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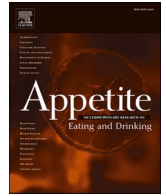
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Effects of Highly Palatable Diet on motivation for food and resistance to punishment in rats: Role of sex and age of exposure

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ABSTRACT

Exposure to highly palatable food is believed to induce behavioral and neurobiological changes that may produce addiction-like behavior and increase the risks of obesity and overweight. Studies in rodents have led to conflicting results suggesting that several factors such as sex and age of exposure contribute to the development of maladaptive behaviors towards food. In addition, it is not clear whether effects of exposure to highly palatable diets (HPD) persist after their discontinuation, which would indicate long-term risks to develop addiction-like behavior. In this study, we investigated the persistent effects of an intermittent 8-week exposure to HPD in male and female rats as a function of age of exposure (adult and adolescent). We found that intermittent exposure to HPD did not alter body weight, but it affected consumption of standard food during the time of exposure in all groups. In addition, in adults, HPD produced a decrease in the initial baseline responding in FR1 schedules, an effect that persisted for 4 weeks in males but not in female rats. However, we found that exposure to HPD did not affect resistance to punishment measured by progressive shock strength break points or motivation for food as measured by progressive-ratio break points regardless of sex or age of exposure. Altogether, these results do not provide support for the hypothesis that intermittent exposure to HPD produce persistent increases in the vulnerability to develop addiction-like behaviors towards palatable food.

1. Introduction

The prevalence of obesity and overweight has dramatically increased worldwide in the last decades (World Health Organization, www.who.int). Although obesity has increased in the entire population, the increases appear more pronounced among children, adolescents and women (WHO, www.who.int), suggesting that developmental and hormonal factors have an impact on this condition. Importantly, obesity is associated with increased mortality, morbidity and impaired quality of life (Abdelaal et al., 2017), and an analysis of societal costs has estimated that its economic impact is on average 1.8% of gross domestic product and may increase up to 3.6% in 2060 (Okunogbe et al., 2021). Therefore, understanding the factors that influence obesity and the underlying biological mechanisms is critical to designing strategies to limit its spread.

In humans, not only continuous consumption, but also cycling between periods of consuming food rich in fat and sugar, separated by self-

imposed periods of dieting with “safe food”, could alter the control of feeding and contribute to the development of obesity and overeating (Dulloo et al., 2015). In rats, several studies have shown that an intermittent access to highly palatable diets (HPD), typically containing medium to high concentrations of fat and sugar, promotes behaviors similar to those found in substance use disorder. For example, intermittent access to HPD produces compulsive behavior in rats (Rossetti et al., 2014), withdrawal-like states characterized by the emergence of negative emotional state and anxiety (Cottone, Sabino, Roberto, et al., 2009; Iemolo et al., 2012), by decreased reward system functioning (Moore et al., 2020) and by impaired memory and hippocampal neurogenesis (Ferragud et al., 2020). Nevertheless, the long-lasting consequences of exposure to HPD on behavior remain unclear, and preclinical studies are essential to determine the behavioral, physiological and neurobiological mechanisms underlying the development of maladaptive behavior and food disorders that lead to obesity (O'Connor & Kenny, 2022).

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Two main measures are often used to characterize addiction-like behavior: excessive motivation measured by progressive ratio (PR) schedules (Hodos, 1961) and compulsivity measured by resistance to punishment (George et al., 2022; Vanderschuren & Ahmed, 2013). Several studies have investigated the effects of exposure to HPD on motivation and compulsivity and have obtained conflicting results. For example, some studies found increases in motivation (Wojnicki et al., 2006; la Fleur et al., 2007; Figlewicz et al., 2013; Garman et al., 2021), some found decreases (Blaisdell et al., 2014; Davis et al., 2008; Tracy et al., 2015; Vendruscolo et al., 2010; Wong et al., 2017) and others found no effect (Jong et al., 2013; Rossetti et al., 2014; Tracy et al., 2015). Similarly, for resistance to punishment, some studies found increases (Johnson & Kenny, 2010; Oswald et al., 2011; Rossetti et al., 2014) and one found no effect (Jong et al., 2013). These discrepancies may be due to differences in the sex and the age of the animals, the duration and type of exposure to HPD prior to eventual discontinuation of HPD, and the PR or punishment procedures used to assess addiction-like responding.

We have recently developed a self-adjusting procedure to investigate punishment in rats (Desmercieres et al., 2022). The progressive shock strength (PSS) procedure, similar to procedures commonly used in humans (Apergis-Schoute et al., 2017; Kanen et al., 2021; Kim & Anderson, 2020), allows individuals to titrate the level of punishment they are willing to receive in order to obtain a reward, and it provides PSS breakpoints that are sensitive to motivation states and to the value of the reward (Desmercieres et al., 2022). This procedure allows a continuous rather than dichotomic evaluation of resistance to punishment and may provide a more precise measure of the effects of exposure to HPD on resistance to punishment.

In this study, we used the PR and the PSS procedures to assess the effects of HPD on motivation and compulsivity for sweet rewards. Because sex is an important risk factor for severe obesity (Hales, 2020), we investigated the effects of HPD in both male and female rats. In addition, because adolescence is a critical period for brain development (Spear, 2000) and a window of vulnerability for the development of maladaptive behaviors including addiction (Chambers et al., 2003) and eating disorders (Klump, 2013), we investigated the effects of exposure to HPD in both adolescent and adult rats.

2. Methods

2.1. Subjects

Sprague-Dawley rats (Janviers Labs) of both sexes were used: 47 adolescents (4 weeks of age at the beginning of the HPD exposure; 155–160 g for females and 130–135 g for males) and 48 adults (10 weeks of age at the beginning of the HPD exposure; 230–250 g for females and 320–350 g for males). All rats were experimentally naïve at the start of the study and housed in pairs throughout the experiments, in

individually ventilated cages (Techniplast, Sealsafe Plus GR900) with two wooden sticks provided for enrichment, in a temperature- and humidity-controlled room kept under reversed light-dark cycle conditions (12 h light-dark cycle, lights off at 7 a.m.). All experiments were conducted during the dark phase and in accordance with European Union directives (2010/63/EU) for the care of laboratory animals and approved by the local ethics committees (COMETHEA; # 2018100509097824).

2.2. General design of the experiment

General design of the experiment is illustrated in Fig. 1. First, rats were exposed to intermittent HPD or control diet for 8 weeks. HPD was discontinued at the end of this period, and standard diet was used for all groups for the remainder of the experiment. Mild food restriction (~15 g/day for females and ~20 g/day for males) was implemented to motivate food responding, limit weight gain and maintain stable behavior until the end of the experiment. Food was given 1 h after the end of the experimental sessions. Rats had unlimited access to water. Starting one week after the last exposure to HPD, rats were allowed to self-administer food pellets for 9 training sessions, and then resistance to punishment and food motivation were measured alternately once per week for 6 weeks. A typical week included 4 training sessions and 1 test session. At the end of the experiment, anxiety levels and sensitivity to pain were measured on the same day.

We performed 4 separate experiments: 1) effects of HPD in adolescent males; 2) effects of HPD in adolescent females; 3) effects of HPD in adult males; and 4) effects of HPD in adult females.

2.3. Exposure to HPD

We used the HPD exposure procedure described by Rossetti et al. which was shown to induce compulsivity for food in female rats (Rossetti et al., 2014). Briefly, for eight weeks in their home cage, HPD rats had access only to highly palatable 5TCY Tablets (Test diets, LabTreat™ OmniTreat™ Enrichment Tablet, 5g/Tablet) during weekends (Friday 6:00 p.m. to Monday 9:00 a.m.) and access to only standard food (4RF21, Mucedola, Italy) for the rest of the week. Control rats had access only to standard food (4RF21, Mucedola, Italy) all days of the week. HPD and standard diets had a similar number of calories (see Table 1) and were given *ad libitum* during the exposure.

Food consumption was measured by weighing food at 1- or 2-day intervals for each cage. The amount of food consumed was divided by the number of animals in the cage and the number of days to obtain an average daily value of food consumption. Food consumption was expressed in kcal/day by multiplying the weight of food eaten by the respective energy density.

