next FR level (Fig. 5D). In contrast, with an increasing ratio to obtain a food reward the effect of higher lever presses in the *fa/fa* rats in comparison to the OR strain was completely changing. In the last acquisition period (PR2) the responding on the active lever presses was significantly greater in OR rats then in the *fa/fa* group (Fig. 5C). These data suggest that rat strains with a genetic predisposition to develop obesity reduce the "wanting" for palatable food when the effort is too high.

The rewarding property of chocolate was tested in the CPP, in which a rat shows a preference for a chamber previously paired with palatable food (1 g chocolate pellets) over a chamber previously paired with regular chow. As indicated in Fig. 5E, all tested strains are able to experience reward from a palatable treat (chocolate) to a similar extent. Nevertheless, although the difference is not significant, OR rats showed the lowest preference for the chocolate-paired chamber in comparison to *fa/fa* with the highest %CPP change (Fig. 5F). As was the case for activity and anxiety-like behaviour studies, these datasets for food motivation and food reward were not linked to the body weight (Table 1). These data indicate that although all strains find chocolate rewarding in the CPP, the *fa/fa* and SD rats show the higher preference. However, when they need to work actively to obtain sucrose pellets, *fa/fa* and SD are the strains less disposed to press the lever. In conclusion, we demonstrate that the motivational property of a palatable food is not necessarily increased in strains with a genetic predisposition to obesity (*fa/fa*) although they find palatable food highly rewarding.

Table 1
Pearson correlation between body weight and behavioural variables within each strain. *p < 0.05.

	Sprague Dawley		Obese Resistant		Obese Prone		*fa/fa*	
	R	p-value	R	p-value	R	p-value	R	p-value
EPM								
Total entries	0.076	0.389	0.004	0.837	0.001	0.915	0.043	0.517
Explorations	0.042	0.521	0.268	0.085	0.016	0.699	0.171	0.181
Time in open arm	0.283	0.092	0.003	0.874	0.014	0.718	0.113	0.284
Time in closed arm	0.222	0.123	0.020	0.660	0.029	0.594	0.351	0.040*
Open Field								
Zone entries	0.288	0.072	0.798	0.010*	0.272	0.082	0.005	0.820
Time in central zone	0.116	0.279	0.132	0.246	0.215	0.123	0.001	0.943
Average velocity	0.299	0.066	0.059	0.448	0.360	0.051	0.003	0.875
Operant boxes								
Active lever presses	0.011	0.743	0.078	0.380	0.094	0.333	0.061	0.439
Sucrose pellets	0.004	0.844	0.020	0.662	0.104	0.308	0.100	0.317
CPP								
% change in preference	0.037	0.550	0.012	0.732	0.155	0.205	0.466	0.510
Novel object task								
Object exploration time	0.037	0.552	0.067	0.416	0.011	0.749	0.009	0.763

