Physiology & Behavior 223 (2020) 112970

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

The effects of novelty on food consumption in male and female rats

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ARTICLE INFO

Keywords:
Novelty
Context
Palatable food
Taste neophobia
Sex differences
Habituation

ABSTRACT

Novelty powerfully impacts feeding behavior and can override homeostatic and hedonic drives, because consumption of a new food could lead to illness or even death. New foods and new feeding environments can decrease or inhibit feeding, but how the two interact and whether there are sex differences has not been determined. The current study examined consumption of a palatable (high sucrose) novel food compared to a familiar food in adult male and female rats that were fed in a familiar or a novel environment. Rats were deprived of food for 20 h prior to each of eight tests. During the first test, male and female rats that were tested in a familiar environment showed robust taste neophobia, as they mainly consumed familiar food. Across repeated tests, these rats increased consumption of the novel food, which indicated that they habituated to the novel taste and developed a preference for the novel food. In contrast, all rats tested in a novel feeding environment ate very little of both foods during the initial test. Across repeated tests, male rats habituated to the novel food faster than females and by the fourth test ate more of the novel than familiar food. In contrast, females showed sustained, suppressed consumption across habituation tests. These results demonstrated robust differences in feeding behavior depending whether rats were fed at home or in a novel feeding environment, and robust sex differences in habituation to eating in a new environment. These findings suggest that novel context has a greater impact on female consumption than male consumption. This difference may be relevant to sex differences in avoidant behaviors in maladaptive circumstances and the development of psychopathology. Therefore, the behavioral profile outlined in this study for consumption under novelty provides an important starting point for investigation of the underlying neural substrates of novelty processing.

1. Introduction

Adaptive reactions to novel stimuli in the environment are essential for survival. Before it can be established whether something is safe or dangerous, novel stimuli are often treated with a level of wariness or avoidance. Initially limiting contact with a novel stimulus allows an accurate assessment of threat level. Once assessed, an animal increases contact, if the novel stimulus is considered innocuous, or continues avoidance, if considered harmful. However, when these avoidant behaviors become maladaptive it can lead to the development of psychopathology.

Appropriate response to new foods is essential for survival because consumption of a new food could lead to illness or even death. A common behavioral reaction to a novel food is a decrease in consumption compared to a familiar food, which is defined as taste neophobia [[5], [7]]. In addition to lower consumption, animals are slower to approach a novel tastant and to express hedonic orofacial responses compared to when it is familiar [5]. A novel feeding environment can also have a great effect on consumption [7]. Studies conducted with mice have found that novel context mediated a decrease in appetitive behavior, as mice placed in a novel open field had longer latencies to consume food [[10], [2]]. Previous research has also shown that environmental cues (both contextual and discrete) that signal danger can override physiological signals and inhibit food intake of a palatable, familiar food in rats after food deprivation [[8], [9], [12]]. Given that feeding environments can have a great impact on the consumption of familiar foods, it is important to determine whether there is an interaction between novel contexts and novel foods. Collectively, prior work suggests that there may be compounding effects of multiple inhibitors, such as novel contexts and novel foods. However, studies into the specific interaction of novel foods and novel environments are lacking. Previous studies have also neglected to compare male and female behavior under these conditions.

Male and females show different consumption patterns based on context. Following contextual fear conditioning, males restricted consumption in the fear context whereas females restricted consumption more generally, in the fear and in a neutral context [12]. This generalization across contexts was attributed to possible higher levels of

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https://doi.org/10.1016/j.physbeh.2020.112970
Received 21 February 2020; Received in revised form 9 April 2020; Accepted 13 May 2020
Available online 26 May 2020

anxiety in females and suggests that context has a greater effect on female feeding behavior. In agreement, sex differences have also been found in context effects on appetitive aspects of feeding behavior, particularly in context-induced renewal of responding to food cues [1]. Overall, previous studies indicate that females and males may have different patterns of consumption in a novel context, particularly in conjunction with the presentation of a novel food.

The current study was designed to determine the impact of novel taste and novel feeding environment independently and together. We characterized feeding behaviors in both sexes to establish whether there are sex differences initially and during habituation to novel taste and novel feeding environment. Food-deprived rats were given access to novel and familiar foods in a familiar or new environment, and their consumption patterns were tracked over eight tests to determine habituation and preferences.

