Research report

Influence of excessive sucrose consumption on exploratory behaviour in rats – Possible implications for the brain reward system

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ABSTRACT

Due to the low cost of production and the strong evolutionary preference for sweet taste in humans, sugar is added to many food products. This leads to often involuntary overconsumption of high amounts of sugar. Yet, growing evidence indicates that high-sugar diets impact brain function and impair cognitive ability. It may be due to physiological changes in specific regions of the brain or/and maladaptive changes in dopamine signalling similar to those observed in the etiology of addiction. In our study, rats from the experimental group were kept on a feeding protocol involving intermittent access to sucrose solution for eight weeks. Then, the animals underwent a spontaneous exploration test in an experimental arena divided into three zones where stationary and movable objects were placed. Studying the rats' exploratory behaviour allowed us to assess the impact of the sucrose diet on a broad spectrum of behaviours related to the general functioning of the organism in its environment. Analyses showed differences in reaction to novelty between different diet groups which had been placed in different experimental setups. Rats from the sugar-fed group responded to change with more pronounced exploratory behaviours directed at the source of the novel stimuli and the surrounding environment. These results may indicate a lower reward value of novelty resulting from diminished responsiveness of the reward system in the sugar-diet group. We have not found evidence for memory and/or learning impairments in rats on the sugar-rich diet.

1. Introduction

Added sugars, mainly sucrose and high-fructose corn syrup, have become major components of the human diet today. Sugar consumption is increasing worldwide (e.g. [1]). This creates a major challenge for social policy and healthcare systems. The obvious effects of sugar overconsumption, such as diabetes or obesity, are widely recognised, while much less attention is paid to cognitive and emotional changes resulting from a diet based on excessive sugar intake.

Due to the low cost of production and the strong evolutionary preference for sweet taste in humans, sugar is added to many food products (including sauces, dairy products, sausage, etc.). This leads to often involuntary overconsumption of sugar. Yet, growing evidence indicates that high-sugar diets (HSDs) profoundly impact brain function and impair cognitive processes even in the absence of extreme weight gain or excessive energy intake [2,3]. The most important fact is that highly palatable foods (e.g. products containing sucrose) are likely to increase dopamine release in the brain reward centres. Over time, regular consumption of this type of food may lead to maladaptive changes in dopamine signalling similar to those observed in the etiology of addiction (e.g. [4]). Furthermore, evidence suggests that under certain circumstances, it can lead to sugar dependency (e.g. [4,5]).

Data from research on rats provide strong evidence that sucrose consumption can lead to deficits in spatial cognition and reward-oriented behaviour (for a review, see [6]). These deficiencies were also observed under conditions of high pre- and postnatal sugar consumption [7,8]. Only a few days of a hypercaloric diet is sufficient to trigger spatial-specific deficits in rats [2,9]. On the other hand, some studies suggest that diet-induced spatial memory deficits are not present when discrete spatial cues are placed in the arena [9]. Such results indicate that hypercaloric diets may impact different aspects of learning and memory and that diet-induced behavioural deficits may become more apparent when task difficulty and cognitive demands are increased. Another study showed that diet-induced obesity resulting from excess sucrose intake, but not fat intake, impairs spatial learning and memory in young animals [10]. Therefore, it is hypothesised that the effect of sucrose on the brain is not directly correlated with excessive caloric intake in general.

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The cognitive changes observed in subjects on high-sucrose diets may be triggered by effects on specific areas of the brain. For example, the hippocampus, which is involved in spatial, contextual, and episodic memory formation, is particularly vulnerable to neuropathological changes following excessive sucrose consumption (e.g., [6,11–16]). In animal studies, markers of neuroinflammation, such as microglia activation measured by IBA-1-immunoreactivity and increased hippocampal levels of pro-inflammatory cytokines such as interleukin(IL)-1β, IL6 and tumour necrosis factor-α (TNF-α), were observed in rats and mice exposed to HSDs [2,13,14]. Furthermore, high-caloric diets have been shown to reduce brain-derived neurotrophic factor (BDNF) mRNA expression in the hippocampus [7,17]. These studies suggest that the mechanisms through which HSDs impair hippocampal neuroplasticity may include increases in pro-inflammatory cytokines, as well as decreases in neurotrophic factor expression, which may ultimately contribute to diet-induced cognitive deficits [18]. In rats, HSDs also led to a decrease in the level of markers of hippocampal neurogenesis in the dentate gyrus [14] and neuroproliferation markers doublecortin and proliferating cell nuclear antigen (PCNA) immunoreactivity [19].

Another area of research on the influence of excessive sugar consumption on behaviour concentrates on addiction-like changes in the brain (mainly in the dopaminergic system - for a review, see [4]; also [5, 20,21]). Dependency based on activation of physio-behavioural systems controlling such areas as metabolism, foraging, and eating to maintain energy homeostasis is sometimes called “natural addiction” in opposition to “drug addiction”, which activates specific system related to their pharmacology [21]. This analogy is based on the propensity of sugar to evoke binging, withdrawal symptoms, and abstinence-induced motivation to gain access to sucrose (e.g., [4]). This motivation occurring during the deprivation period is manifested in rats, among other things, by more frequent lever pressing for sugar [22], increased alcohol consumption in rats with sugar-bingeing [23], and stronger response to sugar-associated cues [24]. In addition, the psychostimulant properties of sugar are evidenced by the observed cross-sensitization with amphetamine [21]. From a behavioural perspective, substance dependence may be detected by measuring symptoms of addiction, such as tolerance (a gradual decrease in responsiveness to the substance resulting in an increased demand for that substance to produce the same effect - [25]) or craving (increased efforts to obtain the substance - [4]).