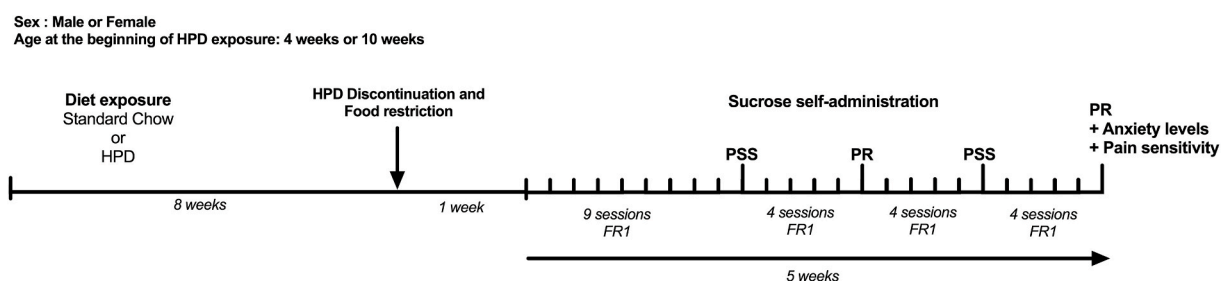


Fig. 1. General Experimental Design. Male or female rats were exposed to HPD or control diet starting at adolescence (4 weeks of age) or early adulthood (10 weeks of age) for 8 weeks according to an intermittent schedule in which HPD was available only on weekends and all rats had access to only standard chow on weekdays. Control rats had access only to standard chow throughout the experiment. After 1 week of discontinuation of HPD and initiation of mild food restriction, sucrose self-administration started with a FR1 schedule for 9 training sessions and then PSS and PR sessions were conducted every fifth day (on Fridays) alternately with interspersed additional FR1 sessions.

Table 1**Diet composition.** NFE = nitrogen-free extracts.

Food	Energy density (kcal/g)	Macronutrient composition (kcal%)					
		Carbohydrate			Fat	Protein	Others (Moisture, Ash and Fiber)
		NFE	Starch	Sucrose			
4RF21 Mucedola (Standard food)	3.150	53.5	42.63	3.68	3	18.5	25
5TCY Test diets (High Palatable Diet)	3.290	63.6	1.38	55.81	22.5	13.9	0
Precision pellet 5TUT 45 mg (Sweet pellets)	3.404	100	0	100	0	0	0

2.4. Food reinforcement Apparatus and training procedure

Experimental chambers (MedAssociates, www.medassociates.com) were enclosed individually in sound-attenuation chests. Each experimental chamber had a recessed food tray, and two levers on the right wall. The floor was constituted by bars that were connected to shockers that could deliver foot-shocks, with electric current set to 0.45 mA. Each chamber was equipped with a food-pellet dispenser, which could deliver 45 mg pellets in the food tray. Experimental events were controlled by computers using MedAssociates interface and Med-PC IV software. Med-PC code used to conduct the procedures is available upon request to the corresponding author. A diode light was present on each lever. One lever was assigned to be the active lever and the corresponding light was used as a conditioned stimulus for food reinforcement. A third diode light was installed on the opposite wall, and its flashing was used as a discriminative stimulus to indicate that food reinforcement would be associated with a foot shock.

The general training consisted of 45-min sessions of a fixed-ratio 1 (FR1) schedule of food reinforcement in which one lever press was required to obtain a 45 mg sucrose pellet. During these sessions, food availability was signaled by turning off the house-light, indicating that a single response on the active lever would immediately result in the delivery of one food pellet, accompanied by flashing of the diode light above the active lever for 2 s. Subsequently, the house light was turned on for an additional 18-sec time-out period, during which responding had no programmed consequences. Following the time-out, a new trial started and the next response on the active lever was again reinforced. Responses on the inactive lever were recorded but never reinforced.

2.5. Self-adjusting progressive shock strength (PSS) procedure

Resistance to punishment was measured in the 10th and 20th session as previously described (Desmercières et al., 2022). In the PSS procedure, active lever presses resulted in the delivery of food rewards and foot-shocks of different strengths. The PSS consisted of steps in which the shock duration was increased each time the animal obtained 2 consecutive rewards at a given duration. The duration of the first step was 0 s (no shock), the second step was a low duration of 0.05 s and subsequent shocks increased of 15% at each step for 20 steps. Thus, the step sequence of durations was: 0, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.12, 0.13, 0.15, 0.18, 0.20, 0.23, 0.27, 0.31, 0.35, 0.41, 0.47, 0.54, 0.62, and 0.71 s. At the beginning of each shock trial, the light on the opposite side of the levers and food tray was switched on and off intermittently for the entire trial for periods of time proportional to the duration of the shock to signal the presence of shock contingencies according to the formulas: Duration On = 0.1 s times step number and Duration Off = 2 s – Duration On. If animals reached the final step, the duration of the shock was not further increased, and all subsequent shocks were set at 0.71 s. If rats did not emit any active response for 5 min, shock duration was reset to 0 s and the shock progression was reinitialized. Thus, animals could at any moment avoid higher strength of shock by limiting the frequency of food reinforcement until shock duration returned to 0 s, which was signaled by the absence of the flashing light. The strength of the shock was measured by the electrical charge in millicoulombs (mC) that an animal would tolerate to obtain

food pellets and was calculated by multiplying the fixed current of the shock (0.45 mA) by the duration in sec. PSS sessions lasted 45 min and the break point was calculated by the cumulated electrical charge sustained during the session.

2.6. Progressive-ratio (PR) schedule

Appetitive motivation was measured in the 15th and 25th session. Under the PR schedule of food reinforcement, the number of responses required to obtain a food pellet increased with each successive food pellet. The steps of the exponential progression were the same as those previously developed by Roberts and colleagues (Richardson & Roberts, 1996) adapted for food reinforcement (Solinas et al., 2003; Solinas & Goldberg, 2005), based on the equation: response ratio = $(5e^{(0.2 \times \text{reinforcer number})}) - 5$, rounded to the nearest integer. Thus, the values of the steps were 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, and 737. Sessions under the progressive-ratio schedule lasted until 10 min passed without completing a step, which occurred within 1 h under basal conditions.

2.7. Anxiety-like behaviors: elevated plus maze

The elevated plus maze (Viewpoint, Lyon, France) consisted of 4 arms (50 cm long X 10 cm wide at 42.5 cm from the floor) separated by a central square (10 × 10 cm) in the middle giving access to all the arms. Two arms had no walls (open arms) and two had black walls on their edges (closed arms). Rats were placed in the central square and their position was recorded automatically and in real time by a camera and video tracking software (Viewpoint, Lyon, France) for 10 min. The software defined four virtual areas corresponding to the arms and a center zone. Anxiety was measured as the time spent in open arms so that more time spent in open arms indicated a lower level of anxiety.

2.8. Pain sensitivity: hot-plate

The hot-plate (Ugo Basile, model-DS 37) was maintained at 48 °C (Deuis et al., 2017). After a habituation of 10 min to the experimental room, animals were placed into a glass cylinder of 25 cm diameter on the heated surface and 47 cm walls. The latency before escape or jumping, was recorded. Experiments were stopped after a cut-off of 120 s to prevent unnecessary pain or tissue damage.

2.9. Statistical analysis

The main hypothesis that exposure to HPD would induce increase resistance to punishment and the analytic plan were specified before the data were collected. Data were analyzed by GraphPad Prism software. Data were checked for normality of distribution using the Shapiro–Wilk test. Data for weight gain and food consumption were assessed by two-way ANOVA for repeated measures followed by Sidak's multiple comparisons post hoc test. PSS data were normalized using natural logarithm transformation as previously done (Desmercières et al., 2022). For baseline measures of PSS and PR break points, we used two-way ANOVA for repeated measures with time and diet as factors followed by Sidak's multiple comparisons post hoc test. When the assumption of sphericity

was violated, data were analyzed using analysis of variance (ANOVA) corrected by the Greenhouse–Geisser method. Anxiety level and pain sensibility were assessed by the non-parametric Mann-Whitney test. Differences were considered significant when $P < 0.05$. All data are freely available on the OSF site (<https://osf.io/3qnbv/>).