2. Materials & methods

2.1. Subjects

Adult male ($n = 16$) and female ($n = 16$) Long Evans rats (Charles River Laboratories; Portage, MI), that weighed 225–250 g upon arrival, were individually housed and maintained on a 12-hour light/dark cycle (lights on 06:00). Males and females were housed in the same colony room on separate shelves. After arrival, subjects were allowed one week to acclimate to the colony housing room before behavioral procedures began, during which they had ad libitum access to water and standard laboratory chow (Purina Lab Diet Prolab RMH 3000; 3.47 kcal/g; 26% protein, 15% fat, 59% carbohydrates), and were handled daily. All housing and testing procedures were in compliance with the National Institutes of Health Guidelines for Care and Use of Laboratory Animals and approved by the Boston College Institutional Animal Care and Use Committee.

2.2. Apparatus

Half of the animals were tested in their Home Cage and the other half were tested in a novel environment (behavioral chamber; plexiglass box (30 × 28 × 30 cm) with grid flooring and a recessed food port (3.2 × 4.2 cm) on one wall; Coulbourn Instruments). Each chamber is enclosed in monolithic rigid foam box). Food was presented in a ceramic bowl.

2.3. Testing procedure

Male and female Long Evans rats were tested for consumption of both novel and familiar foods in either a familiar or novel context. The animals underwent 8 identical testing sessions, each lasting 10 min. Prior to each test all rats were food deprived for 20 h. After each test rats were given ad libitum access to food for at least 24 h before the following test. For each testing session, each rat was presented with two identical bowls. One of the bowls contained 15 g of a familiar food (Rat Chow) and the other contained 15 g of a novel food (Test Diet pellets (TD; 3.44 kcal/g; 21% protein, 13% fat, 67% carbohydrate (all sucrose)).

There were four groups of rats: home cage tested females, home cage tested males, novel context tested females, and novel context tested males. The experiment was conducted in two identical replications with four rats per group. All rats were habituated to transport to the conditioning chamber room, as well as to the ceramic bowls, at least 24 h prior to testing. The weight of both foods was measured following the end of testing to determine how much of each was consumed.

Body weights for each rat was taken in the morning of each test day prior to the testing session. Average body weights were calculated for each group. Due to a suspected technical error on test day 5, body weight measurements for two home cage tested males were replaced with a value calculated by averaging their body weight from the test day before and after.

2.4. Statistical analysis

For each test, consumption levels of each food by experimental groups were analyzed using a mixed model ANOVA with a within-subject factor of food type (novel, familiar) and a between subject factor of group (home cage tested females, home cage tested males, novel context tested females, novel context tested males) and *post hoc* Bonferroni multiple comparisons following significant main effects. Following ANOVAs with significant interactions, simple effects (Bonferroni adjusted) were calculated. Differences between context and sex were analyzed using *a priori* planned orthogonal contrasts. Total consumption during each test (a sum of both foods, novel and familiar) was also analyzed for each group using a univariate ANOVA and *post hoc* Bonferroni multiple comparisons. Differences between context and sex were analyzed using *a priori* planned orthogonal contrasts.

A significance value of $p < 0.05$ was used for all analyses, except for *post-hoc* analyses in which Bonferroni adjusted alpha level was used ($p = 0.05/3 = 0.017$). Data were analyzed for normality using Shapiro-Wilk test. In instances when the data failed normality test, mixed model ANOVA results were confirmed with a non-parametric test. In order to compare the consumption across groups, the difference in the amounts of familiar minus novel food consumed was calculated (the difference score) for each test, and the difference scores were compared across groups with a Kruskal-Wallis between-subjects one-way ANOVA. In two comparisons, the results differed from parametric analysis and those are reported in the results section. In addition, the average rate of change of novel food consumption between the first and last test was calculated for each group ([*grams consumed* T8-*grams consumed* T1]/[8–1]).

3. Results

Male and female rats were exposed to familiar and unfamiliar foods in either their home cage or a novel context for eight testing sessions. Following arrival, males gained weight faster than females, resulting in body weight differences during testing ($p < 0.01$, all tests; Fig. 1). However, there were no differences in body weight between rats tested at home versus novel environment within the same sex; therefore, all consumption results are reported as grams consumed.