It is well known that repeated activation of the reward circuit by abusing a specific substance may lead to neuroplastic changes (i.a. reduction in the density of dopamine receptors). It should be noted that the dopamine system is highly engaged in memory and learning (e.g. [26,27]). It also plays a crucial role in initiating motor actions, as observed in patients with Parkinson’s Disease, and depletion in dopamine in the striatum is associated with disorganization of behaviour [20, 28]. What is more, there is evidence that dopaminergic mechanisms are critical to the process of cognitive shifting and executive control [27]. Thus, chronic consumption of this type of food may lead to maladaptive changes in dopamine signalling similar to those observed in the etiology of addiction [4]. This may be mainly due to a reduction in striatal D2/3 receptor density repeatedly observed in diverse populations of drug users [29,30], which has also been found in obese subjects [31].

Studies conducted to date indicating adverse changes in cognitive functioning have focused on cognitive tasks measuring a narrow and out-of-context range of psychological properties. These included: the Water Morris Maze (spatial memory and learning), object recognition test (memory and learning, progress in reasoning), passive avoidance test (emotional processing), e.g., [11,31–34]; but: [15,32]. Although these studies show a deterioration in selected cognitive skills in many cases, they cannot show how these changes translate into the functioning of an individual in a broader sense. In our project, we intend to identify changes in the overall behavioural characteristics by using a free exploration protocol [35]. Exploration can be defined as behaviour directed toward acquiring information about the environment. Among other things, it allows individuals to prepare for the future (e.g., escape from predators, harsh weather conditions, or ensuring food security). It involves a broad range of behavioural phenomena such as reaction to novelty, risk assessment, arousal, locomotor activity, habituation, memory, etc. Moreover, a novel stimulus (even when it lacks rewarding or biologically beneficial attributes) acts as a positive reinforcer, highlighting its powerful motivational properties (e.g., [36,37]). The organism’s inherent interest in novelty (the main element of exploratory behaviour) is considered to be an evolutionary prerequisite for complex learning, and it guides organisms toward acquiring adaptive behavioural repertoires [38]. On the other hand, high scores on the novelty-seeking scale are considered risk factors for several neuropsychiatric disorders, such as addiction and bipolar disorder [39]. The free exploration test we proposed [35] could allow us to observe how organisms cope with changes in the environment in a comprehensive manner, ensuring that the animals’ stress level remains low and taking into account behavioural dynamics over time. Unlike most behavioural tests, which measure a single behavioural parameter, the free exploration test is based on the assumption that rodents demonstrate their full behavioural spectrum only in a rich testing environment that enables observation of more complex behaviours. From the viewpoint of the theory of levels of integration [40], the study of exploratory behaviour makes it possible to register changes in behaviour occurring at higher levels of integration, without being limited to measuring behaviours or components of behaviour at a lower level of integration/organization. The general hypothesis underlying this approach is as follows:

Changes that occur due to sucrose overconsumption may not be fully manifested in elementary dimensions of behaviour and highly structured cognitive tasks; they may not exceed the registration threshold. However, the presence of these changes in various areas of the organism’s functioning may, as a result of their cumulative effects, lead to changes at higher levels of integration specific for higher forms of functioning, e.g., behavioural styles, behavioural syndrome, etc. Therefore, our research will focus on changes in the overall strategy of coping with challenges posed by the environment and not on selected and highly specific aspects of cognitive and emotional functioning (they will, however, also be controlled throughout the experiment). What is more, this paradigm seems particularly useful for assessing the influence of sucrose overconsumption on reward processing (incentive value of novelty), as well as recognition and memory processing (changes in experimental settings) associated with the brain reward system and the hippocampus, respectively.

To increase the accuracy and reliability of the measurement we decided to provide the test subjects with two levels of novelty as regards its complexity. It is well known that animals prefer more complex environments to simplified or impoverished ones [41–44]. This preference for complexity is most clearly demonstrated when the environmental change takes the form of an increase in complexity [43]. In the experimental setting, complexity may be introduced in numerous forms. In addition to placing novel objects in the experimental arena, we can also provide animals with movable objects. Movability adds a new dimension to the new object and enables animals to control the novel environment to a certain degree. In addition, this specific type of novelty generates sensory stimuli by virtue of its movability, but also as a result of the organism’s own activity. Reaction to this kind of stimuli may differ among individuals with various levels of different psychological properties (e.g. [45]). Therefore, it is possible to hypothesise that the expected neuronal and behavioural changes triggered by excessive sugar consumption (reward processing, specific reactions to varying levels of complexity of the novel stimuli).

Considering the above, the general hypothesis underlying our study is the expectation that high sugar consumption will affect the functioning of the reward system, leading to changes in reaction to novelty and general exploratory behaviour. Excessive sugar consumption will induce a tonic, inter-situational increased and persistent desire for events and stimuli that activate the reward system. The indicators of these changes will be behaviours focused on novelty seeking, lack of
resistance to postponed reinforcements, or high variability in the forms of activity, combined with an increase in the amount of time spent on the exploratory activity.

H1. Sugar consumption will lead to increased exploration after the introduction of novelty. We hypothesise that increased activity directed at the sources of sensory stimulation will be a result of decreased sensitivity to the reward value of novelty due to addiction-like changes in the dopamine system (cf. [39,46,47]).

H2. Sugar consumption will result in a slower rate of habituation to novelty due to learning impairment resulting from excessive sugar consumption having a damaging effect on specific areas of the brain e.g. the hippocampus (cf. [11,31,32]).

H3. Excessive sugar consumption will have a different impact on reactions to various levels of complexity of the novel stimuli. Higher complexity (a movable object) will lead to more intensive exploration by stimulating the reward system to a greater degree than a non-movable object and/or due to a higher level of the reward value of stimulation resulting from the animal’s own activity (cf. [48]).

