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Binge-Eating Behavior in Socially-Isolated Female Mice

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Social isolation and loneliness are strong risk factors for depression. Depression is highly prevalent, comorbid with eating disorders and disproportionately diagnosed in women. Still, the mechanisms underlying these disorders are incompletely understood and understudied in females. We used a social isolation model for depression to investigate its impact on binge eating high-fat food in female mice. Subjects were individually-housed (social isolation) or pair-housed (control) with continuous access to standard chow and allowed 2 hours of access to high-fat food, three times per week. After four weeks, we used the tail suspension test to assess depressive-like behavior and measured food intake during a binge eating test. Individually-housed mice spent more time immobile during the tail suspension test, confirming that isolation induced depressive-like behavior. Both groups of mice consumed similarly large totals of high-fat food after two hours, a majority of which was consumed in the first few minutes of the binge. Interestingly though, after 7.5 and 15 minutes, individually-housed mice consumed significantly more high-fat food than pair-housed mice. Thus, social isolation caused rapid and excessive feeding during the early minutes of binge eating. This model of abnormal feeding behavior in socially isolated female mice may be useful for investigating the brain circuits underlying binge eating.

INTRODUCTION

Depression is a highly prevalent mental disorder affecting 6.7% of adults (16.1 million) in the United States annually (Center for Behavioral Health Statistics and Quality, 2016; Centers for Disease Control and Prevention (CDC), 2010). Eating disorders including binge-eating, anorexia nervosa, and bulimia nervosa affect an estimated 4.6% of the population each year (Le Grange, Swanson, Crow, & Merikangas, 2012). Depression and eating disorders are significantly comorbid conditions (Hudson, Hiripi, Pope, Kessler, & Kessler, 2007). For example, approximately 32.3% of patients with the binge-eating disorder (BED) also meet criteria for major depressive disorder (Hudson, Hiripi, Pope, Kessler, & Kessler, 2007).

Women are nearly twice as likely than men to be diagnosed with depression (Noble, 2005). Women are also more susceptible to developing BED than men (Hudson et al., 2007). In fact, 20 million women in the United States alone suffer from BED, compared to only 10 million men (Striegel-Moore et al., 2009). Still, females are understudied in biomedical research (Beery & Zucker, 2011). These data support an unmet need to study the pathophysiology of BED in females.

Binge-eating is defined as a period of uncontrolled hyperphagia, during which individuals quickly consume excessive amounts of food (Perello, Valdivia, Romero, & Raingo, 2014), often including palatable, high-fat food. Rodents provided with intermittent (as opposed to continuous or daily) access to high-fat food have served an important role in modeling and investigating the pathophysiology of this disorder (Corwin, Avena, & Boggiano, 2011). Mice and rats provided with palatable food rapidly consume upwards to 80% of their daily caloric intake within a 2-hour period of limited access (Bake, Morgan, & Mercer, 2014; Bake, Murphy, Morgan, & Mercer, 2014). This resembles feeding behaviors observed in humans with BED.

Animal studies have also used social isolation to model the depressive-like behaviors observed in humans. This is important because real or perceived social isolation and loneliness are major precipitants of depression (Matthews et al., 2016). Housing mice alone, for example, increase immobility during the tail suspension test (Ieraci, Mallei, & Popoli, 2016), a popular method for assessing behavioral despair and for screening anti-depressant drugs (Castagné, Moser, Roux, & Porsolt, 2010). Consistent with the comorbidity that depression shares with eating disorders in humans, prolonged social isolation has also been shown to increase total food intake in rodents (Perez et al., 1997; Sun et al., 2014; Yamada et al., 2015). Only one study assessed the effects of social isolation on food intake in female mice (Yamada et al., 2015). Importantly though, the effects of social isolation on binge-eating of palatable food in female mice have not yet been investigated.

We used social isolation as a model for depression and investigated its impact on feeding behavior during a test of binge-eating in female mice. Mice were individually housed (social isolation) or pair-housed (control) and provided with intermittent access to high-fat food. After 4 weeks, the tail suspension test was used to measure isolation-induced despair. To examine co-occurring binge-eating behavior in detail, we measured food intake at 5 time

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points: 7.5, 15, 30, 60, and 120 minutes during a binge-eating test.