3. Results

3.1. HPD does not alter body weight but alters food consumption during the period of exposure

Body weight was similar between HPD and control rats during the entire duration of the experiment in all experimental groups. In adult male rats, at the beginning of the experiment, control rats weighed 359 ± 4 g and HPD rats weighed 355 ± 3 g (Fig. 2A) and, at the end of the experiment, 610 ± 8 and 618 ± 9 g, respectively (Fig. 2A) [significant effect of time: $F(1.584, 34.85) = 1966, P < 0.0001$; Greenhouse–Geisser $\epsilon = 0.20$], no effect of diet ($F(1, 22) = 0.03016, P = 0.8637$) and no time \times diet interaction ($F(8, 176) = 0.9746, P = 0.4574$]. In adult female rats, at the beginning of the experiment, control rats weighed 243 ± 3 g and HPD rats weighed 244 ± 3 g (Fig. 2B) and, at the end of the experiment, 309 ± 5 g and 311 ± 4 g respectively (Fig. 2B) [significant effect of time ($F(3.448, 75.86) = 433.6, P < 0.0001; \epsilon = 0.43$), no effect of diet ($F(1, 22) = 0.4023, P = 0.5325$) and no significant time \times diet interaction ($F(8, 176) = 0.4958, P = 0.8581$)]. In adolescent male rats, at the beginning of the experiment, control rats weighed 136 ± 2 g and HPD rats weighed 130 ± 2 g (Fig. 2C) and, at the end of the experiment, 547 ± 6 g and 544 ± 7 g, respectively (Fig. 2C) [significant effect of time ($F(2.295, 50.50) = 7326, P < 0.0001; \epsilon = 0.29$), no effect of diet ($F(1, 22) = 0.4127, P = 0.5272$) and significant time \times diet interaction ($F(8, 176) = 1.993, P = 0.0498$)]. In adolescent female rats, control rats weighed 156 ± 3 g and HPD rats weighed 159 ± 2 g respectively at the beginning of the experiment (Figs. 2D) and 304 ± 7 g and 307 ± 4 g at the end of the experiment (Fig. 2D) [significant effect of time ($F(2.887,$

$60.64) = 499.4, P < 0.0001; \epsilon = 0.36$], no effect of diet ($F(1, 21) = 0.1359, P = 0.7161$) and no significant time \times diet interaction ($F(8, 168) = 0.8958, P = 0.5214$).

During the weekends, when the HPD group had access to HPD and standard food but the control group only had access to standard food, differences in food consumption were observed. In adult male rats, food consumption was similar in the two groups except on the third weekend, where the HPD group had a higher daily consumption (120.2 ± 3.0 kcal/day) compared to the control group (99.5 ± 1.9 kcal/day) (Fig. 3A). Then, both groups stabilized their consumption at about 98 kcal/day (Fig. 3A) [significant effect of time ($F(2.78, 27.75) = 20.48, P < 0.0001; \epsilon = 0.40$), no effect of diet ($F(1, 10) = 2.27, P = 0.16$) and significant time \times diet interaction ($F(7, 70) = 8.43, P < 0.0001$)]. In adult female rats, the HPD group had a higher consumption of food (70.3 ± 1.2 kcal/day) compared to the control group (62.2 ± 0.9 kcal/day) in the first 6 weekends (Fig. 3B). Then, the consumption stabilized and became similar in the two groups at about 60 kcal/day (Fig. 3B) [significant effect of time ($F(3.044, 30.44) = 9.86, P < 0.0001; \epsilon = 0.43$), significant effect of diet ($F(1, 10) = 13.32, P = 0.0046$) and no significant time \times diet interaction ($F(7, 70) = 2.05, P = 0.06$)]. In adolescent male rats, food consumption in the HPD group was higher compared to the control group even though differences at no single time point reached statistical significance (Fig. 3C) [significant effect of time ($F(3.790, 37.90) = 108.4, P < 0.0001; \epsilon = 0.54$), no significant effect of diet ($F(1, 10) = 0.70, P = 0.42$) and significant time \times diet interaction ($F(7, 70) = 6.09, P < 0.0001$)]. In adolescent female rats, HPD and control groups initially had similar daily consumption of food but after 3–4 weeks the HPD group showed higher consumption (70.14 ± 1.6 kcal/day) than the control group (60.26 ± 1.6 kcal/day) (Fig. 3D) [no significant effect of time ($F(3.15, 31.50) = 2.08, P = 0.12; \epsilon = 0.45$), significant effect of diet ($F(1, 10) = 9.3, P = 0.012$) and no significant time \times diet interaction ($F(7, 70) = 0.73, P = 0.64$)].

Concerning daily food consumption during the rest of the week, when all groups had access only to standard food, differences were also

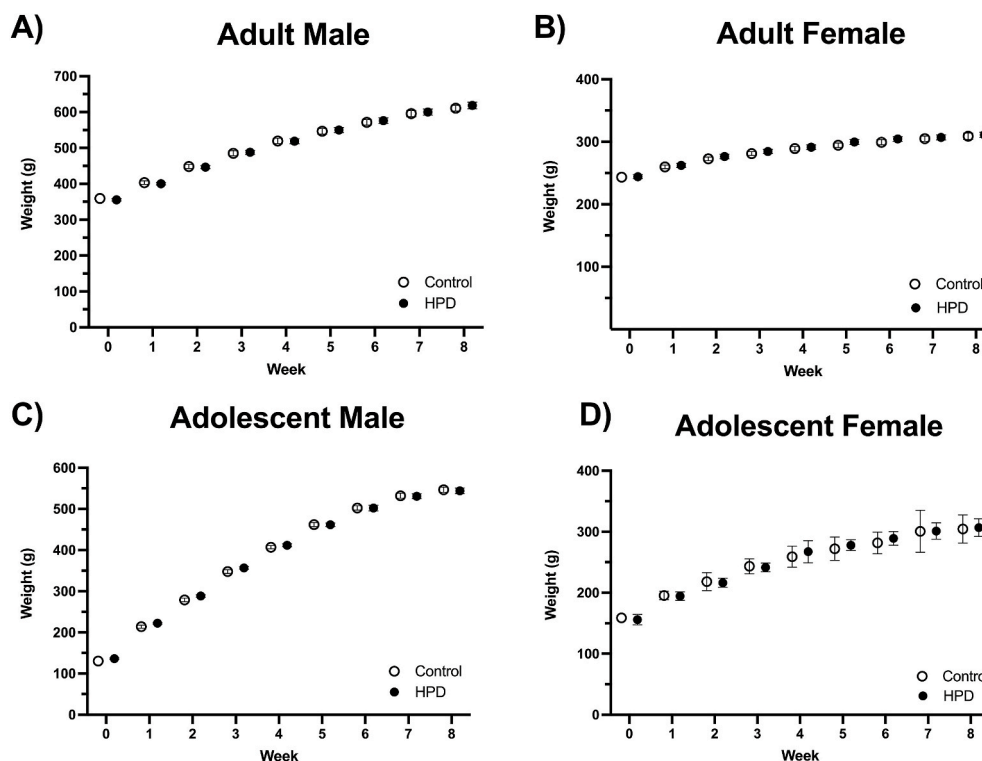


Fig. 2. Effects of HPD on body weight. Time course of body weight during the 8 weeks of exposure to HPD in rats exposed to standard food (Control) or to HPD. A) Adult males (HPD $n = 12$; Control $n = 12$); B) Adult females (HPD $n = 12$; Control $n = 12$); C) Adolescent males (HPD $n = 12$; Control $n = 12$) and D) Adolescent females (HPD $n = 12$; Control $n = 11$). Data are expressed as Mean \pm SEM.