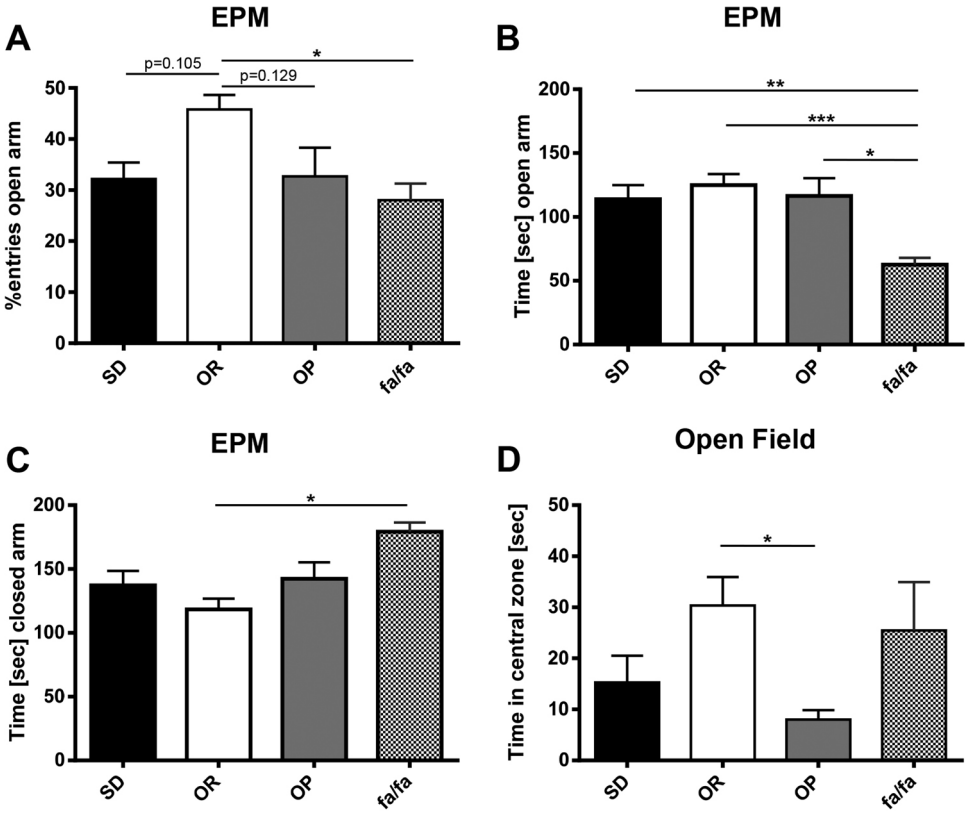

Fig. 3. Parameters linked to anxiety-like behaviour in different rat strains assessed in the elevated plus maze (EPM) and Open Field. (A) Percentage of open arm entries/total entries, (B) time in the open arm (sec), and (C) time in the closed arm (sec), assessed during a 5 min period. (D) Time spent in the central zone of an Open Field arena was recorded for 60 min. Data are expressed as mean + SEM and were analyzed by one-way ANOVA followed by *post hoc* Bonferroni test (A–C) and by Games Howell *post hoc* test (D) at unequal variances (EPM: %entries open arm: $F_{(3,44)} = 3.9$, $p < 0.05$; time open arm: $F_{(3,44)} = 2.7$, $p = 0.0005$; time closed arm: $F_{(3,44)} = 6.3$, $p < 0.005$; Open Field: time in central zone: $F_{(3,44)} = 7.7$, $p < 0.05$); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. N = 12 per group.

3.5. Differences in nucleus accumbens Drd1a gene expression between different strains of rat

We also explored whether the different strains of rats have an altered expression of dopamine-related genes in the NAc, a key node in the dopamine reward signaling pathway. Therefore we analyzed the expression pattern of selected dopamine receptors and enzymes involved in dopamine production and metabolism in the NAc of the different rat strains. The only gene with an altered expression was the *dopamine receptor 1a* (*Drd1a*, Fig. 6) with the highest mRNA expression in OR rats. There was a trend towards significance in expression of *Drd1a* mRNA in OR rats compared to *fa/fa* rats.

In conclusion, we demonstrated that rat strains that differ in their susceptibility to develop an obese phenotype are not characterized by an overall change in the expression of dopamine-related genes in the NAc.