To test these hypotheses, we conducted a two-phase study. First, the animals from the experimental group were maintained on a feeding protocol involving intermittent access to sucrose solution for eight weeks. The control group was kept in standard conditions. Second, all subjects underwent a spontaneous exploration test in an experimental arena divided into three zones. The test started with a series of habituation sessions the purpose of which was to familiarise the animals with the experimental setting. Subsequently, novel objects were placed in one of the zones, and behavioural reactions to the changed environment were measured. What is more, half of the animals encountered a novel stimulus with increased complexity (i.e. a movable object). We believe that this procedure allowed us to induce symptoms of sugar dependency, making it possible to assess a broad range of behavioural changes resulting from the impact of sucrose on its neural substrates.

2. Methods

2.1. Animals

The sample consisted of 49 male Lister Hooded rats. The rats were bred and housed in the vivarium of the Institute of Psychology, Polish Academy of Sciences, Warsaw, Poland. At the beginning of the study, the rats were approx. 30 days old and weighed approx. 140 g.

The rats were housed in groups of 3–4 in Tecniplast® Eurostandard Type IV cages (610 mm × 435 mm × 215 mm) with dust-free softwood granules Tierwohl Super® as bedding. They had ad libitum access to water and standard laboratory fodder (Labofeed H, WP Morawski, Kcynia, Poland). The day/night cycle was set at 12/12 h (lights-on at 8.00 a.m.). The temperature was maintained at a constant 21–23°C, and humidity at 45–60 %. Prior to the experiment, the cages were cleaned once a week. However, in order to ensure that the experimental procedure was not disturbed, the cages were cleaned just before the start of the behavioural test and again after the test was completed.

All the rats were housed, bred and taken care of in accordance with the Regulation of the Polish Minister for Agriculture and Rural Development of 14 December 2016 on laboratory animal care. The experimental procedures were approved by the First Local Committee for Ethics in Animal Experimentation in Warsaw, Poland, permit #1268/2021.

2.2. Feeding protocol

Prior to the experiment, the animals followed two different diet protocols for a period of 8 weeks. One group was given access to sucrose solution for 2 h per day at the beginning of the circadian cycle’s dark (active) phase. During the access to the sucrose solution, no plain water was available to the animals. Sucrose is the most commonly consumed sugar-based sweetener (a disaccharide containing 50 % fructose and 50 % glucose). The animals were given a 10 % sucrose solution, which was similar to the sugar dose found in commercially available sweet beverages and in diets with hidden sugars [49,50]. The Control group followed the standard feeding protocol with ad libitum access to plain water.

Bottles with sweetened water were weighed before and after placing them in the rats’ home cages. Analysis of mean values of a weekly sucrose solution consumption using Anova indicated that the amount of sugar water drank by rats increased significantly during the 8-week feeding procedure (F(7,35) = 16.474; p < 0.001) - Fig. 1. There were no differences in body weight between the groups at the start of the behavioural test (approx. 430 g for an animal).

2.3. Experimental setup and procedure

The measurement apparatus and methods were similar to those used in our previous studies [35,43,51–54]. The experimental chamber (Fig. 2) was a box measuring 800 mm × 600 mm × 800 mm. The chamber was divided into three zones: A, B, and C by two walls running perpendicularly to its longer side. The partition walls between the zones had triangular openings (120 mm × 140 mm) at the bottom, which enabled free movement between the chamber parts. There was a hole curved in the back wall of the chamber that served as an entrance for animals moving from the transporter into the chamber. The front of the chamber was made of transparent plexiglass and it could be lifted to obtain full access to the experimental arena. The entire chamber was covered with a layer of washable varnish. In zones B and C, we placed tunnels (200 mm × 120 mm × 80 mm) made of hardwood covered with washable paint. In contrast to the most frequently used two-dimensional experimental settings, these tunnels provided a complex three-dimensional environment. The central zone (A) was left empty.

At the start of each trial, a transporter (a small cylindrical cage – 60 mm in diameter with doors 120 mm high and 100 mm wide) with the tested animal inside was placed by the entrance to zone A (Fig. 2) behind the wall of the experimental chamber. The entrance door was then lifted, and it was left open until the end of the trial. The animal was free to stay in the transporter or leave it to explore the chamber. The first seven trials were habituation trials during which the apparatus was arranged in the same way. The introduction of novelty (addition of tunnels) took place between trials 7 and 8. The three subsequent trials were conducted with the chamber in this new arrangement (Fig. 3). Each trial was 7 min long and was conducted for each animal once a day.

Two different types of novelty were introduced in trial 8. In the first setting, the novelty was introduced by adding new tunnels to the experimental arena. The second setting had the same configuration of added tunnels, but the top tunnel was built in a way allowing the rats to...
move it like a seesaw (Fig. 3).

Setting 1 - Addition of a stationary novel object in the experimental box (Add group). During the habituation sessions, two tunnels (200 mm × 120 mm × 80 mm) were present in each of zones B and C. They were arranged in the same manner (Fig. 2). On the first experimental day (trial 8), two supplementary tunnels were placed in zone C (Fig. 3). The arrangement of the tunnels in zone B remained unchanged. A total of 27 (12 rats from the sugar diet and 15 rats from the standard diet) rats were used for this condition.

