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RESEARCH ARTICLE

The effect of dietary fat on behavior in mice

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Abstract

Purpose Obesity is linked to cognitive dysfunction in humans and rodents, and its effects can be passed on to the next generation. However, the extent of these effects is not well understood. The purpose of this study was to determine the effect of a prenatal maternal high-fat diet and an individual high-fat diet in inbred mice.

Methods We varied maternal diet and offspring diet to test the hypothesis that a high-fat diet would increase anxiety, reduce activity levels, and impair nest-building. First, we fed a high-fat (HF) or low-fat (LF) diet to genetically identical female Small (SM/J) mice and mated them with LF males. We cross-fostered all offspring to LF-fed SM/J nurses and weaned them onto an HF or LF diet. We weighed the mice weekly and we tested anxiety with the Open Field Test, activity levels with instantaneous scan sampling, and nest building using the Deacon Scale.

Results Diet significantly affected weight, with HF females weighing 28.2 g (± 1.4 g SE) and LF females weighing 15.1 g (± 1.6 g SE) at 17 weeks old. The offspring's own diet had major behavioral effects. HF mice produced more fecal boli and urinations in the Open Field Test, built lower-quality nests, and had lower activity in adulthood than LF mice. The only trait that a prenatal maternal diet significantly affected was whether the offspring built their nests inside or outside of a hut.

Conclusions Offspring diet, but not prenatal maternal diet, affected a wide range of behaviors in these mice.

Keywords Diet · Obesity · Anxiety · Nest · Mice · Activity

Background

Obesity is tightly linked to Alzheimer's disease and other types of cognitive dysfunction in humans [1], and is associated with lower cognitive performance in men based on tests of learning and memory [2]. Obesity's effects in mice include increased anxiety [3, 4], diminished spatial memory [4], reduced object location memory [5], impaired learning of contextual fear conditioning and passive avoidance [6], and increased depressive-like behavior [3].

Compounding its consequences for public health, the effects of obesity are not limited to one generation. In humans, maternal obesity not only raises the risk of obesity and cardiometabolic disease in children [7], it also increases the risk of anxiety, attention deficit hyperactivity disorder, and autism [8]. In rodents, offspring of obese dams exhibit significant deficits in reversal learning accompanied by striatal disturbance [9], as well as long-term impairments in spatial learning [10]. Rats born to obese mothers have been found to have hippocampal inflammation and increased anxiety as adults [11], and female mice born to high-fat-fed mothers exhibit higher anxiety, brain tissue inflammation, and inflammatory cytokines [12].

The effects of a maternal high-fat diet on behavior are complex, and its impact on offspring anxiety is far from resolved in the field (Table 1). In some cases, maternal high-fat diet is credited with increasing anxiety in offspring [11, 12, 14–16], while in others it has been shown to have an anxiolytic effect [17–20]. This discrepancy can be attributed to several causes, including the fact that behavior is a highly variable and notoriously difficult trait to measure—especially anxiety behavior—as well as the multitude of tools being used to measure anxiety and the variability in fat content of diets studied by different researchers.

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Table 1 Compilation of the literature studying the effect of maternal high-fat diet on anxiety in rodent offspring using the Open Field Test and/or the Elevated Plus Maze