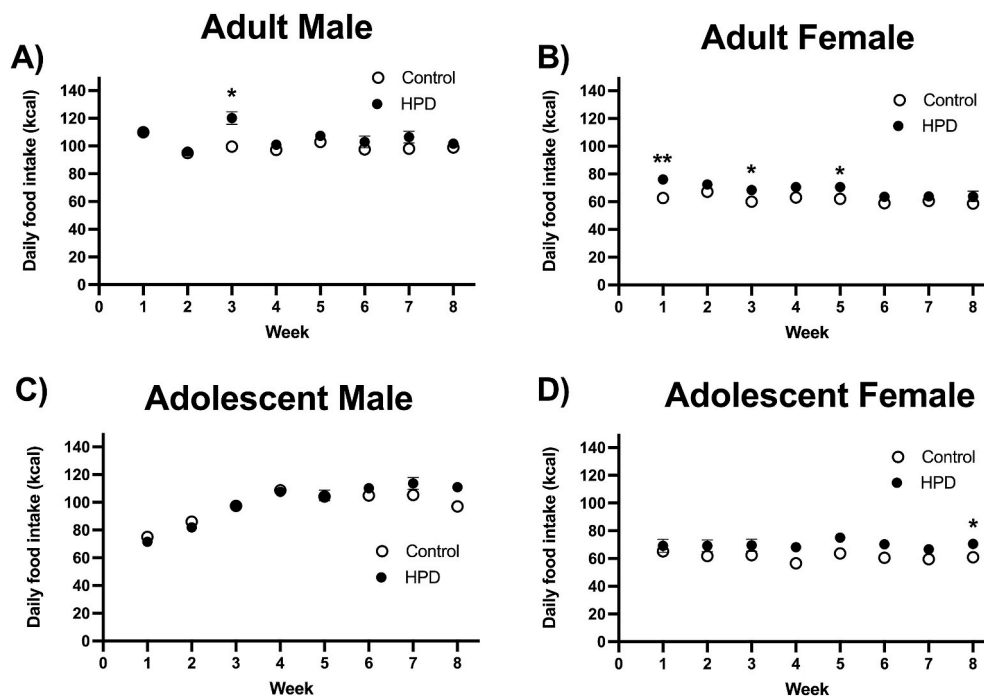


Fig. 3. Effects of HPD on daily food consumption during weekends. Time course of daily food consumption in the weekends during the 8 weeks of exposure to HPD in rats exposed to standard food (Control) or to HPD. A) Adult males (HPD n = 12; Control n = 12); B) Adult females (HPD n = 12; Control n = 12); C) Adolescent males (HPD n = 12; Control n = 12) and D) Adolescent females (HPD n = 12; Control n = 11). Data are expressed as Mean ± SEM of the consumption per cage (n = 6) divided by the number of rats in the cage (2). Two-way ANOVA followed by Sidak Post-hoc: *P < 0.05; **P < 0.01.

observed. In adult male rats, we found that the HPD group had a lower consumption than the control group during weekdays (~97 ± 1.3 kcal/day for controls and ~90 ± 1.7 kcal/day for HPD rats) (Fig. 4A) [significant effect of time (F (2.93, 29.31) = 14.131, P < 0.0001; ε = 0.43),

significant effect of diet (F (1, 10) = 7.75, P = 0.019) and significant time × diet interaction (F (7, 70) = 2.94, P = 0.0094)]. In adult female rats, the HPD group had a lower consumption than the control group (59 ± 1.1 kcal/day for controls and 51.3 ± 1.3 kcal/day for HPD rats)

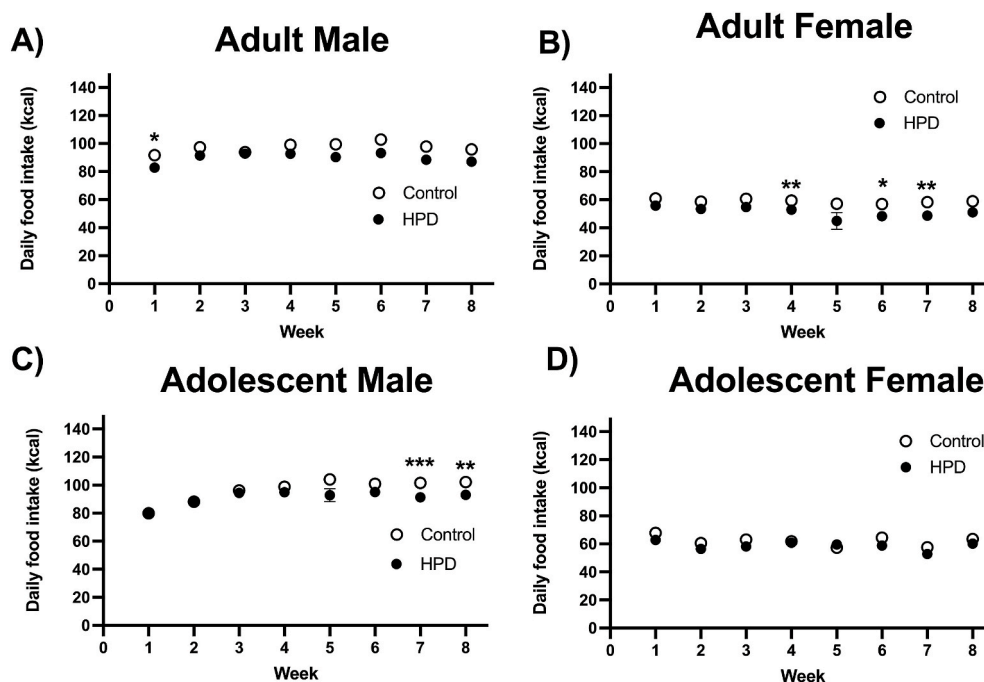


Fig. 4. Effects of HPD on daily food consumption during weeks. Time course of daily food consumption in the week during the 8 weeks of exposure to HPD in rats exposed to standard food (Control) or to HPD. It should be noted that only standard food was available during the week for both HPD and controls. A) Adult males (HPD n = 12; Control n = 12); B) Adult females (HPD n = 12; Control n = 12); C) Adolescent males (HPD n = 12; Control n = 12) and D) Adolescent females (HPD n = 12; Control n = 11). Data are expressed as Mean ± SEM of consumption per cage (n = 6) divided by the number of rats in the cage (2). Two-way ANOVA followed by Sidak Post-hoc: *P < 0.05; **P < 0.01; ***P < 0.001.

(Fig. 4B) [significant effect of time ($F(1.77, 17.68) = 3.98, P = 0.041; \epsilon = 0.25$), significant effect of diet ($F(1, 10) = 18.05, P = 0.0017$) and no significant time \times diet interaction ($F(7, 70) = 0.97, P = 0.46$)]. In adolescent male rats, HPD and control groups initially had similar consumption but in the last 4 weeks, HPD rats had lower consumption than controls (96.5 ± 0.8 kcal/day for controls and 91.3 ± 1.2 kcal/day for HPD rats) (Fig. 4C) [significant effect of time ($F(1.54, 15.45) = 35.07, P < 0.0001; \epsilon = 0.22$), significant effect of diet ($F(1, 10) = 14.99, P = 0.0031$) and significant time \times diet interaction ($F(7, 70) = 4.74, P = 0.0002$)]. In adolescent female rats, HPD and control groups had similar consumption of food throughout the experiment (Fig. 4D) [significant effect of time ($F(4.06, 40.64) = 7.53, P < 0.0001; \epsilon = 0.58$), no significant effect of diet ($F(1, 10) = 2.29, P = 0.16$) and no significant time \times diet interaction ($F(7, 70) = 1.47, P = 0.19$)].

In summary, exposure to intermittent HPD did not result in increases in body weight in any group. When rats had access to HPD, during weekends, they tended to consume less standard food compared to control animals that had access to standard food all the time. More importantly, exposure to HPD during weekends reduced consumption of standard food during the following weekdays.

3.2. Effects of exposure HPD on food taking in the FR1

In the first 5 training sessions in the FR1, we found no difference between HPD and control rats in any condition (Fig. 5) suggesting that operant learning was not affected by exposure to HPD.

In contrast, in adult male rats, baseline responding in the FR1 schedule (the average number of active responses in the last 3 sessions before PSS tests) was lower in HPD rats compared to controls in the first weeks after discontinuation of HPD, and then it reached levels similar to controls in the last week (Fig. 6A) [significant effect of time ($F(2.03, 44.69) = 34.86; P < 0.0001; \epsilon = 0.68$), significant effect of diet ($F(1, 22)$

$= 14.24; P = 0.0010$) and significant Time \times Diet interaction ($F(3, 66) = 5.11, P = 0.0030$)]. Similarly, in adult female rats, FR1 baseline responding was lower in the HPD group early after discontinuation of HPD and then returned to control levels (Fig. 6B) [significant effect of time ($F(1.62, 35.64) = 54.62, P < 0.0001; \epsilon = 0.54$), significant effect of diet ($F(1, 22) = 6.35, P = 0.019$) but not significant Time \times Diet interaction]. In adolescent male rats, baseline responding was slightly lower in the HPD group after discontinuation and rapidly returned to control levels (Fig. 6C) [significant effect of time ($F(1.54, 34.00) = 45.69, P < 0.0001; \epsilon = 0.51$), no significant effect of diet and significant Time \times Diet interaction ($F(3, 66) = 2.79; P = 0.047$)]. No difference in baseline responding in the FR1 schedule was found in female adolescent rats (Fig. 6D).

3.3. Exposure to HPD does not produce a persistent compulsion-like alteration in food taking

HPD and control groups showed similar resistance to punishment regardless of sex and age of HPD exposure. In adult males (Fig. 7A), PSS break points were 1.35 ± 0.5 for the control group and 0.80 ± 0.3 mC for the HPD group ($P = 0.09; t = 1.77; DF = 22$); in adult females (Fig. 7B), PSS break points were 1.6 ± 0.4 for the control group and 1.4 ± 0.5 mC ($P = 0.40; t = 0.85; DF = 22$); in adolescent males (Fig. 7C), PSS break points were 1.1 ± 0.2 for the control group and 0.8 ± 0.1 mC ($P = 0.20; t = 1.331; DF = 22$); and in adolescent females (Fig. 7D), PSS break points were 0.5 ± 0.1 for the control group and 0.8 ± 0.1 mC ($P = 0.87; t = 0.16; DF = 21$).