Setting 2 - Addition of a movable novel object in the experimental box (MoveAdd group). During the habituation sessions, two tunnels (200 mm × 120 mm × 80 mm) were present in each of zones B and C. They were arranged in the same manner (Fig. 2). On the first experimental day (trial 8), two supplementary tunnels were placed in zone C (Fig. 3). The tunnel located on top resembled a seesaw – it was the same structure as in the other configurations, but it was placed on four small pegs (which served as pivots) in the middle and at the end of the side walls. The pegs allow for movement only in one direction. This meant that when one end of the tunnel was touching the ‘roof’ of the tunnel underneath it, the other end was raised. The arrangement of the tunnels in zone B remained unchanged. A total of 35 (12 rats from sugar diet and 23 rats from standard diet) rats were used for this condition.

To avoid the deceptive effect of lateralization or visual-auditory cues, the novelty was introduced into the right zone (as described above - zone C) for half of the rats and in the left zone (zone B) for the remaining half (mirror image of Fig. 3).
2.4. Data processing and statistical analyses

We used BORIS software [55] to encode the behaviours on the basis of the recorded material, which made it possible to define selected behaviours and to assess their duration and frequency. We scored the behaviours the animals engaged in during the entire experimental trial. Consequently, we were able to assign specific scores to the time of separate bouts of behaviours, their frequency, and the total time the animals spent engaging in a given behaviour. The following variables were measured: (1) Time spent in the transporter (excluding the latency to leave the transporter); (2) Time spent in the unchanged zone of the chamber; (3) Time spent in the changed zone of the chamber; (4) Frequency of moving between the zones (left/right/transporter) of the chamber; (5) Time spent on contact with the tunnels in the unchanged zone of the chamber; (6) Frequency of contact with the tunnels in the unchanged zone of the chamber; (7) Time spent on contact with the tunnels in the changed zone of the chamber; and (8) Frequency of contact with the tunnels in the changed zone of the chamber.

To enhance the legibility of the results and tables, the habituation phase has been indicated as H, while the test trials have been indicated as T1, T2, and T3, respectively. Novelty (i.e. addition of tunnels) was introduced in the first test trial (T1). Different experimental settings (a movable or stationary tunnel as novel objects) are indicated in the Results as a “move” factor.

The first step of the analysis was to establish a reference value for the test trials. The mean value for the habituation trials H5 to H7 was calculated, and the created variable was labelled as ‘habitation phase’ (phase H). The next step consisted in calculating the Z-scores for phase H within the four experimental samples separately. Next, all values from test trials T1 to T3 were converted to Z-scores on the basis of phase H. The next step consisted in calculating the Z-scores for phase H within the four experimental samples separately. Next, all values from test trials T1 to T3 were converted to Z-scores on the basis of phase H statistics (Mean, StdDev). These data were analysed using a General test trials T1 to T3 were converted to Z-scores on the basis of phase H within the four experimental samples separately. Next, all values from test trials T1 to T3 were converted to Z-scores on the basis of phase H statistics (Mean, StdDev). These data were analysed using a General Linear Model procedure (GLM), with repeated measurements (H, T1, T2, T3) as within-subject factors, as well as diet and setting assignments as between-subject factors. This was followed by PostHoc t-tests with Bonferroni correction for multiple comparisons. Differences were considered significant for p ≤ 0.05.

In addition, partial eta squared (Eta²) obtained in the RM ANOVA were analysed using Kruskal-Wallis ANOVA to compare the effect size of different factors under study. Despite some limitations, partial eta squared (Eta²) is regarded as an effect size measure, which makes it possible to compare the results obtained in different subject groups or even studies. Therefore, we decided to use this measure in order to obtain more detailed information about the effects of experimental factors on rat behaviour in the experimental box. For non-significant effects, the Eta² value was set to “0”.

3. Results

3.1. Time spent in the transporter

The analysis showed an interactive effect of trial and move: F (3135)= 4.429, p = 0.005, Eta²= 0.090; a main effect of trial: F (3135)= 14.041, p < 0.001, Eta²= 0.238; and a main effect of move: F (1.45)= 18.637, p < 0.001, Eta²= 0.293. There was no interaction effect of trial, move and diet or effect of trial and diet or diet and move. There was no main effect of diet either.

A post hoc analysis showed that rats from different groups followed different patterns of reaction to change in the experimental box – Fig. 4. In the setup with added stationary tunnels, there was a decrease in the time spent in the transporter in the first test trial (T1: t = 6.151; p < 0.001). Then the time remained low. In the condition with the added movable tunnel, there was no change in the time spent in the transporter (p > 0.05). Differences between the groups were also observed in individual trials. Rats from the added stationary tunnel setup spent less time in the transporter than their counterparts from the added movable tunnel setup in all test trials (T1: t = 3.935; p = 0.003; T2: t = 3.298; p = 0.005).

Fig. 4. Z-scores for the time spent in the transporter in groups encountering different types of novelty.

3.2. Time spent in the changed zone of the chamber

Mauchly’s test indicated that the assumption of sphericity had been violated (χ2(5) = 17.884, p = 0.003), so the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε = 0.797). The analysis showed an interactive effect of trial and diet: F (2.391,107.604) = 5.010, p = 0.005, Eta² = 0.100. The following main effects were also detected: a main effect of diet: F(1,45) = 11.293, p = 0.002, Eta² = 0.201, and a main effect of trial: F(2.391,107.604) = 60.220, p < 0.001, Eta² = 0.572. There was no interaction effect of trial, group and diet or an interactive effect of move and diet or an interactive effect of trial and move or a main effect of the move.