Study	Species (Strain)	Maternal Diet Conclusion	Length of Time on Maternal Diet	Diet attributes	Offspring sample size
Kang et al. (2014) [12]	Mice (C57BL/6 J)	Induces anxiety	Gestation and lactation	HF (60% cal from fat) vs. C (10% cal from fat)	22 C ♂, 21 HF ♂, 7 HF-C (lactation) ♂, 30 C ♀, 16 HF ♀, 13 HF-C (lactation) ♀
Fernandes et al. (2012) [13]	Mice (C57BL/6 J)	No effect on anxiety (but induced hyperactivity)	6 weeks prior to pregnancy, through lactation	Western diet (16% fat, 33% sugar) vs. C	9 HF ♂, 8 C ♂ (all raised on C diet)
Rodriguez et al. (2012) [17]	Rats (Wistar)	Anxiolytic	Gestation and lactation	HF (25% cal from fat) vs. C (5% cal from fat)	6 HF ♂, 6 C ♂ (all on C diet)
Bellisario et al. (2014) [19]	Mice (P66Shc WT and KO on C57BL/6 J background)	Anxiolytic in daughters, a nxiety inducing in sons	10 weeks prior to mating until 3 days prior to giving birth	HF (58% cal from fat) vs. C (10.5% cal from fat) vs. Standard Diet (17% cal from fat)	15 HF-wt (10 ♀, 11 ♂), 16 C-wt (15 ♀, 10 ♂), $n = 12$ for the OFT
Peleg-Raibstein et al. (2012) [14]	Mice (C57BL/6 N)	Induces anxiety	3 weeks prior to mating, through lactation	HF (60% cal from fat) vs. chow	12 HF ♂, 9 HF ♀, 12 C ♂, 10 C ♀
Bilbo and Tsang (2010) [15]	Rats (Sprague-Dawley)	Induces anxiety	4 weeks prior to mating, through lactation	High-saturated-fat (60% cal from fat) vs. high-trans-fat (60% cal from fat) vs. C (10% cal from fat)	8 per group
Sasaki et al. (2014) [18]	Rats (Long Evans)	Anxiolytic	4 weeks prior to mating, through lactation	HF (60% cal from fat) vs. C (13.5% fat)	13 HF ♀, 7 HF ♂, 13 C ♀, 13 C ♂
Wright et al. (2011) [20]	Rats (Wistar)	Anxiolytic	8 weeks prior to mating, then some switched to chow	Cafeteria diet, different each day, % fat not reported	5–12 per group
Ramírez-López et al. 2016 [16]	Rats (Wistar)	Induces anxiety	8 weeks prior to mating, then throughout pregnancy and lactation	Chow vs. free-choice of chow and cafeteria diet (chocolate) (24.45% cals from fat)	15 from chow moms, 17 from free-choice moms, all weaned on chow

Study	Length of OFT	OFT total distance traveled	OFT Center:total ratio	OFT Time in Center	OFT Rearing	OFT boarder entries	Elevated Plus Maze
Kang et al. (2014) [12]	15 min	Increased in ♂, NS in ♀	NS in ♂, lower in ♀	NA	Increased in both sexes	NA	NA
Fernandes et al. (2012) [13]	10 min	Traveled more	NA	No diff	Increased (in outer part)	NA	NA
Rodriguez et al. (2012) [17]	10 min	No diff in ♂, ♀ not tested	NA	No diff in ♂, ♀ not tested	NA	Increased in HF ♂, ♀ not tested	No diff
Bellisario et al. (2014) [19]	Three 5- min intervals	HF ♂ and daughters traveled less	NA	No diff	NA	NA	HF ♂ more anxious (groomed more, immobile more), ♀ less anxious x(immobile less)
Peleg-Raibstein et al. (2012) [14]	1 h	No diff	NA	NA	NA	NA	More anxious (spent less time in open arms)
Bilbo and Tsang (2010) [15]	NA	NA	NA	NA	NA	NA	SFD and TFD ♂ spent less time in the open arms
Sasaki et al. (2014) [18]	15 min	NA	HF offspring had a higher ratio	NA	NA	NA	HF offspring entered the open arms more
Wright et al. (2011) [20]	5 min	Pregnancy diet: no diff Lactation diet: reduced tot distance traveled in ♂ (no diff in ♀)	NA	♂ entered center sooner (no diff in ♀)	Pregnancy diet: ♂ increased Lactation diet: ♂ decreased (♀ decreased for both)	NA	Reduced locomotor activity in ♂ and ♀, reduced grooming in ♂
Ramírez-López et al. 2016 [16]	5 min	No diff in total distance traveled or mean speed	NA	Offspring from free-choice dams spent less time in center of arena	NA	No diff	Offspring of free-choice dams spent less time in open arms, entered less often into the open arms, spent more time in closed arms and entered the closed arms more

HF High-Fat diet, *C* Control diet, *WT* Wild Type, *KO* Knock-Out, *Cal* Calories, *OFT* Open Field Test, *NS* Not Significant, *diff* difference

While high-fat diet rodent models have been used to study maternal effects on offspring anxiety behavior most commonly, other behaviors have also been studied. Research on the effect of maternal dietary fat on offspring activity levels has yielded mixed results. A maternal high-fat diet was found to decrease locomotor activity in daughters in Sprague-Dawley rats [21], but it increased activity in one of three mouse strains in an Open Field Test and in all three strains during a swim test [22]. More research is needed in order to understand the direction and magnitude of the effect of maternal obesity on offspring activity levels, if indeed there is a consistent effect.