During the Test 1 home cage groups of both sexes ate more of the familiar food compared to the novel, while the novel context tested groups showed no preference and overall suppressed consumption (Fig. 2, Fig 3A). A mixed model ANOVA for food type and testing group found main effects of food type and group ($F(1,28) = 55.410$, $p < 0.001$;

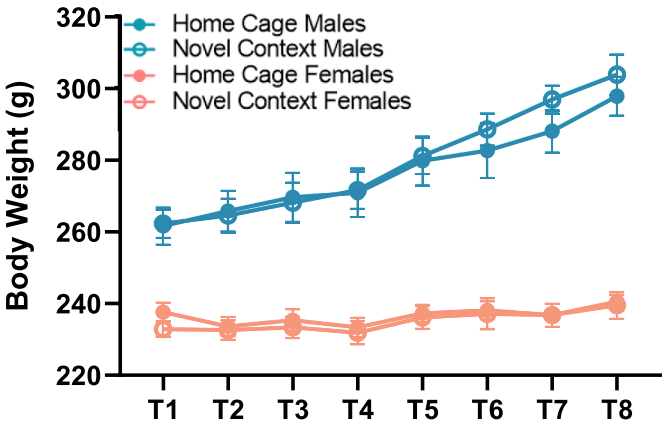

Fig. 1. Body weight averages (mean ± SEM) for each group across testing sessions.