A post hoc analysis showed that rats on different diets demonstrated different patterns of reaction to change in the experimental box – Fig. 5. Rats maintained on the standard diet increased the time spent in the changed zone in the first test trial compared to the habituation phase (T1: t = 6.191; p < 0.001). They also spent more time in this zone in the second (T2: t = 4.315; p < 0.001) and third test trials (T3: t = 6.194; p < 0.001) compared to the habituation phase. There were no differences in the time between test trials. However, in rats from the sugar-fed
group, after an increase of the time spent in the changed zone in the first test trial (T1: \( t = 11.382; p < 0.001 \)), there was a decline of the time in the second test trial (T2: \( t = 5.414; p < 0.001 \)). Then, the time remained constant in the third test trial. There were also differences in the time spent in the changed zone between the diet groups. Rats from the sugar-fed group spent more time in that zone than rats from the standard-diet group in the first test trial (T1: \( t = 4.737; p < 0.001 \)).

### 3.3. Time spent in the unchanged zone of the chamber

Mauchly’s test indicated that the assumption of sphericity had been violated (\( \chi^2(5) = 21.726, p < 0.001 \)), so the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (\( \varepsilon = 0.807 \)). The analysis showed an interactive effect of trial and diet: \( F(2.422,108.987) = 3.947, p = 0.016, \eta^2 = 0.081 \), a main effect of diet: \( F(1,45) = 24.349, p < 0.001, \eta^2 = 0.351 \), a main effect of move: \( F(1,45) = 11.075, p = 0.002, \eta^2 = 0.198 \), and a main effect of trial: \( F(2.422,108.987) = 12.155, p < 0.001, \eta^2 = 0.213 \). There was no interaction effect of trial, group and diet or effect of trial and group.

A post hoc analysis showed that rats on different diets demonstrated slightly different patterns of behaviour – Fig. 7. Rats maintained on the standard diet moved between the zones less frequently in the first test trial (T1: \( t = 4.351; p < 0.001 \)), and less frequently in the third test trial (T3: \( t = 5.240; p < 0.001 \)) compared to the first test trial, and again decreased in the third test trial (T3: \( t = 4.130; p = 0.002 \)). In rats from the sugar-fed group, the frequency of moving between the zones decreased in the first test trial (T1: \( t = 4.008; p = 0.003 \)). Then, the frequency increased in the second test trial (T2: \( t = 5.238; p < 0.001 \)), and then remained the same in the third trial (\( p > 0.05 \)). There was a difference in the frequency of moving between the zones in the third test trial between the diet groups. Rats from the sugar-fed group moved between the zones less frequently than rats from the standard-diet group (T3: \( t = 4.735; p < 0.001 \)).

A post hoc analysis showed that rats from different conditions also demonstrated different patterns of behaviour – Fig. 8. In the setup with added stationary tunnels, there was a decrease in the frequency of moving between the zones in the first test trial (T1: \( t = 5.923; p < 0.001 \)), then an increase in the second test trial (T2: \( t = 6.317; p < 0.001 \)), and again a decrease in the third test trial (T3: \( t = 5.727; p < 0.001 \)). In the setting with the added movable tunnel, there was no change in the test trials compared to the habituation phase (T1: \( p > 0.05 \)). However, a change between the first and third test trials was observed (\( t = 3.560; p = 0.014 \)), with higher frequency in the third test trial. A comparison of the frequency levels in the individual trials indicated that rats from the added movable tunnels setting moved between the zones more frequently than rats from the other setup in the third test trial (T3: \( t = 5.038; p < 0.001 \)).

### 3.4. Frequency of moving between the chamber zones (left/right/transporter)

The analysis showed an interactive effect of trial and diet: \( F(3135) = 8.055, p < 0.001, \eta^2 = 0.152 \); an interactive effect of trial and move: \( F(3135) = 11.212, p < 0.001, \eta^2 = 0.199 \); and a main effect of trial: \( F(3135) = 16.342, p < 0.001, \eta^2 = 0.266 \); a main effect of diet: \( F(1,45) = 5.123, p = 0.028, \eta^2 = 0.102 \); and a main effect of move: \( F(1,45) = 6.032, p = 0.018, \eta^2 = 0.015 \). There was no interaction effect of trial, group and diet and no interactive effect of diet and move.

### 3.5. Time spent on contact with the tunnels in the changed zone of the chamber

Mauchly’s test indicated that the assumption of sphericity had been violated (\( \chi^2(5) = 19.001, p = 0.002 \)), so the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (\( \varepsilon = 0.822 \)). The analysis showed an interactive effect of trial, diet and group: \( F(2.467,111.015) = 3.926, p = 0.016, \eta^2 = 0.080 \); an interactive effect

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**Fig. 6.** Z-scores for time spent in the unchanged zone of the chamber in the sugar-fed and standard-diet groups.

**Fig. 7.** Z-scores for the frequency of moving between the chamber zones in the sugar-fed and standard-diet groups.
of trial and diet: $F(2.467,111.015) = 3.894, p = 0.016$, $\eta^2 = 0.080$; an interactive effect of trial and move: $F(2.467,111.015) = 3.820, p = 0.018$, $\eta^2 = 0.078$; an interactive effect of diet and move: $F(1,45) = 16.795, p < 0.001$, $\eta^2 = 0.272$; a main effect of diet: $F(1,45) = 9.428, p = 0.004$, $\eta^2 = 0.173$, a main effect of move: $F(1,45) = 15.804, p < 0.001$, $\eta^2 = 0.260$, and a main effect of trial: $F(2.467,111.015) = 62.019, p < 0.001$, $\eta^2 = 0.580$.

Post hoc analyses showed differences in reaction to change between different diet groups subject to different conditions during the experiment – Fig. 9. Rats from the group maintained on the standard diet spent more time exploring the added stationary tunnels in the first test trial ($T1: t = 4.112; p = 0.008$), and that amount of time remained high in consecutive test trials. A similar pattern was observed in the rats maintained on the standard diet which encountered the added movable tunnel ($T1: t = 5.550; p < 0.001$). There were no differences between these two groups in the individual trials.