Another interesting avenue to explore in terms of maternal diet is its effect on nest-building behavior. After observing that high-fat-fed mice seemed to build lower-quality nests, we became interested in quantifying the difference to test it statistically. Nests are important for murine thermoregulation, and both males and females build them [23]. Mouse pups especially rely on nests to reduce heat loss, as they are born hairless [24]. Nest quality affects mouse fitness, as illustrated by Lynch [25] who found that mice selected for poor nest building became less fertile over 15 generations, whereas mice selected for good nest building became more fertile and increased their litter size and body weight. More recent research has shown that nest size is correlated with locomotor activity [26], and that mice in cages lacking enrichment materials for nest building produced pups that weighed less and had lower survival rates to weaning age [27]. Several quantitative trait loci have been identified that contribute to genetic variation in nest building [28]. In addition to genetics, nest building is affected by hormones [29] and lesions on the hippocampus, which are also both known to be disrupted by obesity [30, 31].

We tested the hypotheses that an individual's high-fat diet would: (1) increase anxiety (shown by a reduced center:total distance ratio and higher levels of rearing, urination, and fecal boli production in the Open Field Test); (2) reduce activity levels (shown by increased time performing inactive behaviors such as sleeping and resting and decreased time performing self-maintenance, exploring, and social interaction behaviors during instantaneous scan sampling sessions); and (3) impair nest-building ability (shown by a lower score on the Deacon Scale). We also tested the hypothesis that a maternal high-fat diet would affect anxiety, activity levels, and nest building in the same manner in offspring.

Methods

Animal rearing

We studied the inbred SM/J mouse strain from The Jackson Laboratory (Bar Harbor, Maine) which we have previously shown is hyper-responsive to the same high-fat (HF) diet used in this study [32–34]. Using a strain with maximal response to an HF diet increases our power to detect effects.

At 3 weeks of age, 30 male mice and 10 female mice born at Loyola University were weaned onto a low-fat (LF) diet, and 20 female mice were weaned onto an HF diet to create an F_0 generation. We used twice as many HF females as LF females because they had a lower rate of successful pregnancy. In the LF diet, 15% of the calories came from fat (Research Diets D12284), whereas 42% of the calories came from fat in the HF diet (Harlan Teklad diet TD.88137) (Table 2). We have studied these mice using these particular diets for 20 years [32–34]. We chose the LF diet instead of regular chow because it was specifically designed to match the HF diet as closely as possible in terms of nutrients and calories (the HF diet has 18.95 kJ per gram, whereas the LF diet has 16.99, as determined based on Atwater factors by the manufacturers). These diets are in line with what humans consume, with fat intake ranging from 28.5–46.2% of total energy in Europe [35] and 34% in the United States [36]. Procedures followed the institutional and national guidelines for the care and use of animals, and all experimental procedures were approved by the Institutional Animal Care and Use Committee at Loyola University (protocol #1188). Each day, animal welfare was assessed by the facility staff and researchers, and a veterinary technician was in the facility for consultation and further assessment. There were no known adverse events observed in the mice other than weight gain in the HF-fed mice, which was intended. The behavior assays were selected because they are minimally invasive and minimally disruptive to the animals.