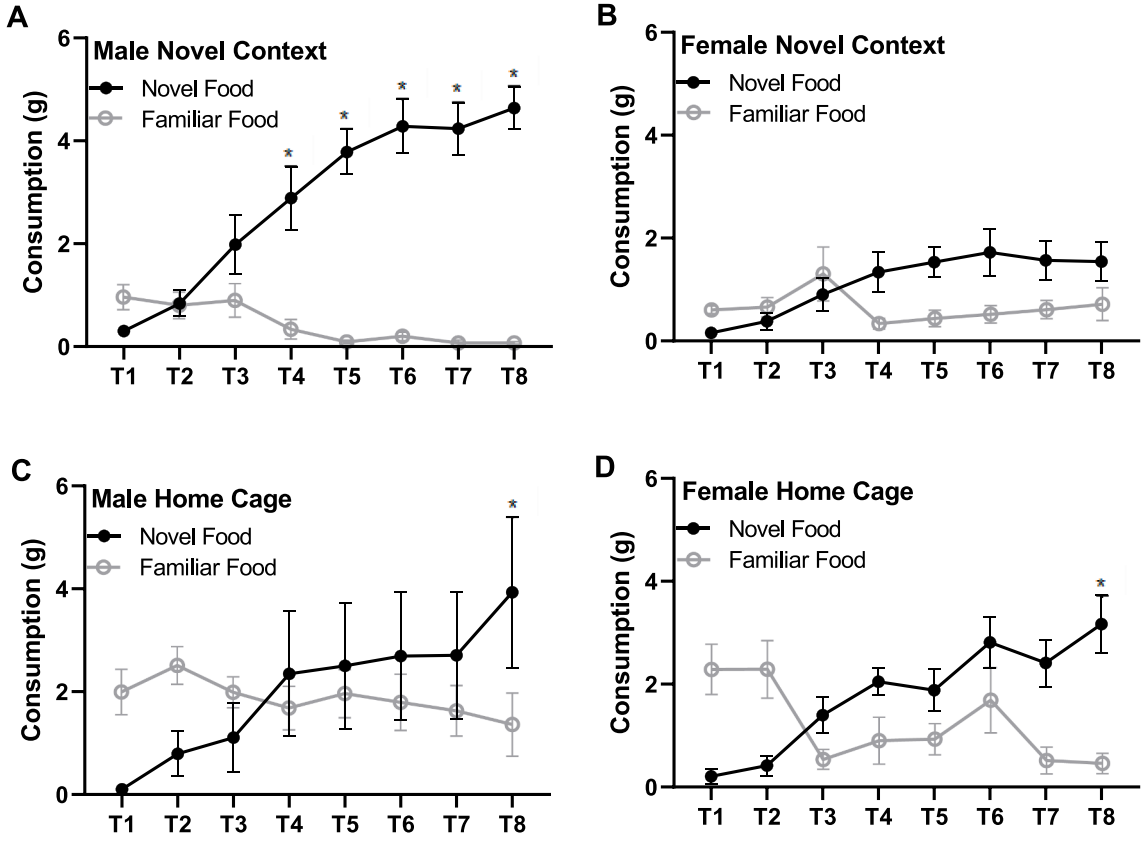

Fig. 2. Familiar & Novel food consumption across tests. Graphs show consumption in grams (mean ± SEM) of each food type for Males tested in a novel context (A), Females tested in a novel context (B), Males tested in home cage (C), and Females tested in home cage (D). Asterisks indicate a significant difference in consumption between food types.

F(3, 28) = 4.138, $p = 0.015$) as well as a food type *by* group interaction (F(3, 28) = 6.103, $p = 0.002$). Male and female groups that were tested in home cages showed a higher consumption of familiar food than of the novel food. This was supported by significant simple effects (Bonferroni adjusted) within each sex (F(1, 28) = 30.99, $p < 0.001$; F (1,28) = 37.395, $p < 0.001$). Group differences found by the ANOVA in consumption of novel food versus familiar food was further supported with a Bonferroni *post hoc,* which found a significant difference in

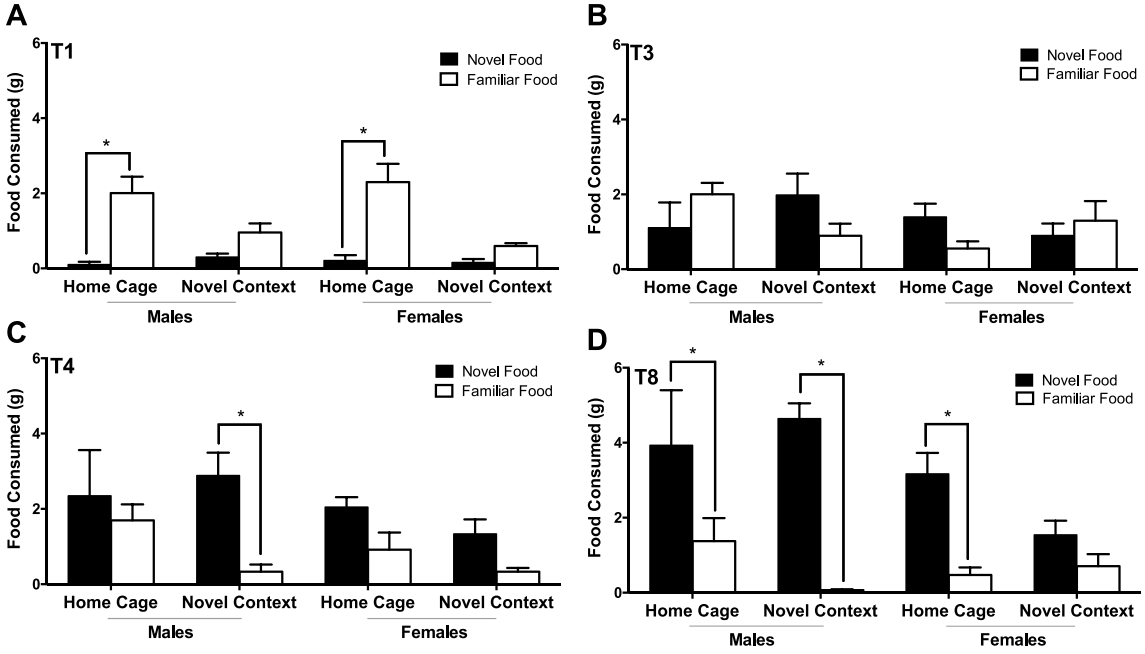

Fig. 3. Familiar & Novel food consumption across tests. Graphs show consumption in grams (mean ±SEM) during Test 1(A), Test 3 (B), Test 4 (C), and Test 8 (D). Asterisks indicate a significant difference in consumption between food types.