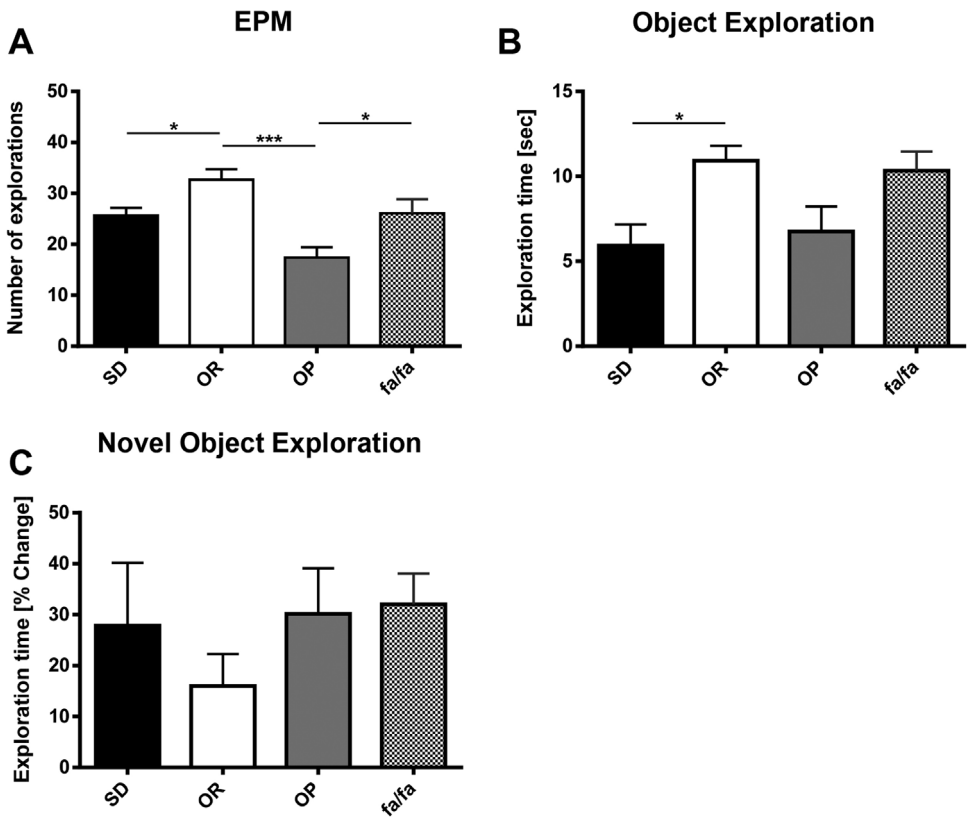

Fig. 4. Parameters linked to exploration in different rat strains. Exploratory behaviour of different rat strains in an elevated plus maze test presented as (A) number of explorations. (B) Exploration time for an object and (C) change (%) in exploration time during a novel object recognition task. Data represent mean ± SEM and were analyzed by one-way ANOVA followed by *post hoc* Bonferroni test (EPM: number of explorations: $F_{(3,44)} = 8.5$, $p < 0.0005$; Object Exploration: exploration time: $F_{(3,44)} = 4.3$, $p = 0.01$); $^{*}p < 0.05$, $^{***}p < 0.001$. N = 12 per group.

4. Discussion

These studies were designed to explore and compare the behavioural phenotype (i.e. exploratory-, anxiety-, reward-linked, and cognitive behaviours) of different strains of rats that are commonly used in obesity research. An overarching question is whether strains that differ in their genetic predisposition to develop obesity also differ in their expression of these behavioural traits. We found that many of the parameters measured in the behavioural tests did differ between strains, with similar phenotypes in the obesity-susceptible models OP and *fa/fa*, and both models systematically differ from the OR strain. The two obesity models generally corroborate one another, while data from the SD rats tend to be an average of the behaviour of the other three groups. This is consistent with the literature, as SD rats can be identified as obesity-prone and obesity-resistant by exposure to high-fat diet and this cannot be distinguished in the current study. Importantly, these differences in behaviour in the different rat strains could not be accounted for by differences in body weight for almost all of the parameters measured in the behavioural tests. Collectively our data suggest that genetic factors likely account for differences in the expression of exploratory, anxiety-like, reward and cognitive behaviours between different strains of rats commonly used in obesity research and that these are not secondary to development of obesity.

In the current study we only measured total body weight to determine the level of obesity and used this parameter to calculate for possible correlations with the assessed behaviours. Previous studies in the OP and OR strain had shown that the increased body weight in OP rats correlates with a difference in body fat content and other parameters of the metabolic syndrome [11] and similar effects were also published for the *fa/fa* rats in comparison to lean controls (8–10). Therefore, we conclude that total body weight serves as a representative trait to reflect the status of obesity in the models used in the current experiments. However, it would be interesting in future studies to combine the different behavioural traits with additional measurements of obesity e.g. adiposity, metabolic function, and circulating hormones influencing hunger satiety and to compare these results with the actual data.