The F_0 mice were weaned onto an HF or LF diet at 3 weeks of age and raised for 7 weeks on that diet, while being housed with one other mouse of the same sex and diet. They were then mated, with one male and one female housed per cage. When the female was determined to be pregnant by abdominal palpation, the male was removed from the cage. To avoid confounding the prenatal and postnatal maternal obesity effects, all pups were cross-fostered within 24 h of birth to an LF-fed SM/J nurse. Half of the pups from each litter were weaned

Table 2 Composition of diets

Component	High-fat diet	Low-fat diet
Energy from fat, %	42	15
Casein, g/kg	195	197
Sugars, g/kg	341	307
Corn starch, g/kg	150	313
Cellulose, g/kg	50	30
Corn oil, g/kg	0	58
Hydrogenated coconut oil, g/kg	0	7
Anhydrous milk fat, g/kg	210	0
Cholesterol, g/kg	1.5	0
Kilojoules per gram	18.95	16.99

onto an HF diet and the other half onto an LF diet. This resulted in four F_1 diet treatment groups: HF-HF, LF-HF, HF-LF, and LF-LF, where the first diet listed is the mother's diet and the second is the offspring's diet (Fig. 1). Ten offspring of each sex were randomly assigned to each diet treatment group, for a total of 80 F_1 mice. After weaning, each mouse was housed in corn-based bedding with one other mouse of the same sex and diet in a cage that contained a wooden gnawing block (Bio Serve), a red privacy hut (Alt Design), a 2″ × 2″ cotton nestlet for nesting material (Ancare), and food and water ad libitum in a 12 h light, 12 h dark cycle. We took advantage of the fact that SM/J mice have forced heterozygosity (A^w/a) at the agouti locus; since half of the mice are tan and the other half are black, we housed each mouse with a mouse of a different color in order visually differentiate between the two mice in each cage for behavioral analysis. The mice were weighed weekly and were sacrificed via CO_2 asphyxiation at 17 weeks of age for a different experiment [37].

Our sample size gave us 80% power to detect differences of 0.4 residual standard deviation units ($p = 0.05$), which we deemed sufficient based on other studies of rodent anxiety in the literature.

Open field test

The Open Field Test was conducted using a 17.5″ (L) × 13″ (W) × 15″ (H) opaque plastic box with a grid on the floor that subdivided it into 48 rectangles measuring 2.16″ × 2.18″ (Fig. 2). The mice were brought into the testing room at 9:00 AM and allowed to acclimate, with testing beginning at 11:30 AM. No cage changes were performed within 24 h prior to testing to avoid impacting mouse behavior, and the entire arena was sanitized with 70% ethanol after each mouse was tested. A 10 week old F_1 mouse was then placed into a corner of the arena, and the mouse's movements were observed and video recorded for 5 min by 2 female researchers concurrently. Any discrepancies in observations by the 2 researchers were corrected by reviewing the video. We note that the researchers were female because Sorge et al. [38] showed that male experimenters induce a stress response in mice and rats, which includes increasing fecal boli production. The following measurements were collected: the number of times the mouse reared, the number of times it crossed any of the 8 squares in

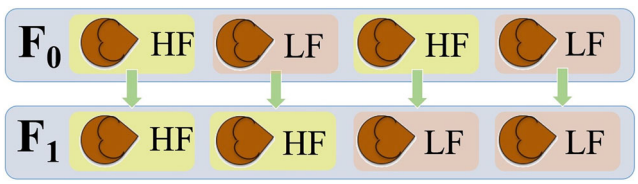

Fig. 1 Breeding design. HF = High-fat diet, LF = Low-Fat diet

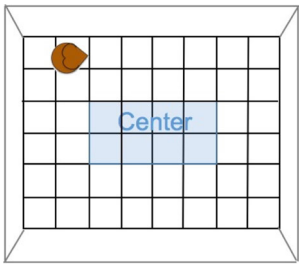

Fig. 2 Diagram of the Open Field Test arena

the center of the arena, the total number of squares it crossed, the number of times it urinated, and the number of fecal boli it produced. The number of squares crossed was determined by reviewing the video of the session, because the mice often moved too quickly to accurately count with the unaided eye. The center:total distance ratio was calculated by dividing the number of center squares crossed by the total number of squares crossed. A low center:total distance ratio and high levels of rearing, urination, and fecal boli production were interpreted as indicators of anxiety [39].