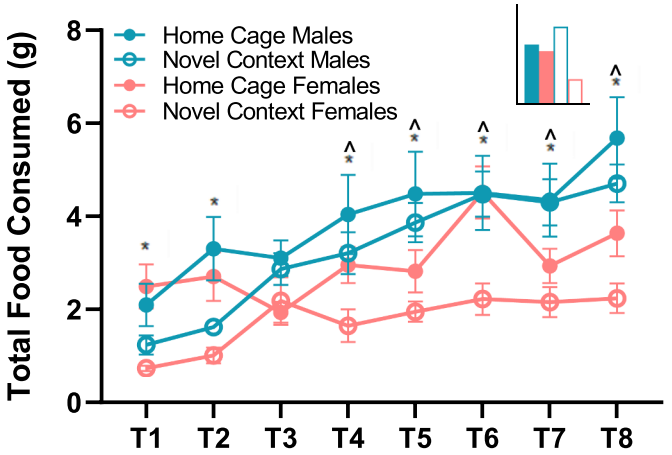

Fig. 4. Total food consumed in grams (mean ± SEM) by groups across testing sessions. Astericks indicate a significant difference in consumption between groups. Symbol ^ indicates a significant difference in consumption between sexes. Bar graph (insert in upper right) shows average rate of change in Novel food consumption across tests.

consumption patterns between the home cage tested female and novel context tested female groups ($p = 0.022$).

There were overall differences in total consumption in different contexts. *A priori* planned contrasts of context and sex showed a significant difference between groups tested in home cage and groups tested in a novel context in average consumption of each food ($p = 0.002$), but no difference of sex ($p = 0.6$). A comparison of total food consumption across groups revealed that females tested in novel context ate the least and that was significantly less than males tested in home cage ($p = 0.004$; Fig. 4).

The pattern of consumption during Test 2 was similar to that of Test 1. Home cage groups ate more of the familiar than the novel food and novel context groups ate similar low amounts of both foods (Fig. 2). A mixed model ANOVA with factors for food type and group found a main effect of both food type and group ($F(1,28) = 15.312$, $p = 0.001$; $F(3,28) = 4.247$, $p = 0.004$) and a food by group interaction ($F(3, 28) = 4.06$, $p = 0.016$). Bonferroni post hoc comparison found a significant difference in consumption between home cage tested males and novel context tested females ($p = 0.006$). A test of simple effects confirmed that both home cage tested males and females consumed more of the familiar food than the novel food (males $F(1,28) = 12.467$, $p = 0.001$; females $F(1,28) = 14.720$), $p = 0.001$). There was no difference in consumption of the two foods in either male or female novel context groups (males, $p = 0.93$; females, $p = 0.59$). Context based differences in consumption were further supported by an a priori planned significant contrast for testing context ($p = 0.001$). The analysis of group differences in total consumption levels (one-way ANOVA; $F(3,28) = 5.459$, $p = 0.004$) found that males tested in home cage consumed more than females tested in novel context ($p = 0.007$, Bonferroni adjusted; Fig. 4). However, non-parametric analysis using Kruskal-Wallis between-subjects one-way ANOVA of the difference scores (see Statistical Analysis) did not yield a significant main effect of group for Test 2 ($x^2(3) = 6.778$, $p = 0.079$.

During Test 3 most rats ate similar amounts of both foods (Figs. 2, 3B). This was confirmed with an ANOVA that found no main effect of food type or group ($F(1,28) = 2.99$, $p = 0.095$; $F(3,28) = 1.245$, $p = 0.31$). However, there was a significant interaction of food type by group ($F(3,28) = 4.678$, $p < 0.01$). Analysis of simple effects revealed that males tested in home cage consumed more of the familiar food than the novel food ($F(1,28) = 15.537$, $p < 0.01$). Post hoc multiple comparisons found no differences between groups ($p > 0.05$, all). Total consumption was similar across all groups ($F(3,28) = 2.102$, $p = 0.123$; Fig. 4).

In Test 4 novel context tested males consumed more novel food than familiar food (Fig. 2A), whereas all other groups ate similar amounts of both foods (Fig. 2B-D, Fig. 3C). The results of a mixed model ANOVA revealed a main effect of both food type and group ($F(1,28) = 7.489$, $p = 0.011$; $F(3,28) = 3.259$, $p = 0.036$), but no interaction ($F(3,28) = 0.733$, $p = 0.54$). A Bonferroni post hoc comparison yielded group differences in consumption between males tested in home cage and females tested in novel context ($p = 0.028$). Results of a test of simple effects showed males tested in novel context consumed more of the novel than familiar foods ($F(3,28) = 6.887$, $p = 0.014$). Our a priori planned orthogonal contrasts, showed no significant difference between contexts ($p = 0.062$), but showed significant difference of sex ($p = 0.036$). Non-parametric analysis did not reveal a greater difference in consumption for novel context males compared to other groups until Test 5 (Group: $x^2(3) = 19.409$, $p < 0.001$; Dunn-Bonferroni post hoc: novel context males vs. novel context females $p = 0.033$, novel context males vs. home cage females $p = 0.006$).