In the current study we found that OR rats expressed the greatest number of open arm entries/total entries in the elevated plus maze. While this parameter suggests that anxiety-like behaviour in the OR rats is lower than the other groups, it is difficult to separate this behavioural construct from the changes in activity and/or exploratory behaviour (which were also higher for these rats than for the other strains). We noted that Zucker *fa/fa* rats appeared to display anxiety-like behaviour in the EPM but not in the Open Field, possibly indicating that this strain is less skillful at moving into the open arms, relative to other strains. Surprisingly few studies have explored anxiety-like behaviours in obese strains of rats. One study found that OP rats had an increase in anxiety-like behaviour relative to OR rats (assessed as activity in the central region of the Open Field), without any difference in locomotor activity (activity in the peripheral part of the Open Field) [15]. These tests, unlike ours, were performed in OP and OR rats withdrawn from a high fat-high sucrose diet, for which the OP rats appear to be especially vulnerable to express an anxiety-like phenotype.

Another question that we have addressed here is whether different strains of rats differ regarding their ability to experience reward from a palatable food and/or express reward behaviour for it. Food reward was tested in the condition place preference (CPP) test in which rats show preference for a chamber previously coupled to a treat (in this case chocolate) over a chamber coupled to chow:

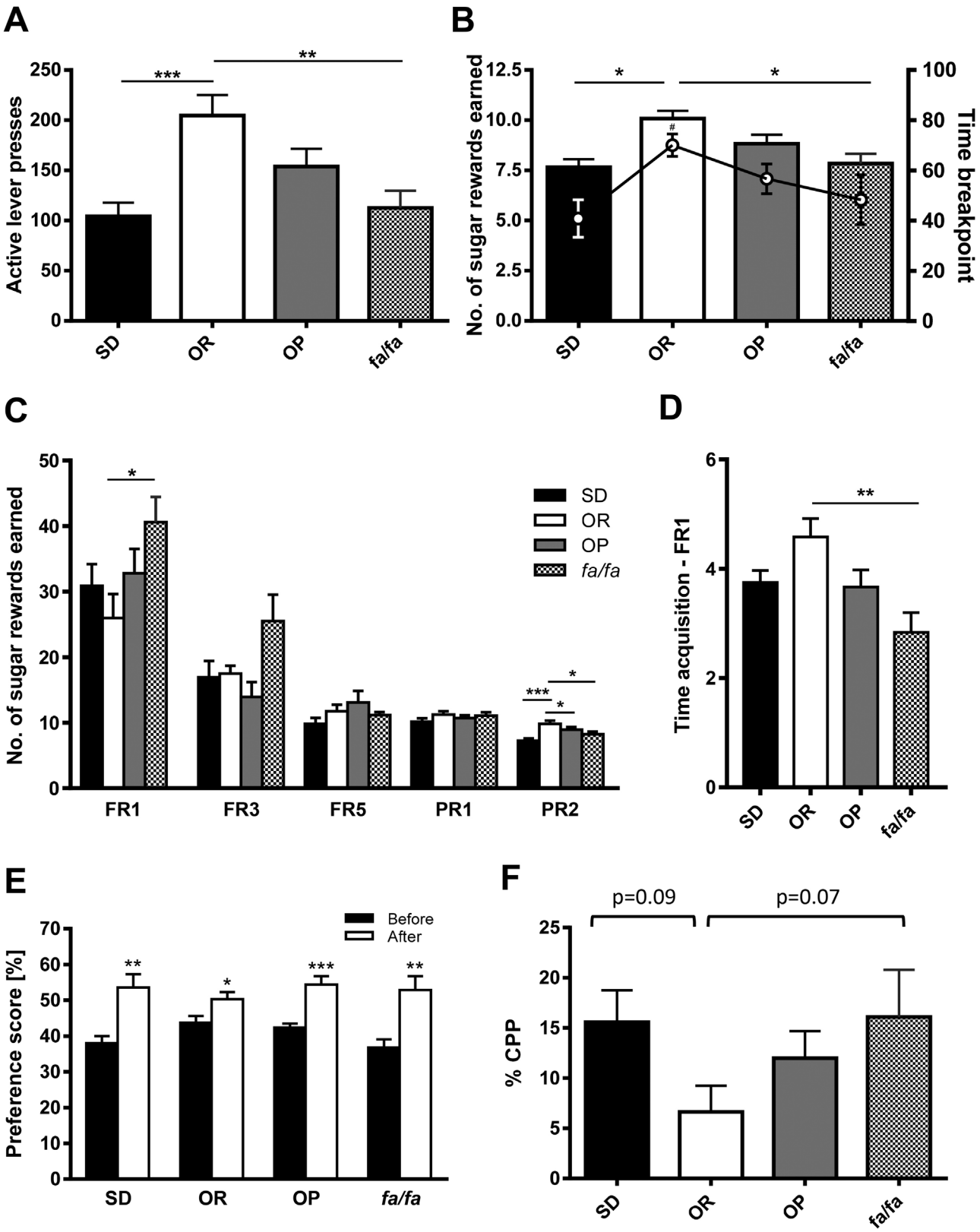