MATERIALS AND METHODS

All procedures were carried out in accordance with the guidelines established by the National Institutes of Health for the Care and Use of Laboratory Animals and were pre-approved by the Institutional Animal Care and Use Committee of Western Connecticut State University.

Mice Husbandry

A total of n = 21, C57BL/6 female mice were purchased from Charles River Laboratories at 8 weeks of age. Mice were group housed and habituated to a 12h light/dark cycle for at least 1 week prior to experimentation. The experimental room was maintained at a temperature between 23-28°C. Food and water were provided *ad libitum*.

Social Isolation

Mice were randomly divided into two groups (independent variables): individually-housed (IH; n = 9) and pair-housed (PH; n = 12) mice. Individually-housed mice were isolated in single, separate cages for 4 weeks. Pair-housed mice lived two per cage the entire time, controlling for food intake and body weight gain in mice living in a social environment.

Binge-Eating Test

Mice were introduced to palatable, high-fat food (Research Diets, Inc.; 60% fat, 5.24kcal/g metabolizable energy) before experimentation to eliminate neophobia. During experimentation, both individually-housed and pair-housed mice had access to high-fat food for 2 hours, 3 times per week (Monday/Wednesday/Friday). Mice were not food deprived and had ad libitum access to standard chow (Purina LabDiet #5001; 3.34kcal/g metabolizable energy) at all other times. This model of intermittent access to high-fat food reliably produces binge-type feeding in non-food restricted rats and mice (Dimitriou, Rice, & Corwin, 2000). To examine binge-consumption patterns in detail, food intake was measured at 5-time points: 7.5, 15, 30, 60, and 120 minutes. Data from the three days (Monday/Wednesday/Friday) were averaged for each mouse. It is not possible to measure the food intake of individual mice when living together. So, for pair-housed mice, the total food intake per cage was divided by two to calculate the food intake per mouse for each cage, as performed by (Yamada et al., 2015). In other words, for n = 12 pair-housed mice, there were n = 6 data points.

Tail Suspension Test

After 4 weeks, the tail suspension test was used to investigate depressive-like behaviors induced by isolation. Mice were individually suspended by their tail 30cm above the table with adhesive tape, in such a position that mice could not escape or hold on to nearby surfaces. Since C57BL/6 mice have a tendency to climb up their tail, a 4cm plastic straw was passed through their tail as a "climb stopper," as performed previously by Can et al., 2012. Mice were suspended for 6 minutes and were video recorded. Mobility and immobility times were manually evaluated from the video files. Mobility was defined as movement of mouse limbs, shaking of the body, and escape-related behaviors. Immobility was defined as no movement of all four limbs, small movements of front limbs (but not involvement with back limbs), twitches, and pendulum like swings. Videos were randomly scored by a blinded reviewer to eliminate bias. Total immobility time and the latency to immobility were used as measures of depressive-like behavior.

Binge-Eating Test

A two-way repeated measures Analysis of Variance (ANOVA) followed by Bonferroni post tests were used to assess differences in food intake during binge-eating between individually-housed and pair-housed mice over time. Independent t-tests were used to compare total caloric intake as well as total immobility time and latency to immobility between groups during the tail suspension test. Differences between means were considered to be statistically significant if $p < 0.05$.

RESULTS

Tail Suspension Test

Results from the tail suspension test are displayed in Figure 1. PH mice exhibited an average of 204.5 ± 9.8 seconds of total immobility time whereas IH mice had an average of 240.3 ± 8.6 seconds of total immobility time (Figure 1A). A t-test revealed this difference to be statistically significant ($p < .05$). In addition, IH mice had a shorter latency to immobility time in comparison to PH mice. IH mice reached immobility within an average of 10.4 ± 1.2 seconds, while the latency for PH mice averaged 26.4 ± 6.3 seconds (Figure 1B). The difference in latency to immobility between groups was also statistically significant ($p < 0.05$).