Ethogram data

After noticing that mice on an HF diet seemed to be less active than LF mice, we resolved to investigate the possible difference quantitatively. A behavioral ethogram was created by listing all mouse behaviors witnessed during 5 h of observation, for a total of 19 behaviors (Table 3). The offspring were observed 3–4 times per week, and the observation times were categorized as a morning session (between 8:00 and 10:30 am) or an afternoon session (between 2:30 and 5:00 pm). Each session consisted of 20 observations by instantaneous scan sampling, with each observation of an individual mouse at least 1 min apart. Specifically, a researcher marked on the ethogram checklist which behavior the mouse was performing at the instant it was observed, then moved on to the next mouse, and continued until all of the mice had been observed once. The observer then returned to the first mouse and checked off its behavior again, completing this cycle 20 times. The mice were observed for an average of 26 sessions between the ages of 3–14 weeks. Since some mice were observed more than others, we calculated an average of each behavior per age period for analysis (3–5, 6–8, 9–11, and 12–14 weeks). The cages were rearranged randomly on the rack each week so the order of observation varied.

Many of the behaviors were performed too infrequently to detect a difference due to diet. For example, behaviors observed less than 1% of the time were: motionless but alert, being groomed, rearing, drinking, gnawing, digging, carrying, nesting, allogrooming, running, fighting, and mounting. To incorporate these rarer behaviors into the analysis in a more meaningful way, in addition to analyzing the individual

Table 3 Mouse behaviors

Behavior Category	Factor	Age 3–5 weeks	Age 6–8 weeks	Age 9–11 weeks	Age 12–14 weeks
Self Maintenance	High fat avg	7.3%	6.2%	6.7%	10.0%
(drinking, eating, autogrooming)	Low fat avg	12.1%	10.8%	14.6%	13.4%
	Offspring Diet	**0.00864**	**0.0134**	**8.00E-04**	**8.60E-04**
	Nurse	**0.00178**	**0.00066**	0.36662	**0.00126**
Inactive	High fat avg	80.6%	81.7%	75.4%	79.8%
(resting, motionless but alert,	Low fat avg	75.8%	77.3%	67.5%	66.6%
sitting, sleeping)	Offspring Diet	0.10326	0.64236	**2.58E-02**	**0.00001**
	Nurse	**0.00002**	**0.02823**	**0.00897**	**1.17E-06**
Explore	High fat avg	11.5%	11.8%	16.9%	10.1%
(gnawing, digging, carrying, nest	Low fat avg	11.7%	11.7%	17.7%	19.8%
arrangement, climbing, running,	Offspring Diet	0.56773	0.51643	0.17565	**0.00012**
rearing, walking)	Nurse	**0.00002**	0.1679	**0.00005**	**2.96E-09**
Social Interaction	High fat avg	0.7%	0.3%	1.0%	NA
(allogrooming, being groomed,	Low fat avg	0.4%	0.3%	0.2%	NA
fighting, mounting)	Offspring Diet	0.12934	0.50193	**0.00333**	NA
	Nurse	0.17361	0.57082	0.10353	NA
	Sample size	*n* = 36 HF	n = 35 HF	n = 36 HF	n = 35 HF
		40 LF	40 LF	35 LF	38 LF

This table indicates how the 19 ethogram behaviors were grouped into 4 larger 645 behavioral categories. It shows the average percent of time that mice on a high-fat diet and mice on a low-fat diet mice were observed performing behaviors in each of these summary categories at each of the four age periods. Since sex did not have a significant effect on the behaviors, males and females were analyzed together. The p-values are from an ANOVA showing the effect of nurse ID and offspring diet on the summary ethogram categories. Nurse ID affected how often the mice performed self-maintenance, inactive, and exploration behaviors throughout their lives. Offspring diet affected self-maintenance behaviors throughout life, and inactive, exploration, and social interaction behaviors later in adulthood

Statistically significant effects of offspring diet and nurse are bolded

behaviors, we grouped them into four larger behavior categories: Self Maintenance (drinking, eating, and autogrooming), Inactive (sleeping, resting, motionless but alert, and sitting), Explore Cage (walking, climbing on the ceiling bars, gnawing, digging, carrying, nest arrangement, running, and rearing), and Social Interaction (allogrooming, being groomed, fighting, and mounting) (Table 3). We calculated the average percent of time a mouse spent performing behaviors in these four categories during each of the four age periods that each animal was observed in. The Social Interaction category could only be analyzed for the first three age periods, because the mice were housed individually after 12 weeks of age to measure nest building ability.