Although in rats maintained on the sugar diet there was also an increase in the time spent on contact with the tunnels in the first test trial in the setting with stationary tunnels ($T1: t = 10.600; p < 0.001$), as well as in the setting with movable tunnels ($T1: t = 5.479; p < 0.001$), there were differences in the amount of time between individual trials. Rats from the group confronted with a stationary tunnel spent more time exploring the tunnels in the changed zone than rats which encountered a movable tunnel in the first ($T1: t = 4.621; p < 0.001$), second ($T2: t = 4.745; p < 0.001$), and third test trials ($T3: t = 4.743; p < 0.001$).

In addition, rats from the sugar-fed group spent more time exploring the stationary tunnels than rats from the standard-diet group in the first ($T1: t = 5.855; p < 0.001$), and in the second test trial ($T2: t = 3.759; p = 0.028$). There were no differences between the diet groups in the see-saw-tunnels setup.

### 3.6. Time spent on contact with the tunnels in the unchanged zone of the chamber

The analysis showed only a main effect of trial: $F(3135) = 6.713$, $p < 0.001$, $\eta^2 = 0.130$, and a main effect of move: $F(1,45) = 14.621$, $p < 0.001$, $\eta^2 = 0.245$.

In general, rats from the added movable tunnel setup spent more
time on contact with tunnels in the unchanged zone than rats from the other setup ($t = 3.824; p < 0.001$).

### 3.7. Frequency of contact with the tunnels in the unchanged zone of the chamber

The analysis showed an interactive effect of trial and diet $F(3135) = 3.539, p = 0.016$, $\eta^2_p = 0.073$, a main effect of trial: $F(3135) = 4.072, p = 0.008$, $\eta^2_p = 0.083$, and a main effect of diet: $F(1,45) = 16.102, p < 0.001$, $\eta^2_p = 0.264$, and a main effect of move: $F(1,45) = 6.588, p = 0.014$, $\eta^2_p = 0.128$. There was no interaction effect of trial, move and diet or effect of trial and move. No interactive effect of move and diet was found either.

A post hoc analysis showed differences between the rats on different diets in terms of their reaction to change in the experimental box. Rats maintained on the standard diet interacted with the tunnels in the unchanged zone in the third test trial less frequently than in the habituation phase (T3: $t = 3.576; p = 0.014$). There were no differences between the first and second test trials or between the second and third trials. In rats maintained on the sugar-rich diet, there were no changes in the frequency of interactions with tunnels in the unchanged zone ($p > 0.05$) throughout the experiment. The diet groups differed in terms of frequency of contact with the tunnels across individual trials. Rats from the sugar-fed group demonstrated a higher frequency than rats from the standard-diet group in the second test trial (T2: $t = 3.610; p = 0.011$) and the third test trial (T3: $t = 3.773; p = 0.006$).

Moreover, rats from the group which encountered the movable tunnels had contact with the tunnels in the unchanged zone more frequently than the rats from the added stationary tunnel setup ($t = 2.567; p = 0.014$).

### 3.8. Frequency of contact with the tunnels in the changed zone of the chamber

The analysis showed only a main effect of the trial: $F(3135) = 7.126, p < 0.001$, $\eta^2_p = 0.137$. There was no interaction effect of trial, group and diet or effect of trial and group or effect of trial and diet. No main effects of group or diet were found either.

A post hoc analysis showed a significant increase in the frequency of interaction with the tunnels in the changed zone in the third test trial (T3: $t = 4.608; p < 0.001$) compared to the habituation phase. There were no changes between the first, second and third test trials.

### 3.9. Effect size analysis

Kruskal-Wallis ANOVA showed ($H = 22.004, df = 6, p < 0.001$) that the effects associated with the experimental factors ranked differently, with the Trial factor ranking first, followed by Move and Diet factors. Table 1 shows the median of $\eta^2_p$ for experimental effects.

### 3.10. Summary of results

Rats maintained on the sugar diet differed substantially from rats maintained on the standard diet in terms of their reaction to change introduced in the experimental chamber. Subjects from the sugar-fed group increased their activity in the zone where the change was introduced in the first test trial, but then the activity decreased and remained at a low level in the third test trial. On the other hand, subjects from the standard-diet group increased their activity after the introduction of novelty, but the activity remained high until the end of the experiment. All groups of rats increased their contact with the tunnels in the changed zone after new tunnels had been introduced. However, rats from the sugar-fed group spent more time exploring the stationary tunnels than investigating the movable tunnels across all test trials. In addition, the sugar-fed group spent more time exploring the stationary tunnels compared to the standard-diet group (in the first and second test trials). In addition, the increase in the duration of contact with the tunnels after the introduction of novelty was much higher in the sugar-fed group than in the standard-diet group (in the stationary tunnels setting).

Differences were also observed in the unchanged zone of the box. Animals from the standard-diet group decreased the amount of time spent in this zone after the novelty had been introduced in the other zone, while their activity remained low in the subsequent trials. Animals from the sugar-fed group maintained the same level of activity in the unchanged zone despite the introduction of novelty in the other zone. In general, rats from the sugar-fed group spent more time in the unchanged zone than the rats from the standard-diet group (in the second and third test trials). Moreover, the frequency of contacts with the tunnels in the unchanged zone was higher in rats from the sugar-fed group (in the second and third test trials).

Rats from different diet groups differed slightly in terms of the frequency of moving between the chamber zones. Both groups decreased the rate of moving between the zones in the first test trial and then increased it in the second trial, but the animals from the control group again decreased the frequency in the third trial, while the rats from the sugar-fed group maintained the same frequency as in the second test trial.