Fig. 5. Parameters linked to food-motivated behaviour (lever-pressing for sucrose in a progressive ratio task) and food reward (%CPP) in different rat strains. Number of (A) active lever presses and (B) sugar rewards earned in an operant lever-pressing paradigm as well as time of break point reached. (C) Average number of responses during the different training sessions and (D) time (number of FR1 sessions) needed to pass the FR1 session criteria (50 presses on active lever). (E) Preference scores for the conditioned compartment before and after conditioning in each strain and (F) %CPP change for a chamber paired to palatable food (chocolate pellets) in different strains. Data represent mean ± SEM and were analyzed by one-way ANOVA followed by *post hoc* Bonferroni test and by Games Howell *post hoc* test at unequal variances (OC: active lever presses: $F_{(3,44)} = 8.5$, $p < 0.0005$; no. of sugar rewards earned: $F_{(3,44)} = 4.3$, $p = 0.01$ (B); $F_{(3,44)} = 2.8$, $p < 0.05$ (FR1, C); $F_{(3,44)} = 7.3$, $p < 0.0005$ (PR2, C); $F_{(3,44)} = 5.2$, $p < 0.005$ (D); time break point: $F_{(3,44)} = 3.0$, $p < 0.05$) and Student's *t*-test was used to compare the preference score before and after conditioning; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{\#}p < 0.05$ difference to SD. N = 12 per group.

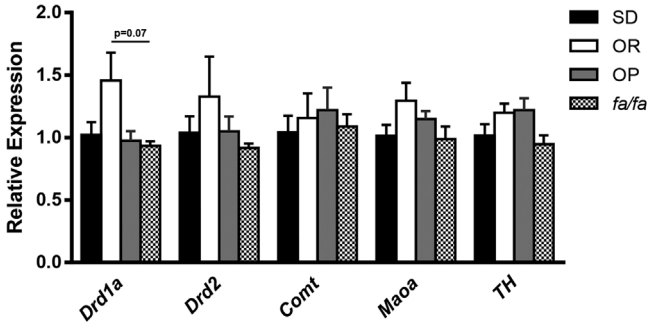

Fig. 6. Gene expression of dopaminergic genes in the NAc. Gene expression pattern of selected dopamine receptors and enzymes involved in dopamine production and metabolism in the NAc of different rat strains. Data represent mean ± SEM. N = 12 per group.