Nest quality

At 13 weeks of age, each mouse was housed alone in a fresh cage and given a 2″ × 2″ cotton nestlet between 10:00 to 11:00 am. Twenty-four hours later, the nest was photographed and rated for quality using the Deacon Scale, which ranges from 1 to 5 [23]. A Deacon score of 1 indicates a poor quality nest, where over 90% of the nestlet remains unused; a score of 2 means that 50–90% of the nestlet is still intact; 3 indicates the nestlet is mostly shredded but there is no identifiable nest site; 4 means that more than 90% of the nestlet is torn and the nest walls are higher than the mouse's body; and a score of 5 is a near perfect nest (Table 4) [23]. Since there was a privacy hut in the cage, we also noted if the nest was built inside of the hut or outside of it.

Statistical analysis

In each of the three assays, we measured the effect of offspring diet and maternal diet on behavior. In testing for anxiety, the response variables for the Open Field Test were the center:total distance ratio and the number of times each mouse reared, urinated, and produced fecal boli. In the test of activity via instantaneous scan sampling, there were 19 response variables for each of the four age periods: the proportion of time each mouse spent performing each of the 19 behaviors per session during that age period. In the test of nest quality, the response variable was the mouse's nest quality score based on the Deacon Scale.

In each of the three behavioral assays, the data were not normally distributed, as determined by the Shapiro-Wilk test of normality. Because the data had a non-normal distribution, we randomized the relevant phenotypes (the behaviors) over the factors to obtain a null distribution of ANOVA parameters under the hypothesis of no treatment effects. Using just the offspring of HF mothers, we randomized the trait values 9999 times,

Table 4 Deacon Scale to measure nest quality, from Deacon et al. [23]

Deacon Score	Description of Nest
1	Over 90% of the nestlet remains unused
2	Between 50 and 90% of the nestlet is still intact
3	Nestlet is mostly shredded but there is no identifiable nest site
4	More than 90% of the nestlet is torn and the nest walls are higher than the mouse's body
5	A near perfect nest

then tested the difference between the LF and HF offspring using a 2-sample t-test for each behavior and compared the t-test statistic from the observed values to those of the randomized values. We performed another randomization to determine if offspring sex had a significant effect on behavior. We repeated this procedure for the offspring of LF mothers. The t-test statistics for each of these tests were normally distributed, even though the raw data was not. The *p*-values from the randomization procedure were nearly identical to those resulting from the ANOVA. Since the ANOVA was so robust to the non-normally distributed data, we proceeded to analyze the data with a General Linear Model and report the p-values resulting from the GLM.

For each of the three assays (Open Field Test, ethogram, and nest quality) we used SYSTAT (Version 12) to test the full model, which included the effects of maternal diet, offspring diet, offspring sex, nurse ID, parity, and their two- and three-way interactions. For the ethogram data we also included observation time of day and age period in the model (this was not necessary for the Open Field Test or nest quality traits because those assays were conducted on each mouse only once, at the same time of day when the mice were all the same age). We then ran a reduced model for the ethogram data that included just nurse, offspring diet, and age period, since those were the only three variables with a statistically significant effect. We also performed a principal components analysis for each of the three behavioral assays.