3.4. Exposure to HPD does not produce persistent alterations in motivation for food

HPD and control groups showed similar motivation for sucrose

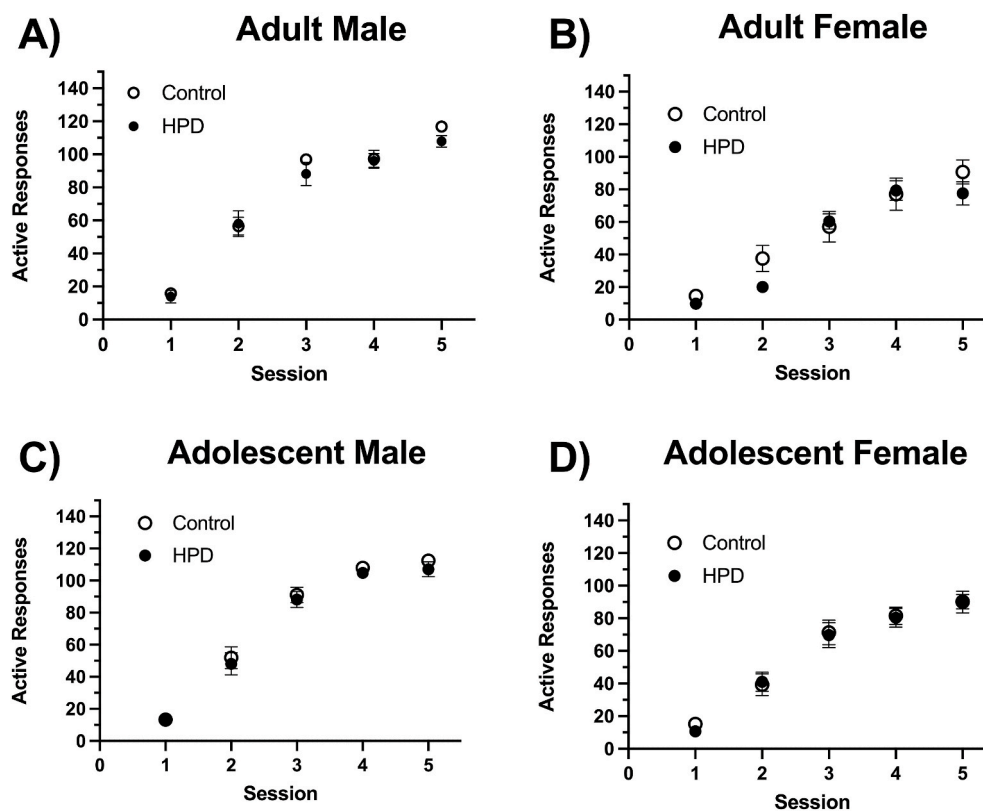


Fig. 5. Effects of HPD on initial FR1 training. Food self-administration in the first five FR1 sessions in rats exposed to standard food (Control) or to HPD. HPD was discontinued 7 days before the start of FR1 training. A) Adult males (HPD $n = 12$; Control $n = 12$); B) Adult females (HPD $n = 12$; Control $n = 12$); C) Adolescent males (HPD $n = 12$; Control $n = 12$) and D) Adolescent females (HPD $n = 12$; Control $n = 11$). Data are expressed as Mean \pm SEM.

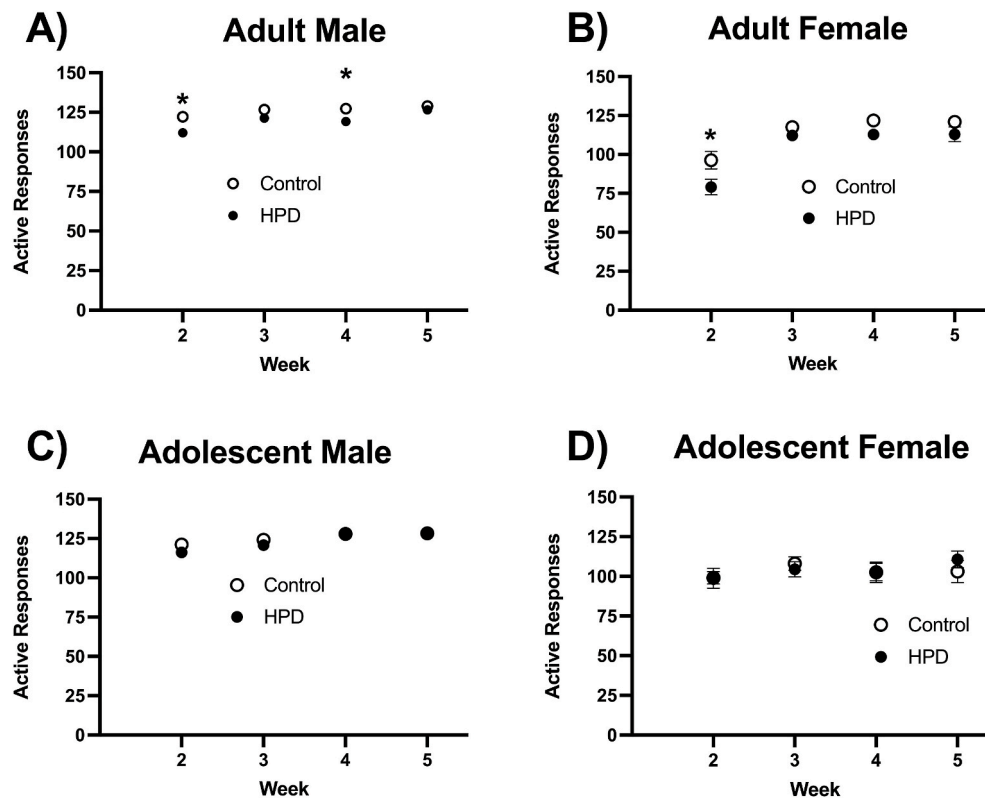


Fig. 6. Effects of HPD on baselines in the training FR1 sessions during the experiment. Baseline responding in FR1 session throughout the experiments in rats exposed to standard food (Control) or to HPD. A) Adult males (HPD $n = 12$; Control $n = 12$); B) Adult females (HPD $n = 12$; Control $n = 12$); C) Adolescent males (HPD $n = 12$; Control $n = 12$) and D) Adolescent females (HPD $n = 12$; Control $n = 11$). Data are expressed as Mean \pm SEM. Two-way ANOVA followed by Sidak Post-hoc: * $P < 0.05$.

pellets regardless of sex and age of HPD exposure. In adult males (Fig. 8A), PR break points were 581 ± 45 for the control group and 480 ± 42 active responses for the HPD group ($P = 0.12$; $t = 1.64$; $DF = 22$); In adult females (Fig. 8B), PR break points were 413 ± 64 for the control group and 407 ± 45 active responses for the HPD group ($P = 0.93$; $t = 0.08$; $DF = 22$); in adolescent males (Fig. 8C), PR break points were 397 ± 38 for the control group and 380 ± 45 active responses for the HPD group ($P = 0.77$; $t = 0.30$; $DF = 22$); and adolescent females (Fig. 8D), PR break points were 236 ± 32 for the control group and 188 ± 35 active responses for the HPD group ($P = 0.34$; $t = 0.99$; $DF = 21$).

3.5. Effects of exposure to HPD on anxiety-like behaviors and pain sensitivity

For anxiety-like behavior, HPD and control groups spent similar times in the open arms regardless of sex or age of HPD exposure. In adult male rats, the HPD group spent 88 ± 54 s in the open arms and the control group 94 ± 31 s (Fig. 9A) [no effect of diet (Mann-Whitney $U = 38.50$; $P = 0.053$)]. In adult female rats, the HPD group spent 77 ± 20 s in the open arms and the control group with 115 ± 33 s (Fig. 9B) [no effect of diet (Mann-Whitney $U = 59$; $P = 0.48$)]. In adolescent male rats, the HPD group spent 54 ± 12 s in the open arms and the control group 58 ± 12 s (Fig. 9C) [no effect of diet (Mann-Whitney $U = 69.50$; $P = 0.90$)]. In adolescent female rats, the HPD group spent 107 ± 14 s and the control group with 142 ± 25 s (Fig. 9D) [no effect of diet (Mann-Whitney $U = 48$, $P = 0.29$)].