Binge-Eating Test

High-fat food intake for PH and IH mice during 2 hours of limited, intermittent access is displayed in Figure 2. Mice consumed high-fat food for the duration of the 2-hour binge-eating test (Figure 2A). Time significantly influenced the quantity of food consumed ($p < 0.001$). All mice consumed most of their 2h intake within the first 7.5 minutes (Figure 2A; $p < 0.05$). Specifically, PH mice consumed 43.86 ± 3.00% whereas IH mice ate 53.69 ± 1.30% of their 2-hour intake within the first-time interval. Groups of mice behaved differently over time [Time * Treatment $F(4,52) = 6.431$, $p < 0.001$]. IH mice ate comparatively more than PH mice during the 0-7.5min interval (4.20 ± 0.16kcal vs. 3.38 ± 0.16kcal, $p < 0.05$) but less during 30-60 min interval (0.53 ± 0.05kcal vs. 1.02 ± 0.13kcal; $p < 0.05$) (Figure 2A).

Socially isolated, (IH) mice consumed a total of 7.84 ± 0.29kcal while PH mice ate 7.79 ± 0.32kcal during the average 2-hour binge-eating session (p = n.s., Figure 2B). Although total high-fat food intake was similar, treatment significantly influenced cumulative high-fat food intake over time [Time * Treatment [$F(1.997, 25.965) = 5.558$, $p = 0.01$]. IH mice consumed a significantly greater cumulative amount of high-fat food compared to PH mice at 7.5 and 15 minutes ($p < 0.05$). While PH mice consumed an average of 3.38 ± 0.16kcal and 4.48 ± 0.18kcal of high-fat food, IH mice consumed an average of 4.20 ± 0.16kcal and 5.24 ± 0.20kcal of high-fat food at the 7.5-minute and 15-minute time

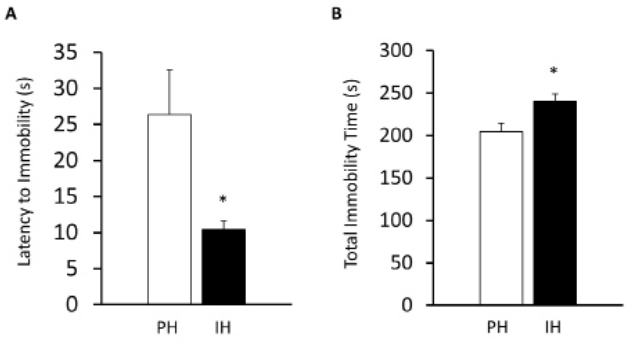

Figure 1. Social isolation produced depressive-like behavior in the tail suspension test. A) Average latency to immobility during the tail suspension test. B) Total time spent immobile during the tail suspension test. Values are means ± SE, n = 6 Pair-Housed (PH); n = 9 Individually-Housed (IH);T-test; *$p < 0.05$.

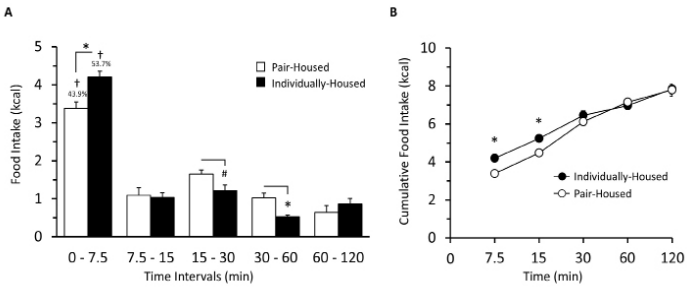

Figure 2. Social isolation caused a brief period of hyperphagia during early minutes of the binge eating test. A) Absolute high-fat food intake within indicated time intervals during the 2-hour binge eating test. B) Cumulative high-fat food intake during the 2-hour binge eating test. Values are means ± SE; n = 6 Pair-Housed (PH); n = 9 Individually-Housed (IH); 2 – Way RM-ANOVA w/ Bonferroni posttest *$p < 0.05$, #$p < 0.10$ compared with PH group; †$p < 0.05$ compared with other time points. Percentages above bars refer to kilocalories consumed in the 7.5 minute bin relative to 2h intake.

points, respectively (Figure 2B).