In terms of total consumption, there was an emerging sex difference as females tested in novel context ate less than males tested in home cage (Fig. 4). A Bonferroni post hoc, following a significant one-way ANOVA for group ($F(3,28) = 3.259$, $p = 0.036$), confirmed a signficant difference in consumption between home caged tested males and novel context tested females ($p = 0.01$).

Consumption patterns during Test 5 through 7 were similar to patterns during Test 4; only novel context tested males consumed more of the novel than familiar food (Fig. 2A). For Test 5, there was a significant main effect of food type and group ($F(1,28) = 11.799$, $p = 0.002$; $F(3, 28) = 3.99$, $p = 0.017$), but no interaction ($F(3,28) = 2.495$, $p = 0.08$). Bonferroni post hoc comparisons showed group differences between males tested in home cage and females tested in novel context ($p = 0.021$). There were significant simple effects, when food type consumption was examined within each group, for males tested in novel context ($F(1,28) = 16.435$, $p < 0.001$), but no other group (male home cage, $F(1,28) = 0.338$, $p = 0.57$; female home cage, $F(1,28) = 1.057$, $p = 0.31$; female novel context, $F(1,28) = 1.456$, $p = 0.24$). Using a priori contrasts, we additionally found significant difference of sex ($p = 0.003$).

In terms of total consumption during Test 5, males consumed more than females within each context (Fig. 4). Analysis of total consumption revealed a significant main effect of group ($F(3,28) = 3.99$, $p = 0.017$) with significant contrasts for sex within home cage tested groups and novel context tested groups separately ($p = 0.045$; $p = 0.022$).

During Test 6 (Fig. 2) there was a main effect of both food type and group ($F(1, 28) = 11.12$, $p = 0.002$; $F(3, 28) = 3.994$, $p = 0.017$), but no significant interaction. Bonferroni post hoc comparisons showed a significant group difference between females tested in home cage and females tested in novel context ($p = 0.049$). There were marginally significant group differences between males tested in home cage and females tested in novel context ($p = 0.051$) and between males and females tested in novel context ($p = 0.055$). As in Tests 4 and 5, males tested in novel context continued to consume more novel food than familiar ($F(3,28) = 13.951$, $p = 0.001$).

Total consumption differed between sexes in novel context, where males consumed more than females (Fig. 4). Analysis using a priori contrasts, showed significant difference of sex within novel context tested groups ($p = 0.009$). Total consumption analysis also showed that both males and females tested in home cage and males tested in novel context ate significantly more than females tested in novel context ($p = 0.02$, $p = 0.045$, $p = 0.05$, respectively).

Similar patterns continued during Test 7 (Fig. 2) with a main effect of food type and group ($F(1,28) = 16.896$, $p < 0.001$; $F(3, 28) = 4.170$, $p = 0.015$). Bonferroni post hoc comparisons showed group differences between males tested in home cage and females tested in novel context ($p = 0.039$) and between males and females tested in novel context ($p = 0.045$). There was also a significant contrast of sex, regardless of context ($p = 0.002$). An analysis of total consumption levels showed

that females tested in novel context ate significantly less overall than males tested in home cage and males tested in novel context ($p = 0.012$, $p = 0.035$, respectively; Fig. 4).

In the final test session, Test 8, all groups except novel context tested females consumed more novel food than familiar (Figs. 2, 3D). This was confirmed with main effects of both food type and group ($F(1, 27) = 25.087$, $p < 0.001$; $F(3, 27) = 5.936$, $p = 0.003$). Novel context tested females ate similar amounts of both foods, differing significantly from consumption patterns of both novel context and home cage tested males ($p = 0.004$, $p = 0.02$). The remaining three groups consumed more novel food than familiar; males tested in home cage ($F(1, 27) = 5.233$, $p = 0.03$), females tested in home cage ($F(1, 27) = 6.628$, $p = 0.016$), and males tested in novel context ($F(1, 27) = 19.156$, $p < 0.001$). A priori planned contrasts revealed a significant different between contexts ($p = 0.001$), but no overall difference of sex.