In the hot plate test, in adult males, HPD and control groups had similar latencies to escape (93 ± 6 s for the control group and 91 ± 9 s for the HPD group) (Fig. 10A) [No effect of diet (Mann-Whitney $U = 67$; $P = 0.78$)]. In adult females, HPD and control groups had similar latencies to escape (100 ± 9 s for the control group and 92 ± 7 s for the HPD group) (Fig. 10B) [no effect of diet (Mann-Whitney $U = 56$; $P = 0.35$). In

adolescent males, latencies to escape were lower in the HPD group compared to the control group (109 ± 9 s for the control group and 84 ± 9 s for the HPD group) suggesting that, in males, exposure to HPD during adolescence produces long-lasting increases in pain sensitivity (Fig. 10C) [significant effect of diet (Mann-Whitney $U = 36$; $P = 0.03$)]. In adolescent females, HPD and control groups had similar latencies to escape (79 ± 13 s for the control group and 67 ± 14 s for the HPD group) (Fig. 10D) [no effect of diet (Mann-Whitney $U = 49$; $P = 0.30$).

4. Discussion

In this study, we investigated the effects of intermittent exposure to HPD on subsequent food self-administration and notably, on indexes of compulsivity and of excessive motivation. We found that, irrespectively of sex or age, when rats had access to HPD, their pattern of food intake was altered with decrease of consumption of standard chow in between periods of HPD exposure. In addition, in adult but not adolescent rats, exposure to HPD produced a transient decrease in self-administration of sucrose under FR1 schedule. However, HPD did not produce long-lasting alterations in compulsivity or motivation for food which suggests that this exposure to HPD does not increase persistently the risk to develop addiction-like behavior towards food.

Exposure to highly processed food rich in fat and sugar is believed to be a major risk factor to develop excessive eating, overweight and obesity (O'Connor & Kenny, 2022). In our study, intermittent exposure to HPD diet did not induce increase in body weight suggesting that exposure to HPD is not necessarily obesogenic. This is consistent with previous reports showing no increase in body weight in animals exposed intermittently to HPD (Cottone, Sabino, Roberto, et al., 2009; Ferragud et al., 2020; Rossetti et al., 2014). On the other hand, exposure to HPD altered the pattern of food intake during the period of exposure in all groups. In particular, consistent with previous studies (Cottone, Sabino,

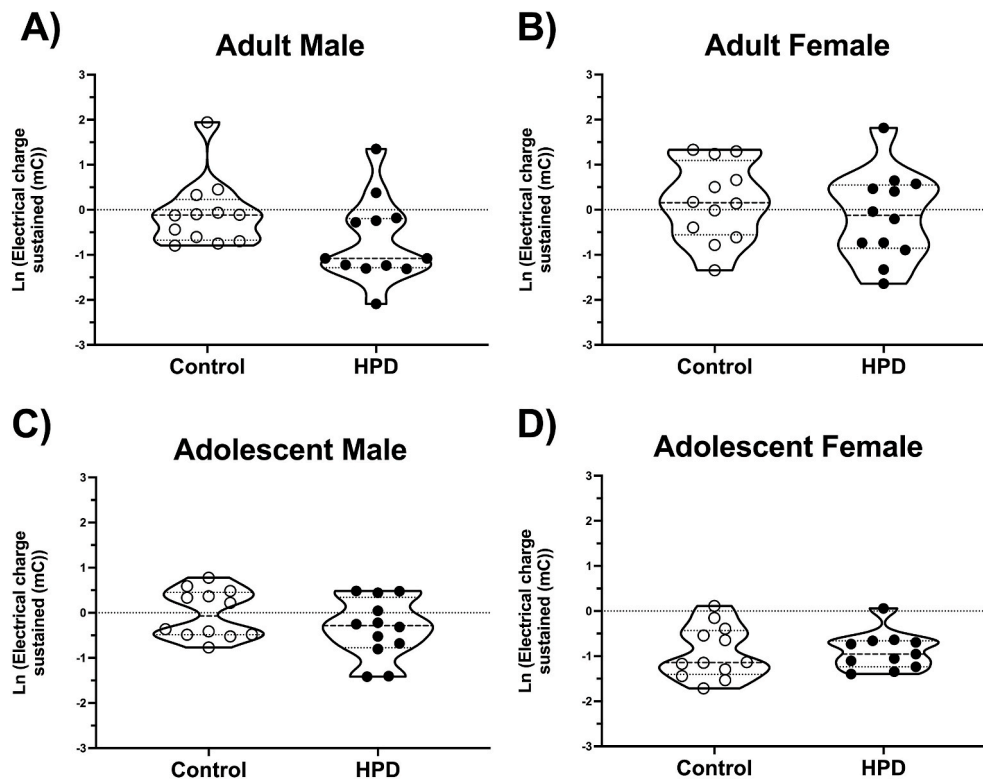


Fig. 7. Effect of HPD on resistance to punishment in the PSS procedures. Natural logarithm of electric charge sustained in the PSS tests in rats exposed to standard food (Control) or to HPD. A) Adult males (HPD $n = 12$; Control $n = 12$); B) Adult females (HPD $n = 12$; Control $n = 12$); C) Adolescent males (HPD $n = 12$; Control $n = 12$) and D) Adolescent females (HPD $n = 12$; Control $n = 11$). Data represent the average of two sessions and are expressed as Mean \pm SEM. Student unpaired T-Test: all comparisons are not significant.

Roberto, et al., 2009; Ferragud et al., 2020; Rossetti et al., 2014), HPD rats showed a decrease in consumption of standard food suggesting a negative contrast effect with intermittent exposure to high palatable food during weekends decreasing the rewarding effect of normal food during weekdays. Alternatively, this decrease could be due to compensatory reduction of feeding due to excessive consumption during weekends or it could be a manifestation of HPD-induced anxiety-like behavior. Female rats (adult and adolescent) consumed more HPD food compared to control groups confirming that, in females, HPD is more rewarding than standard food. In contrast, adult and adolescent male rats showed mixed results in HPD consumption with low initial consumption, probably due to novelty avoidance, followed by a slight increase in consumption in the subsequent weeks. Thus, male rats appear less sensitive to the effects of HPD than female rats.

Resistance to punishment is often used as an index of compulsivity for food or drugs. In many procedures used in rodents, punishment is provided as foot shocks of a fixed intensity that allows characterizing animals as resistant (i.e. compulsive) or sensitive (i.e. non-compulsive) (George et al., 2022; Rossetti et al., 2014; Vanderschuren & Ahmed, 2013). However, using an arbitrary threshold forces a dichotomic classification ignoring the fact that compulsivity may be a more continuous phenomenon. To avoid this problem and to better mimic tests of compulsivity used in humans (Apergis-Schoute et al., 2017; Kanen et al., 2021; Kim & Anderson, 2020), we recently developed a self-adjusting procedure that allows individual animals to titrate the level of shock that they are willing to tolerate to obtain rewards (Desmercieres et al., 2022). Consistent with our previous study, we found that compulsivity measured by the PSS break point is log-normally distributed, suggesting that resistance to punishment is a continuous rather than dichotomic phenomenon regardless of sex or age of exposure to HPD. In addition, we found that, regardless of sex or age of exposure to HPD, PSS and PR do not correlate (Fig. S1), confirming that they measure independent

biobehavioral processes.

Previous studies have found that exposure to high fat and sugar diet increases (Garman et al., 2021; la Fleur et al., 2007; Reichelt et al., 2016; Wojnicki et al., 2006), decreases (Blaisdell et al., 2014; Davis et al., 2008; Tracy et al., 2015; Vendruscolo et al., 2010) or has no effect (Jong et al., 2013; Mitra et al., 2009; Rossetti et al., 2014) on motivation for food measured by progressive ratio schedules. Some of these discrepancies could be due to the sex of animals since most studies investigated only males or only females. One study directly investigated male and female rats exposed to sucrose and found increased motivation in females but decreased motivation in males (Reichelt et al., 2016). Differently from that study, we found that HPD did not change motivation for sweet sucrose rewards in males or females exposed to HPD as adults or adolescents. Another possible reason for these discrepancies is the age at which animals were exposed to HPD because most studies investigated the effects of exposure at adolescence or adulthood separately. Indeed, adolescence is a window of vulnerability for the development of maladaptive behaviors including addiction or food-related disorders (Larsen & Luna, 2018; Murray & Chen, 2019) and it has been shown that exposure to obesogenic diets during adolescence leads to increased (Figlewicz et al., 2013) or decreased (Vendruscolo et al., 2010) motivation compared to exposure at adulthood. However, in our study, motivation for sweet food was equally unaffected by exposure during adolescence or adulthood.