Results

Weight

Offspring diet had a significant effect on weight within 1 week of being weaned onto it, with HF-fed mice weighing significantly more than LF-fed mice at 4 weeks of age ($p = 5.6 \times 10^{-10}$). The weight difference increased with age, and by the time the mice were 17 weeks old, the HF females weighed 28.2 g (± a standard error of 1.4 g), the LF females weighed 15.1 ± 1.6 g, the HF males weighed 33.4 ± 1.7 g, and the LF males weighed 19.3 ± 1.6 g, with a strongly statistically significant effect of diet ($p < 0.0001$). We considered the HF-fed mice to be obese when their mean was 3 standard deviations apart from the LF-fed mean. The mean of the HF males was 3.7 standard deviations apart from the mean of the LF males at 10 weeks of age, and 5.2 standard deviations apart at 17 weeks of age. The mean of the HF females was 3.2 standard deviations apart from the LF females mean at 10 weeks of age, and 4.8 standard deviations apart at 17 weeks of age. The HF mice had significantly higher serum levels of triglycerides, glucose, insulin, and leptin at 17 weeks of age than the LF mice [37]. Maternal HF diet did not affect the weight of the sons or LF daughters, but did significantly increase the weight of HF daughters at 9-weeks of age ($p = 0.041$) and beyond [37].

Open field test

Offspring diet had a significant effect on the Open Field Test traits. The principal components analysis revealed that PC1 accounted for 98.3% of the variance and was dominated by rearing in the Open Field Test (with a weight of greater than 0.99). PC2 accounted for 1% of the variance and was dominated by fecal boli production and urination (which were weighted 0.82 and 0.56 respectively), with rearing and center:total distance weighted negatively. Despite the strong variation in rearing, this trait was not significantly affected by diet. This is likely due to diet having an opposite affect depending on sex. HF females reared more than LF females (13.06 times versus 9.47 times), but HF males reared less than LF males (9.11 times versus 11.63).

Urination frequency and fecal boli production, the drivers of variation in PC2, were affected by diet in the same direction in males and females. The general linear model revealed that offspring diet was significant on a multivariate level ($p = 0.028$), as well as for the individual traits of urination frequency ($p = 0.007$) and fecal boli production ($p = 0.042$). The average urination frequency was 3.8 times higher in HF females than LF females, and 1.9 times higher in HF males compared to LF males (Fig. 3a). The average fecal boli production was more than 4 times higher in mice on an HF diet than those on an LF diet for both sexes (Fig. 3b).

There was little effect of prenatal maternal diet on anxiety. The general linear model of the Open Field Test traits indicated that maternal diet had borderline significance on the multivariate level ($p = 0.054$) and was not significant for any of the individual traits. However, a maternal HF diet did appear