Females tested in novel context had the lowest total consumption compared to all other groups (Fig. 4). Analysis of total consumption using a one-way ANOVA yielded a significant main effect of group ($F(3,28) = 12.771$, $p = 0.003$). A post hoc Bonferroni test found that females in novel context ate significantly less than males tested in home cage and males tested in novel context ($p = 0.004$, $p = 0.02$, respectively). Similarly, the females tested in novel context had the lowest rate of change in novel food consumption across the first and last test (0.197) compared to all other groups (Novel Context Males: 0.62; Home Cage Females: 0.422; Home Cage Males: 0.477) (Fig. 4).

4. Discussion

In this study, we investigated how novelty impacts food consumption in males and females. We behaviorally characterized the effects of novel food and novel feeding environment and their interaction by tracking consumption across multiple tests until habituation. To our knowledge, this is the first study to examine the interaction of novel foods and novel environments in male and female rats. We found sex differences when animals were tested in a novel environment. Female rats tested in a novel context did not habituate to the novel food, or to the new environment, as they consumed small amounts of both foods across all tests. In contrast, all other groups increased consumption of the novel food and by the final testing session all showed preference for the novel food.

In the current study, we chose a palatable novel food to encourage habituation to a novel taste. The novel food (TD pellets) was calorically similar to the familiar food (standard chow), but had high sucrose content, making it sweet tasting. Previously, we compared rats' preference for TD pellets to other high-sugar/high-fat (Oreos, Nabisco), high-sugar/low-fat (Lucky Charms, General Mills), and low-sugar/high-fat (Cheetos, Frito Lay) palatable foods [11]. The highest preference score was for TD and the high-sugar/ high-fat food (equal), based on the amounts consumed during 30 min tests (5 g of single food given, test order counterbalanced). Nevertheless, in the current study, females fed TD pellets in the novel context showed slow and subdued signs of habituation through eight exposures, compared to their male counterparts.

Our results are in agreement with prior studies that examined taste and context exposure separately. Reilly and colleagues outlined the course of taste neophobia, with rats showing lower intake on initial presentations of a novel saccharin solution that increased over time, with the number of licks increasing in cluster size (an index of palatability) across each trial [5]. Our findings are generally consistent with an increase in preference over time, however we tested rats across multiple sessions and did not observe an emergence of preference for the novel taste until test 4. Additionally, the timeline for increased consumption of the novel food varied based on testing context. Males in the novel context showed preference much earlier (Test 4) than home cage tested groups (Test 8).

A previous study examining the effect of context habituation length on novel food intake found a similar effect. Male rats who were habituated for 5 days instead of 25 were faster to increase novel saccharine solution intake in a preference test [7]. Our males tested in a novel context began to show preference for the novel food faster than the home cage tested males who were tested in a familiar context. Our study also included female rats, who, when tested in home cage, also showed increased preference for novel food by the final testing session. Females tested in a novel context were the only group that did not show that pattern.

Novelty effects on feeding behavior have been previously used as behavioral models of depression and anxiety [10]. In general, greater cessation of feeding behavior has been considered to indicate greater depression or anxiety. However, there are procedural differences in terms of the type of novelty (food, feeding environment) and in behavioral measures (consumption and/or latency to approach food) [[10], [2]]. Prior work often used these behaviors to determine the efficacy of anxiolytic drugs, typically in preparation that examined either the effects of novel foods or novel feeding environments. Studies that have examined the effects of novel contexts on consumption have noted longer latencies to consume familiar food, however, this was observed within a single testing session rather than across multiple presentations [[10], [2]]. In the current study, novel context attenuated overall consumption during initial exposure similarly for males and females, but only males developed a preference for the novel food across multiple testing sessions while females did not.