Furthermore, rats from the stationary tunnels setting reduced their frequency of moving between the zones after the introduction of the novel tunnels then increased the frequency in the second test trial and decreased it again in the last test trial. Rats from the movable tunnels setting did not change their behaviour in this respect after the introduction of novelty, but the frequency in the third trial was higher than in the first trial.

There was also a difference in the amount of time spent in the transporter between the experimental setups. Rats from the stationary tunnels group decreased the amount of time spent in the transporter in the first trial and generally spent less time in the transporter than the rats from the other setup.

The effect size analysis has shown that the experimental factors directly linked to the test arena arrangement (Trial and Move) play a major part in explaining the variability in the rats’ behaviour. This seems in line with current knowledge about environmental control over behaviour. The Diet factor was placed in the third position, with the median $\eta^2_p = 0.137$. The Diet factor, together with the interaction of Diet and Trial factors, explained more than twenty percent of behaviour variance ($\eta^2_p = 0.217$), which should be considered to be a powerful effect.

### 4. Discussion

Studying intrinsically motivated exploratory behaviour makes it possible to formulate hypotheses about the effects of experimental manipulation on reward-mediated neural pathways. This approach is based on the assumption that neophilic preferences, which are the main feature of exploration, reflect the reward value of novelty [37]. The mesolimbic dopamine system plays a major role in the processing of the reward value of novelty, as it does for drugs of abuse and other forms of reinforcement [56]. Novel stimuli are known to excite dopamine neurons [57] and boost signalling in brain regions receiving dopaminergic input [58]. Therefore, the influence of factors affecting the reward

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**Table 1**

Median of $\eta^2_p$ for experimental effects.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial</td>
<td>8</td>
<td>0.225</td>
</tr>
<tr>
<td>Move</td>
<td>8</td>
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</tr>
<tr>
<td>Diet</td>
<td>8</td>
<td>0.137</td>
</tr>
<tr>
<td>Trial x diet</td>
<td>8</td>
<td>0.08</td>
</tr>
<tr>
<td>Diet x move</td>
<td>8</td>
<td>0.0</td>
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<tr>
<td>Trial x move</td>
<td>8</td>
<td>0.0</td>
</tr>
<tr>
<td>Trial x move x diet</td>
<td>8</td>
<td>0.0</td>
</tr>
</tbody>
</table>
system may well be detected in the analysis of reactions to novelty (cf. [59]). As mentioned in the Introduction, excessive sugar consumption may lead to changes in the dopamine system similar to those observed in drug addiction (e.g. [4]). We hypothesised that the influence of a sugar-rich diet on the brain reward system may be assessed by analysing exploratory behaviour.

The results of our study showed differences in reactions to novelty between different diet groups in different experimental settings. It is known that the introduction of novelty into a familiar environment triggers a range of investigative behaviours directed at the source of the novel stimuli [37]. In our experiment, the animals maintained on a sugar-rich diet responded to the change in the experimental box by spending three times more time exploring the novel stimulus than the animals from the standard-diet group. The amount of time spent in the changed zone of the experimental arena was also much higher but contrary to the standard-diet group, which spent more time in the changed zone throughout the consecutive sessions, the rats from the sugar-fed group spent less time in that zone in the next test session and maintained this lower level in the third test trial. These results may indicate a lowered reward value of novelty in the sugar-fed group, which needed to spend more time on exploration in order to reach the same level of reward as the other group. This argument is supported by the fact that the diminished responsiveness of the reward system was detected in the pre-test period when the rats kept on the sugar-rich diet increased their sugar intake in the course of eight weeks. In addiction studies, this reaction is considered to be a sign of tolerance to substances consumed in excessive amounts and cravings (e.g. [25], for a review, see [4]). This strong reaction to novelty may also suggest a cross-sensitisation effect of sugar, eliciting a stronger response to the rewarding stimulus (cf. [60,61]). However, sensitisation often involves increased motor activity (e.g. [4]), which was not observed in our study as measured by the frequency of moving between the zones of the experimental box. Both groups demonstrated similar levels as regards this behaviour until the last test trial when the rats from the standard-diet group reduced the frequency of moving between the zones of the experimental arena, but the rats from the sugar-fed group exhibited a sustained high frequency of this behaviour. This effect, however, seems to be consistent with the overall higher demand for stimulation in the sugar-fed group.

Enhanced reactions to the introduction of novelty may also be explained in terms of heightened impulsivity in the sugar-fed group (cf. [62]). Impulsive behaviours in chronic drug abusers have been found to be associated with reduced dopamine D2/3 receptors in the striatum (e.g. [63]), and a low D2/3 receptor availability was correlated with lower latency to come into contact with the novel object, which is consistent with higher novelty-reactivity and potentially higher impulsivity [64]. Again, these insights point to the dopaminergic system being involved in establishing differences in behaviour between the different diet groups.

Reaction to novelty is not the only element of exploratory behaviour that can be empirically observed. As proposed by Berlyne in his classical work [65], exploration involves not only specific exploration directed at a wide range of stimuli. This type of behaviour enables an ongoing revision of mental maps and enhanced certainty as to the information obtained from the environment, which seems to be a crucial adaptive function [66]. In our study, rats from the sugar-fed group reacted to the introduction of novelty with a high rate of object exploitation, but at the same time, they did not reduce the time spent in the unchanged zone of the experimental box and maintained a relatively high rate of contacts with the unchanged tunnels in the test trials. This may indicate a higher level of divisive exploration in this group, suggesting a higher demand for updated information about the environment. Although the neural substrates of this distinctive type of exploration are yet to be identified, it may be hypothesised that the hippocampal regions involved in memory processing are employed. Based on our findings, it is unclear if this behaviour relates to memory malfunction in sugar-fed rats. It is also likely that this kind of “broad” exploration constitutes, to some degree, a source of reward to the animals and that it is influenced by the same changes in the reward system as the reaction to the object’s novelty. Some support for this point of view is provided by Ricker and colleagues [67], who claim that a self-directing choice behaviour can lead to the choice itself becoming a reinforcing factor.