they spent more time in the treat-paired chamber even when no chocolate was offered. OR rats showed the lowest preference for the chocolate-paired chamber in comparison to *fa/fa* with the highest %CPP, but the difference did not reach statistical significance, suggesting that all rats are able to experience reward from a palatable treat to a similar extent. This finding is in line with a previous study showing that OP and OR rats are able to acquire CPP for high caloric snack foods [16]. Despite this, differences did emerge regarding their motivation for sucrose. To assess motivation, rats were trained to press a lever for sucrose pellets and once trained they were exposed to a progressive ratio schedule in which they had to work increasingly hard (by pressing a lever more) to obtain a subsequent pellet [17]. The leaner OR rats stand out from the other strains by displaying the most motivated behaviour for sucrose. These findings resonate with a previous study showing obese rats (both dietary and genetic) display less motivated behaviour than their leaner counterparts [18]. Interestingly, and in contrast to the final PR test session, when we analyzed the food-motivated behaviour during the acquisition period the obese susceptible *fa/fa* rats had higher rates of responding than the OR group. When the work requirements increased (PR test sessions) the *fa/fa* strain stopped to press for a sucrose reward whereas the OR rats were still willing to work for palatable food. Collectively, these data suggest that rat strains with a genetic predisposition to develop obesity reduce the "wanting" for palatable food when the effort is too high, as seen with the progressive ratio task. This observation is supported by a previous study where instrumental responding was analyzed in obesity-prone compared to obesity-resistant rats. When work requirements were relatively low the obesity-prone rats pressed the lever for food more often than obesity-resistant rats whereas the break points during progressive ratio testing were only slightly elevated [19]. In line with the food-motivated behaviour data, when the effort to obtain a reward is low, as tested with the CPP for a chocolate treat, the *fa/fa* rats tend to have the highest food-reward [18]. However, it is very unclear how genetic predisposition to obesity would be expected to impact on food motivation as the existing literature would indicate that it could be attenuated [18], increased [20,21] or even have little effect [19,22]. Our studies highlight the fact that genetic background should be taken into consideration when exploring food-motivated behaviour.

Regarding exploratory behaviour, the OR rats showed a higher number of explorations (open and closed arms) in the EPM and during the first part of the object exploration test. Interestingly, when one object was replaced, the OR rats were the strain showing less exploration compared to any other group. The latter data does not necessarily mean that the OR rats have a lower novelty-seeking trait. The deficit could be due to the difference in their exploration between the first and second object. The OR rats seems to persist in exploring the first object as much as the second. The object recognition task not only involves the detection and encoding of a novel object [23] but also measures memory of a familiar object [24]. Therefore our data could also be interpreted as a deficit in working memory, which is needed to identify which object is "novel".

Given that the mesoaccumbal dopamine system is a key neural pathway involved in food reward and motivation [25,26], we also explored the expression of dopamine-linked genes in the nucleus accumbens. We did not find any significant difference in the expression of any of the genes studied between strains. Nevertheless, it should be stated that we detected a trend toward an increased expression of the D1 receptor in OR rats and this is in line with data from Robinson et al., 2015, where they measured lower mRNA levels of D1R in rats susceptible to diet-induced obesity [27]. In addition, we also studied the locomotor activity after dopaminergic activation with a D2 receptor agonist and 60 min after injection of quinpirole the response did not differ between the strains. However, when we analyzed the time course after application the sensitivity to the D2 receptor agonist was the highest in the OP rats in comparison to all other strains tested. Similar results have been shown in a recent study where it was also reported that obesity-prone rats were more sensitive to the D2 receptor-mediated effects of quinpirole [28]. Most studies to date have analyzed changes in dopamine signaling after long-term exposure to palatable food in different models of dietary obesity and demonstrated a suppressed central dopamine system [29–32]. Therefore, it would be also interesting in future experiments to use the models of the current study to characterize the predetermined alterations in the central dopamine pathway in more detail.

For almost all of the behavioural tests explored here, differences in body weight could provide a confounding factor that impacts on many of the parameters measured, irrespective of genotype. Body weight differences clearly exist between the different rat strains at baseline. "Within-strain" correlation analysis did not find evidence that body weight contributed significantly to variance for almost all of the parameters in any of the strains. The only exception was the parameter "number of zone entries" in the Open Field, for which there was a significant positive correlation with body weight for the OR strain.

Our data suggest that OR rats have a higher locomotor activity than the other strains and have a higher level of exploration in the EPM relative to SD rats. These behavioural constructs may contribute not only to the leaner phenotype of the OR rats but also to their performance in some of the behavioural tests.

In conclusion, rat strains that differ in their genetic predisposition to develop obesity also behave differently. Moreover, differences in weight within strains did not explain differences in behaviours, suggesting that weight status does not impact behaviour.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2017.02.037.

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