Concerning compulsivity measured as resistance to punishment, the results in the literature also show contrasting results with some studies reporting increases (Oswald et al., 2011; Rossetti et al., 2014) and another reporting no change (Jong et al., 2013) after exposure to HPD. In addition, Johnson and Kenny found that prolonged access to an obesogenic diet blunted the ability of an aversive conditioned stimulus to disrupt food seeking and taking (Johnson & Kenny, 2010). In the present study, using the PSS procedure we found no effect of exposure to

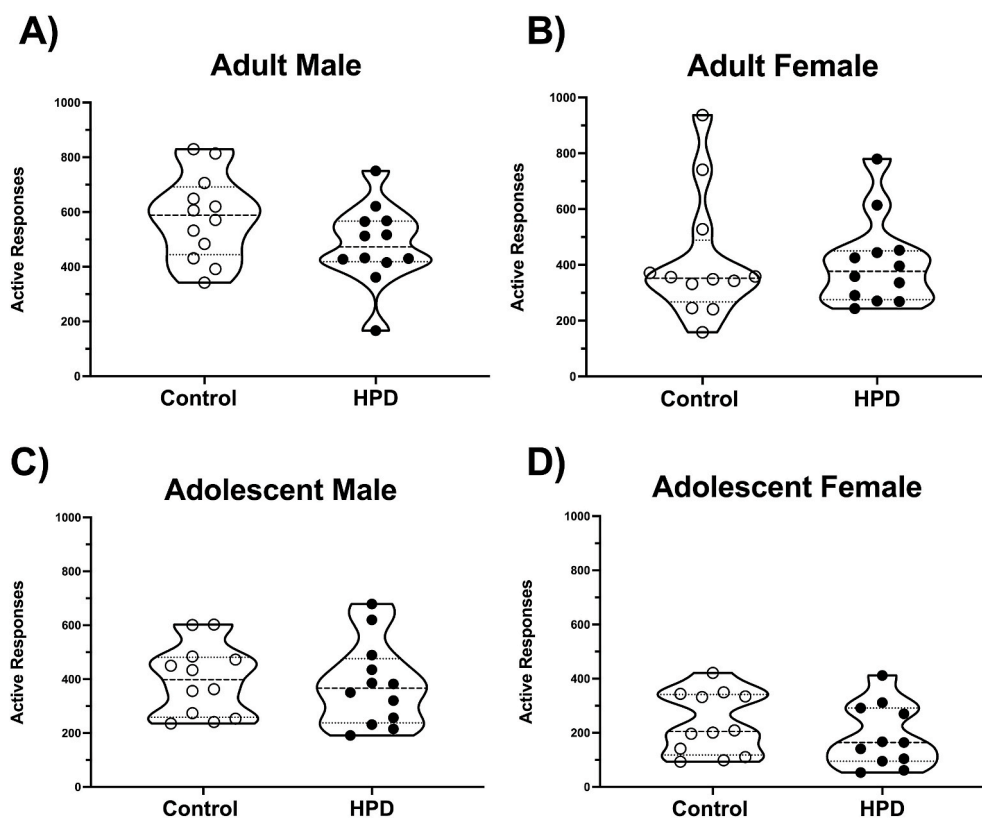


Fig. 8. Effect of HPD on food motivation in the PR procedure; Total active responses in the PR tests in rats exposed to standard food (Control) or to HPD. A) Adult males (HPD $n = 12$; Control $n = 12$); B) Adult females (HPD $n = 12$; Control $n = 12$); C) Adolescent males (HPD $n = 12$; Control $n = 12$) and D) Adolescent females (HPD $n = 12$; Control $n = 11$). Data represent the average of two sessions and are expressed as Mean \pm SEM. Student unpaired T-Test: all comparisons are not significant.

HPD on compulsivity regardless of sex and age of exposure to HPD suggesting that these factors may not explain by themselves the differences in the literature.

In this study, we wanted to investigate the long-lasting rather than the immediate consequences of exposure to obesogenic diets because if diet-induced dysfunctions persist even when bad eating habits are changed for healthier ones, this would have more serious consequence on health. For this reason, after 8 weeks of exposure to HPD, rats were switched to normal chow for several days before being tested for excessive motivation or resistance to punishment. This may have contributed to the lack of effects of HPD since other studies have found that some of the deleterious effects of obesogenic diets tend to disappear upon discontinuation (Boitard et al., 2016; Carlin et al., 2016; Rabasa et al., 2016). However, other studies have found long-lasting effects of obesogenic diets on motivation (Garman et al., 2021; Reichelt et al., 2016; Tracy et al., 2015; Vendruscolo et al., 2010). Our protocol of exposure was based on the one used by Rossetti et al. (2014) except that we used Sprague-Dawley instead of Wistar rats and we discontinued HPD diet several days before the beginning of operant training. Whereas Rossetti et al. found increases in resistance to punishment in females exposed to HPD at adolescence (Rossetti et al., 2014), we did not find significant difference in any of our comparisons. Future studies are needed to determine whether these discrepancies are due to rats' strain or to discontinuation of HPD exposure.

In humans, obesity is often associated with increased anxiety (Fulton et al., 2022; Garipey et al., 2010) but the causal relationship between the two conditions remains unclear. Some studies in rats have shown that HPD increases anxiety-like behavior in some tests (Cottone, Sabino, Steardo, & Zorrilla, 2009; Duthheil et al., 2016; Sivanathan et al., 2015), but little or no effect has been described in the elevated plus maze (Duthheil et al., 2016; Rossetti et al., 2014; Sivanathan et al., 2015).

Consistent with these latter findings, we found no significant effect of HPD on anxiety in the elevated plus maze in any group although there was a general trend for increased anxiety in all groups and the trend almost reached significance in the adult male groups. It should be considered that in the present study, anxiety was only evaluated at the end of food self-administration sessions, corresponding to several weeks after exposure to HPD. In contrast, in the study of Cottone et al. the HPD-induced increases in anxiety-like behavior in the elevated plus maze was measured 24–26 h after the last access to HPD (Cottone, Sabino, Steardo, & Zorrilla, 2009). Therefore, we cannot exclude that exposure to HPD induced anxiety at an earlier time point, which disappeared over time.

In humans, obesity is also associated with pain and the relationship appears bidirectional (McVinnie, 2013). According to a recent meta-analysis, experiments in rodents mostly found a decrease in pain sensitivity in a variety of conditions and tests (Marques Miranda et al., 2021). However, some studies have also found increased pain sensitivity (Roane & Porter, 1986; Song et al., 2017; Tramullas et al., 2016) and this may be due to inflammatory processes (Song et al., 2017; Tramullas et al., 2016). In our study, consistent with these latter studies, we found a general trend for increased sensitivity to pain in all groups that reached statistical significance in adolescent male rats. Again, pain sensitivity was measured at the end of the experiments and therefore, it is possible that HPD produced a temporary increase in pain sensitivity which tended to recover upon discontinuation of HPD. Interestingly, if pain sensitivity was increased by exposure to HPD during the first days/weeks of withdrawal, this did not affect PSS breakpoints. This confirms previous studies (Degoulet et al., 2021; Desmercieres et al., 2022; Li et al., 2021) showing that pain sensitivity does not play a major role in resistance to punishment.