Fewer studies have examined novelty effects on food consumption in both sexes and potential differences when a novel food is consumed in a familiar versus novel environment. The current results indicate that there is a cumulative effect when novel foods are consumed in a novel environment, and that together they lower total consumption more than each separately. Previous work showed that unconditioned fear (electric footshock) decreased the consumption of a novel taste [5], aligning with our observation that the novelty effects may be cumulative, especially if the underlying mechanisms are related to anxiety or fear states. In our study, the compounding effect of novelty had a stronger impact on females, as females fed a novel food and in a novel context showed sustained low total consumption. While females in novel context increased novel food consumption over time, their consumption was less than half of any other group and they never reached the levels of habituation seen in other groups. Their average rate of change for novel food consumption was also the lowest of any group. Conversely, males tested under the same conditions were the first of any testing group to show increased consumption of the novel food. The average rate of change for novel context tested males was the highest of any group. The faster increase in consumption in males could be due to faster habituation or greater preference for the palatable food or both.

Interestingly, rats tested in a familiar context were slower to increase consumption of the novel food and did not show preference until the final testing session, and average rate of change of novel food consumption was similar for males and females. This delay is likely due to the strong association previously established between home cage (familiar context) and the consumption of their usual rat chow (familiar food). Additionally, slower increase in consumption for home cage males could be related to the greater individual variability for this group. Four of the eight males tested at home (all from one replication) never showed any preference for the novel food. These four males also had lower total consumption suggesting that perhaps they experienced higher levels of aversion or stress that drove their food avoidance.

Sex differences in states akin to anxiety or depression may driving the low consumption we observed in females but not males tested in a

novel context. In agreement with this hypothesis, a previous study using a different model of anxiety, social separation, found greater effects on food consumption for females. Following social separation, female Syrian hamsters showed an increased latency to consume food compared to their male counterparts [13]. Differences in consumption patterns of male and female rats have been noted, particularly when they are tested in settings that are presumed to induce a state akin to fear or anxiety. In one study, when presented with a tone that signals a footshock (fear cue), female rats maintained inhibition of consumption much longer than males [8]. This aligns with the current finding that the females tested in novel context, instead of habituation, show sustained inhibition of consumption. The difference in the male versus female response to novel food and environment may indicate differences in adaptivity. Sustained low consumption in novel context could serve as protective measure in females. On the other hand, the resistance to habituation overtime can become disadvantageous and even dangerous.

The mechanisms underlying habituation may be related to extinction processes. Fear habituation and extinction circuits have been shown to partially overlap, at least in males [4]. For females there is evidence of an effect of estrous cycle on extinction learning, as rats in proestrus (high estradiol) show better extinction [6]. Therefore, estradiol levels may impact habituation, similar to the effects on extinction. In this study, we did not monitor estrous cycling in females in order to avoid the potentially stressful effects of that procedure on food intake. Interestingly, total consumption for home cage tested females varied across tests in a manner that may suggest differences in cycling estrogen (lower consumption compared to male counterparts in test 5, the same in test 6, and again lower in test 7). We did not observe similar variability in the novel context tested females, however that could be due to their consistent low total consumption. Of note, estrous cycle effects on consumption are typically observed over a 24-hour period [3]. Our testing sessions were short (ten minutes) and may have been too brief to capture an effect of estrous stage on total intake.

In conclusion, our study revealed robust sex differences in food consumption under novelty. Rats of both sexes increased consumption of the novel food overtime in a familiar environment, indicating similar habituation to novel taste and similar preference for the novel palatable food. In a novel environment, males habituated to a novel food faster than females, who showed sustained, suppression of consumption across multiple exposures. These results demonstrated that novel context has a greater effect on female's consumption compared to males. The differences in how novelty impacts consumption in males and females may be relevant to sex differences in avoidant behaviors [14] in maladaptive circumstances and the development of psychopathology. Research investigating novelty processing can provide insight to underlying behavioral and neural mechanisms and aid in the development of treatment for avoidance-based neuropsychiatric disorders. This behavioral preparation is therefore is a valuable model to test neural substrates for adaptive habituation and novelty processing.

Funding

This work was supported by the National Institutes of Health, NIDDK grant R01DK085721 to GDP.

Declarations of Competing Interest

None.

Acknowledgments

We thank Laura Buczek and Michelle Fitzpatrick for technical support and research assistance and Dr. Ehri Ryu for advice regarding statistical analysis.

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