Total Caloric Intake and Weight Change

Total kilocalories consumed from high-fat food and standard chow, as well as weight change during the week of testing are displayed in Figure 3. PH mice consumed a sum of 23.07 ± 1.50kcal from high-fat food (during binge-eating sessions) compared to 23.53 ± 0.86kcal for IH mice (Figure 3A; p = n.s.). Kilocalories consumed from standard chow (between binge-eating sessions) summed 30.61 ± 1.24 kcal for IH mice, which was more than the 25.16 ± 1.60kcal intake for PH mice (Figure 3A; $p < 0.05$). Total caloric intake (high-fat food + standard chow) for IH mice was 54.12 ± 1.64kcal, which was also greater than the 48.23 ± 3.10kcal total for PH mice (Figure 3A; $p < 0.05$). PH and IH mice gained similar grams of body weight measuring 0.44 ± 0.49g and 0.49 ± 0.29g, respectively (Figure 3B; p = n.s.).

DISCUSSION

After 4 weeks, socially-isolated female mice exhibited depressive-like symptoms including longer immobility time and shorter latency to immobility during the tail suspension test. Isolated females also consumed more food than pair-housed mice during the earliest minutes of binge-eating. These results not only indicate that the comorbidity of depression and binge-eating can be modeled in female mice, but also that social isolation can produce rapid and excessive feeding at the onset of a binge-eating episode.

Binge-eating is conventionally assessed by measuring food intake during a 2-hour test session (Perello et al., 2014; Wolfe, Baker, Smith, & Kelly-Weeder, 2009). Our results, however, demonstrate the benefits of measuring consumption at earlier time points. Mice ate a majority of high-fat food during the first minutes of the binge-eating session. In fact, socially-isolated mice consumed 53.7% of their 2-hour total caloric intake within the first 7.5 minutes of the binge-eating session. For comparison, pair-housed mice consumed 43.9% of their 2-hour caloric intake at the same time point. Though the absolute food intake between IH and PH mice was only significantly different for the 0-7.5-minute interval (Figure 2A), the cumulative intake during the first 15 minutes was also different (Figure 2B). Measuring food intake at these early time points helped detect a difference in eating behavior which otherwise would have been missed had we only measured food intake after 2 hours, when total food intake was no different between IH and PH mice.

It is interesting to think about why food intake by isolated mice differed from pair-housed mice only at the earliest (7.5 and 15-minute) time points. One explanation could simply be because the capacity of a mouse stomach is limited (McConnell, Basit, & Murdan, 2008). So, there is an upper limit to how much food (volume) a mouse can physically consume in a short period of time. Given the hyperphagia we observed in the socially isolated mice, they would have reached this limit sooner. Results from studies in mice and rats performed by Bake et al. (2014) also support this by showing that animals with scheduled access to high-fat food approached a state of satiety in approximately 15 minutes, once they had consumed upwards of about 60% of their 2-hour high-fat food intake. Consistent with this, we observed that IH and PH mice consumed 67.1% and 57.7% of their 2-hour intake after 15 minutes. Mice may have reached a state of satiety, limiting observation of any differences in feeding behavior afterwards.

The initial motivation for isolated mice to more quickly consume the high-fat food could be linked to anticipatory behavior. Mice with intermittent exposure to food rewards show increased food anticipatory activity (locomotor and rearing activity) in advance of receiving the food (Bake, Murphy, et al., 2014). Social isolation may have increased anticipation for high-fat food, motivating mice to eat an increased amount in a shorter period of time. In the future, closer inspection of food anticipatory activity could help to confirm this.