However, these findings hold true only with regard to the stationary tunnels’ setting. Novelty may also involve changes in the complexity of the environment, with more complex objects eliciting a stronger exploratory reaction [43,54]. The magnitude and scope of this reaction depend on the degree of change in the environment. It is characterised by the discrepancy between a single exposure to a stimulus and subsequent exposures. Therefore, an increase in complexity should lead to increased exploration. In our study, the introduction of a movable tunnel did not elicit an enhanced exploratory response in the sugar-fed group, and the rats’ activity was similar to the activity of the standard-diet animals. It is possible that the movable object (placed on top of the two-level tunnel structure and moving unexpectedly like a seesaw when a rat interacted with it or climbed on top of it) may have evoked a source of stress. Even though the animals could recognise the stimulus as novel, they may have chosen not to approach or investigate it. Neophobia (fear of novelty) can suppress neophilia if the degree of novelty is too high or if some other factors generate high levels of fear, as demonstrated in the theoretical analysis by R. Hughes [37]. It seems that this fear reaction was more pronounced in the sugar-diet group, resulting in the reward value of novelty being reduced. The reasoning about the fear response to a movable novel object is also supported by the differences in time spent in the starting box between the groups encountering different types of novelty. The starting box is a safe, familiar environment, and the time spent in it may be considered a measure of anxiety (cf. [68]). In our study, the rats exposed to novelty in the form of a seesaw tunnel spent more time in the starting box in all test sessions, which may suggest that this change increased the level of neophobia. However, the fear response hypothesis does not support the earlier assumption about increased impulsivity in the sugar-fed group. Stressful situations should lead to more impulsive behaviours and higher novelty/sensation seeking in this group (cf. [69]).

Another aspect of neural changes resulting from excessive sugar consumption, which can be discussed by analysing exploratory behaviour, is memory processing. Numerous studies point out to the hippocampus as the region significantly affected by sugar-rich diet (e.g., [6, 11–16]). It has been suggested that relationships between hippocampal memory processes and neotic preferences may specifically involve novelty detection in which current experiences are compared with encoded information about past events [70–72]. If such comparisons result in a discrepancy between the stored data and the current event, the detection of novelty would be registered and addressed with specific behaviour [73]. Hippocampal impairment may lead to interference with information comparison and thus with the ability to detect or respond to novelty [74,75]. Another sign of properly functioning memory is the relatively quick pace of habituation to change. Our analyses have not shown any negative changes in memory processing. Habituation to the experimental setup was similar in animals from both diet groups, as measured by the reduction in the time spent investigating the novel tunnels in the successive test trials. The rapid decrease in time spent in the changed zone of the experimental arena seems to support this conclusion. It is not necessarily in conflict with previous findings, as the memory functions of the hippocampus are mainly associated with its dorsal region, in contrast to the regulation of fear or anxiety-like response associated with the ventral region of the hippocampus [75]. It may be speculated that different regions of the hippocampus respond differently to excessive sugar consumption (cf. [76]). However, not all studies on the effect of sucrose consumption have detected impairments in all memory functions [77,78]. What is more, some findings indicate that sucrose overconsumption may lead to improved learning in the
To sum up, the results of our study seem to corroborate previous findings about the significant influence of intermittent sucrose consumption on the brain reward system. Despite a subtle manipulation of the feeding protocol, which can be roughly compared to humans eating a few sweets every day, the observed behavioural changes in the experimental group seem very significant. As illustrated by effect size analyses, the diet factor is responsible for over 20% of the behavioural variance. All animals were kept in very similar conditions. There were no differences in the animals’ weight and overall characteristics. It looks, therefore, that even in the test situation, where the proximate/situational factors play major roles, subjects following the sugar-rich diet behaved significantly differently compared to their counterparts maintained on the standard rat diet. On the other hand, we have not found any evidence for memory and/or learning impairments in rats on a sugar-rich diet. It may be suggested that the dosage of sucrose proposed in our feeding scheme was too low or that sucrose consumption has a limited damaging effect on the hippocampus. Nonetheless, studying exploratory behaviour allows us to assess the impact of a sucrose-rich diet on a broad spectrum of behaviours related to the general functioning of the organism in its environment. Our study has contributed to the existing body of knowledge about the influence of diet on cognitive and emotional development.

Yet, the presented above analyses of results have some important limitations. Our study aimed to pinpoint the relation between sugar overconsumption and its behavioural correlates. Further, we attempted to interpret this result and suggest possible neural mechanisms. However, it should be noted that by proposing hypotheses about changes in neural functioning we built on the previous extensive literature on the subject. We are aware that this approach does not exhaust the subject and requires further research into neural processes and changes involved in excessive sugar consumption.

CRediT authorship contribution statement

KM - Conceptualization; Funding acquisition; Methodology; Project administration; Resources, Supervision; Validation; Writing – original draft; Writing – review & editing; WP - Conceptualization; Data curation; Formal analysis; Methodology; Validation; Visualization; Writing – original draft; Writing – review & editing; AC and KG - Conceptualization; Investigation. All authors reviewed and accepted the manuscript.

Declaration of Competing Interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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Appendix 1. Descriptive data for analysed variables

<table>
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<tr>
<th>Trials</th>
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<th>T2</th>
<th>T3</th>
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<td>Mean</td>
<td>Stdev</td>
<td>Mean</td>
<td>Stdev</td>
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