Important issues in the investigation of the effects of HPD on brain

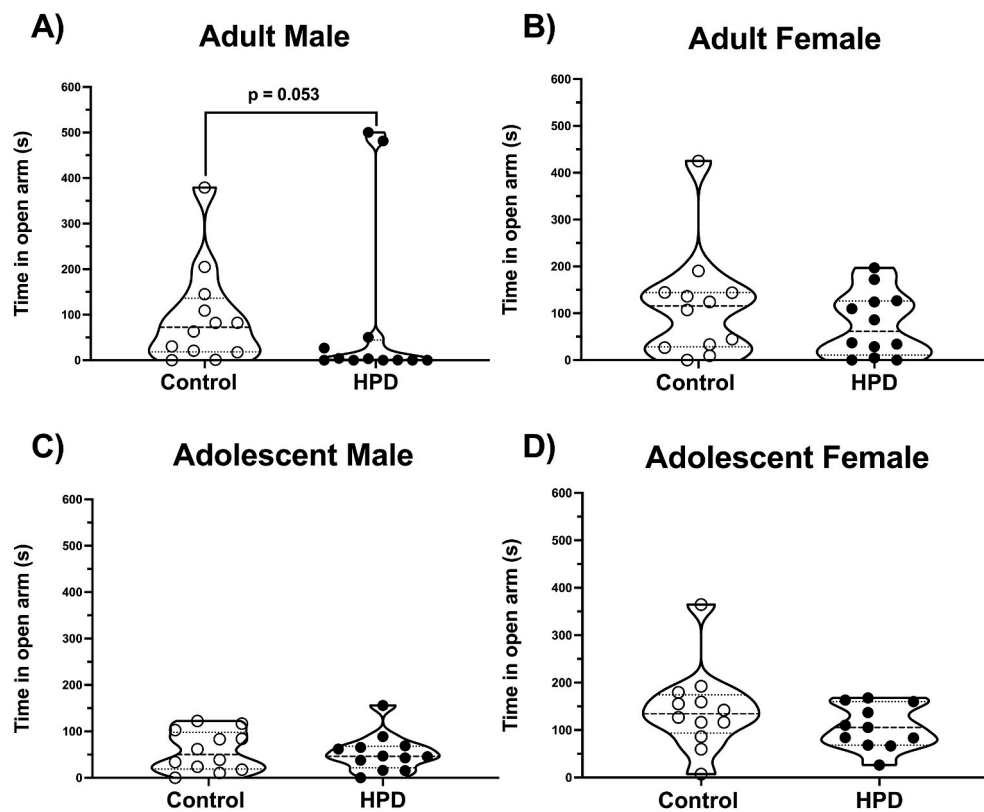


Fig. 9. Effects of HPD on anxiety-like behavior. Time spent in second in the open arms of an elevated plus maze in rats exposed to standard food (Control) or to HPD. A) Adult males (HPD $n = 12$; Control $n = 12$); B) Adult females (HPD $n = 12$; Control $n = 12$); C) Adolescent males (HPD $n = 12$; Control $n = 12$) and D) Adolescent females (HPD $n = 12$; Control $n = 11$). Data are expressed as Mean \pm SEM. Mann-Whitney Test.

and behavior are both the actual composition of diets and the protocol of exposure. The actual composition of the diet is an important determinant of the effects of HPD. Certain diets include percentage of fat as high as 70% (Touati et al., 2011) whereas the type of food used in this study should be considered as medium fat (13.9%), high sugar (63.6%) diet. Among HPD studies, some used enriched tablets similar to ours (Cottone, Sabino, Roberto, et al., 2009; Iemolo et al., 2012; Rossetti et al., 2014), others used sucrose solutions (Reichelt et al., 2016; Vendruscolo et al., 2010; Wong et al., 2017) and others different kinds of commercial or home-made sugary and fatty diets (Johnson & Kenny, 2010; Jong et al., 2013; la Fleur et al., 2007; Oswald et al., 2011; Tracy et al., 2015). Also, in the literature, the duration of HPD exposure varies from a few days (Oswald et al., 2011), to several weeks and up to six months (Blaisdell et al., 2014), and the timing of the exposure could be continuous (Blaisdell et al., 2014; Davis et al., 2008; Figlewicz et al., 2013; Garman et al., 2021; la Fleur et al., 2007; Tracy et al., 2015; Vendruscolo et al., 2010), intermittent with periods of several days (Cottone, Sabino, Roberto, et al., 2009; Iemolo et al., 2012; Johnson & Kenny, 2010; Rossetti et al., 2014), or a few hours per day (Reichelt et al., 2016; Wojnicki et al., 2006; Wong et al., 2017). Another issue to take into consideration to understand the conflicting results in the literature is whether rats are housed in group such as in this study or isolated. Whereas social isolation allows more precise assessment of individual consumption, it can also increase food consumption (Morgan & Einon, 1975), food reward and motivation (Mastrogiovanni et al., 2021) in rodents and in humans (Hanna et al., 2023; Tomova et al., 2020). It is difficult to determine which procedure better mimics human exposure to HPD because human food selection and intake varies enormously not only in different cultures and in different socio-economical classes, but also within them and in many instances even within the same person at different times. Whereas all these differences in experimental protocols are likely to account for the

discrepancies in the literature, we should probably embrace the richness provided by this variety because findings that are consistent among different procedures are likely to have the highest translational value for human condition.

In conclusion, in this study we found that intermittent exposure to HPD did not produce persistent effects on motivation and compulsivity to consume sweet food regardless of sex or age of exposure. This lack of persistent effects, together with the great number of contradicting results found in the literature suggests that the consequences of exposure to HPD on the development of food addiction are at best limited and subtle.

Ethical statement

All experiments were conducted during the dark phase and in accordance with European Union directives (2010/63/EU) for the care of laboratory animals and approved by the local ethics committees (COMETHEA).

CRediT authorship contribution statement

Stevenson Desmercieres: Writing – original draft, Investigation, Formal analysis, Data curation. **Virginie Lardeux:** Writing – review & editing, Methodology, Investigation. **Jean-Emmanuel Longueville:** Writing – review & editing, Visualization, Software, Data curation. **Emilie Dugast:** Investigation, Methodology, Writing – review & editing. **Nathalie Thiriet:** Conceptualization, Supervision, Writing – review & editing. **Marcello Solinas:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – original draft.

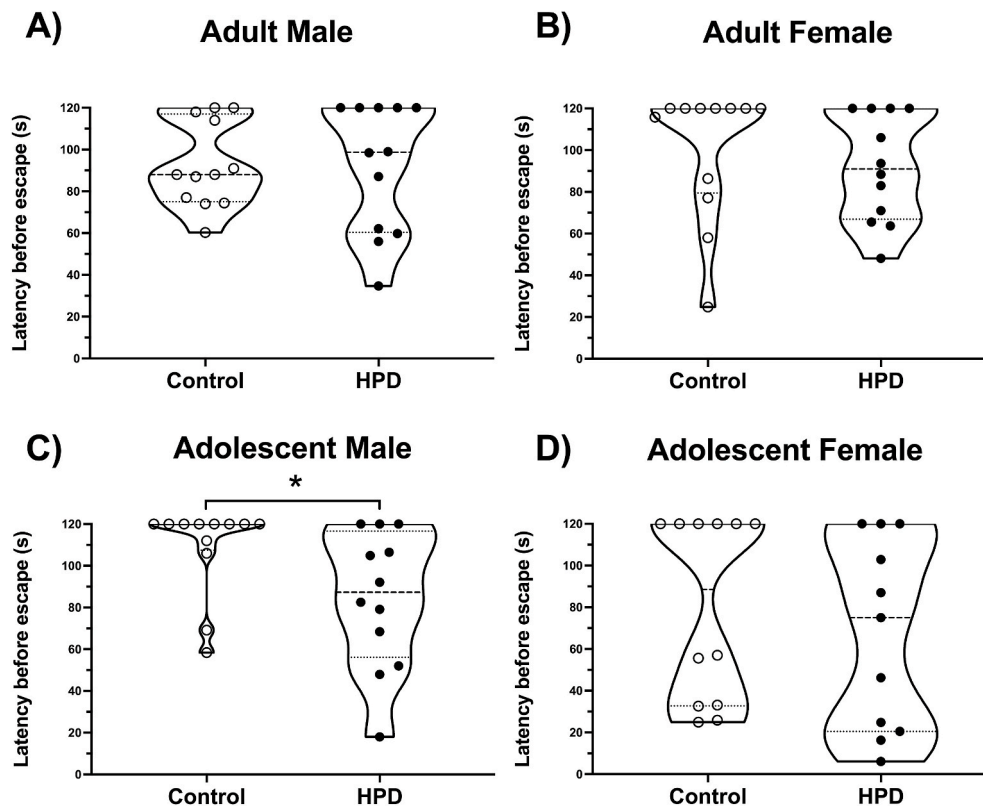


Fig. 10. Effects of HPD on pain reactivity by hot plate test. Latency in second before escape in the hot plate test in rats exposed to standard food (Control) and rats exposed to HPD. A) Adult males (HPD $n = 12$; Control $n = 12$); B) Adult females (HPD $n = 12$; Control $n = 12$); C) Adolescent males (HPD $n = 12$; Control $n = 12$) and D) Adolescent females (HPD $n = 12$; Control $n = 11$). Data are expressed as Mean \pm SEM. Mann-Whitney test: * $P < 0.05$.

Declaration of competing interest

The authors declare no competing interests.

Data availability

I have included a link to the OSF site where data are freely available.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.appet.2024.107340>.

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