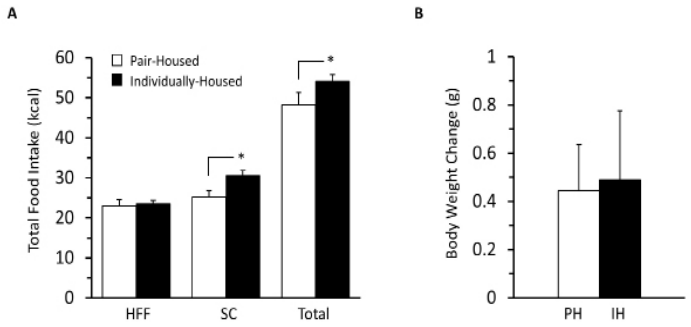

Figure 3. Social isolation increased caloric intake without impacting body weight. A) Kilocalories consumed from high-fat food (HFF) intake during binge eating test, standard chow (SC) intake between binge eating tests, and total intake for the week. B) Grams of body weight changed from Monday to Friday, during testing of binge eating. Values are means ± SE; $n = 6$ Pair-Housed (PH); $n = 9$ Individually-Housed (IH); T-test $*p < 0.05$.

Binging on palatable foods is also motivated by reward, which is regulated by dopaminergic pathways in the brain (Bello & Hajnal, 2010). Dysregulation of dopamine signaling from reduced social interaction could also contribute to binge-eating phenotypes. Similar to drug addiction, anticipation and consumption of high-fat food stimulates dopamine release in brain reward centers (De Macedo, De Freitas, & Da Silva Torres, 2016). Socially isolated rats are more vulnerable to addiction due to neurobiological adaptations in brain reward circuits (Whitaker, Degoulet, & Morikawa, 2013). Combined with intermittent, limited exposure to a high-fat food reward, the resulting adaptations from social isolation could lead to a more "compulsive" behavior and a loss of control over the intake of food at the onset of binge-eating.

Perceived loss of control is an important but subjective evaluation used during diagnosis of binge-eating episodes in humans. Individuals suffering from BED describe loss of control as a feeling of helplessness and despair (Wolfe et al., 2009). This defining characteristic of binge-eating is difficult to measure in rodents and is therefore considered a critical limitation for animal models of eating disorders (Perello et al., 2014). By measuring alterations in high-fat food consumption at early time points during binge-eating, though, our experiment and results may offer insight in the design of future studies aiming to characterize loss of control and/or compulsive feeding behavior in mice.

We found that both socially-isolated and pair-housed mice gained the same amount of body weight, despite increasing overall caloric intake (Figure 3). This is consistent with literature demonstrating that social isolation can modify feeding habit without changing body weight. In rats, 6 but not 3 weeks of isolation increased bodyweight though both increased weekly food intake (Nakhate, Kokare, Singru, & Subhedar, 2011; Perez et al., 1997). In mice, neither 2 nor 8 weeks of isolation changed body weight in adult mice despite altering feeding (Sun et al., 2014; Yamada et al., 2015). Only after 13 weeks of continuous social isolation has body weight and fat mass increased with increased feeding (Sakakibara et al., 2012). Many weeks of altered food intake appear to be required to modify body weight. It is also important to notice that though binge-eating episodes may be present in overweight and obese humans, only about 40% of binge-eaters are overweight or obese (Hudson et al., 2007).

Individually housing mice in separate cages is typical in experiments assessing the behavior of individual mice. We showed here that isolating female mice influenced feeding and affective behavior. Experimenters using research designs that include housing female mice individually should be careful to account for the possibility that social isolation may have inadvertent experimental consequences and change the very behavior they aim to study. Since our study only looked at female mice, it is not clear whether the same precautions need to be taken for males. In the future, it would be interesting to investigate whether male mice are also susceptible to changes in feeding behavior induced by social isolation. It could be that, regardless of the sex, socially isolated mice suffer binge-eating more than pair-housed mice. Or, perhaps females are more sensitive to social isolation, representative of the sex differences in the prevalence of depression and eating disorders in humans.

Overall, as a model for depressive-like behavior and altered binge-eating, our results demonstrate that social isolation can be useful for studying the biological pathways relevant to these disorders in humans. For example, new research has identified Dasotraline, a dopamine and norepinephrine reuptake inhibitor, as a possible therapeutic drug for individual suffered from BED (Navia et al., 2017). In the future, our model of social isolation and binge-eating may be helpful to identify and screen new treatments such as these for women.

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