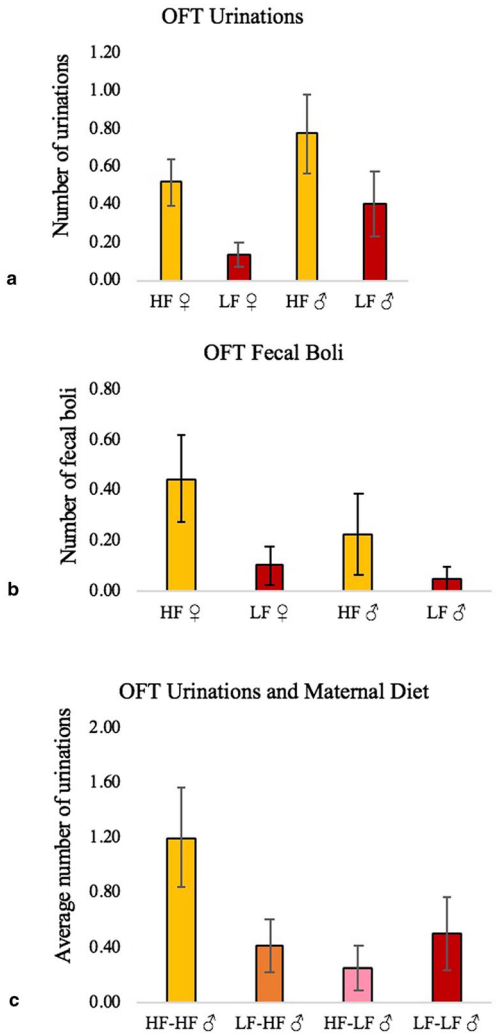

Fig. 3 **a** In the Open Field Test, high-fat male mice born to high-fat mothers (HF-HF ♂) had a borderline significant elevation in anxiety through increased urinations compared to high-fat males born to low-fat mothers (LF-HF ♂) (p = 0.058). **b** High-fat mice also produced more fecal boli than low-fat mice (p = 0.042). **c** Independent of maternal diet, high-fat mice urinated more than low-fat mice (p = 0.007). Sample size: HF diet ♀ (*n* = 20), LF diet ♀ (n = 20), HF diet ♂ (n = 20), LF diet ♂ (n = 20), HF-HF ♂ (*n* = 10), LF-HF ♂ (n = 10), LF-LF ♂ (n = 10), HF-LF ♂ (n = 10). Error bars represent ± a single standard error, HF = high-fat diet, LF = low-fat diet, OFT = Open Field Test

to increase urination frequency in HF sons (1.2 average urinations) compared to LF sons (0.42 average urinations), with a t-test showing borderline significance ($p = 0.058$) (Fig. 3c).

Ethogram data

Testing the full model revealed that offspring diet ($p = 4.60 \times 10^{-8}$), age period ($p = 2.08 \times 10^{-10}$), and nurse ID ($p = 0.01$) all had a statistically significant effect on offspring activity as assessed by the ethogram traits. Since sex did not have a significant effect, the males and females were analyzed together. Maternal diet also did not have significant effect. The fact that nurse ID was significant means that even though all pups were cross-fostered to genetically identical LF-fed SM/J nurses, the nurses differed in some other way that had a lasting impact on their fostered offspring, no matter what diet the offspring or the biological mother had. We included nurse ID in the model to control for it.

The most commonly observed behaviors in the mice were sleeping (62.5% of the time), autogrooming (7.4%), climbing (7.4%), walking (5.9%), and eating (3.7%). The results of the principal components analysis indicated that PC1 (which explained 80.9% of the variance) was dominated by sleeping (0.90), with resting (0.002) and being groomed (0.00075) weighted in the same direction and all other behaviors in the opposite direction. PC2 (which explained 7.7% of the variance) was dominated equally by resting (0.43) and autogrooming (0.43), with motionless but alert making a minor contribution (0.10). In PC2, eating (0.72), climbing on the ceiling bars (0.23), and sleeping (0.18) were weighted in the opposite direction.

We also grouped the behaviors into four larger categories for analysis: self-maintenance, inactive, explore cage, and social interaction. HF offspring spent less time performing self-maintenance behaviors than LF mice at all four age periods (Fig. 4a). Although they differed in self-maintenance early on, significant differences in other behaviors did not manifest until later in life. The mice spent an equal amount of time exploring the cage until 12–14 weeks of age, when LF mice increased their time exploring and HF mice decreased it ($p = 0.0001$). This change meant that HF mice explored only half as often as LF mice in adulthood (Fig. 4b). While spending less time performing self-maintenance and exploration behaviors than LF mice, the HF mice spent more time being inactive as adults. Mice on an LF diet became more active with age (they were inactive 75.8% of the time at 3–6 weeks old, and 66.6% of the time at 12–14 weeks old), whereas mice on an HF diet never increased their activity levels (they were inactive 80% of the time at both 3–6 weeks and 12–14 weeks of age) (Fig. 4c). The difference in activity levels between the two diet groups became detectable at 9–11 weeks of age ($p = 0.026$), but weight differences were detectable at 4 weeks, indicating that the reduced activity levels followed the weight gain from an HF diet. Neither group of mice performed social interaction behaviors frequently, but at 9–11 weeks of age the HF mice performed them significantly more often than LF mice (1% of the time versus 0.2% of the time, $p = 0.003$) (Fig. 4d). This was not due to differences in time spent fighting or mounting, but rather due to the HF mice spending more time grooming each other ($p = 0.005$) and being groomed ($p = 0.025$).

The differences in the four behavior summary categories appear to be primarily driven by a significant difference in the following individual behaviors at 12–14 weeks of age: sleeping (Fig. 5a), climbing on the ceiling (Fig. 5b), and walking (Fig. 5c), with HF-fed mice sleeping more and climbing and walking less than LF-fed mice.

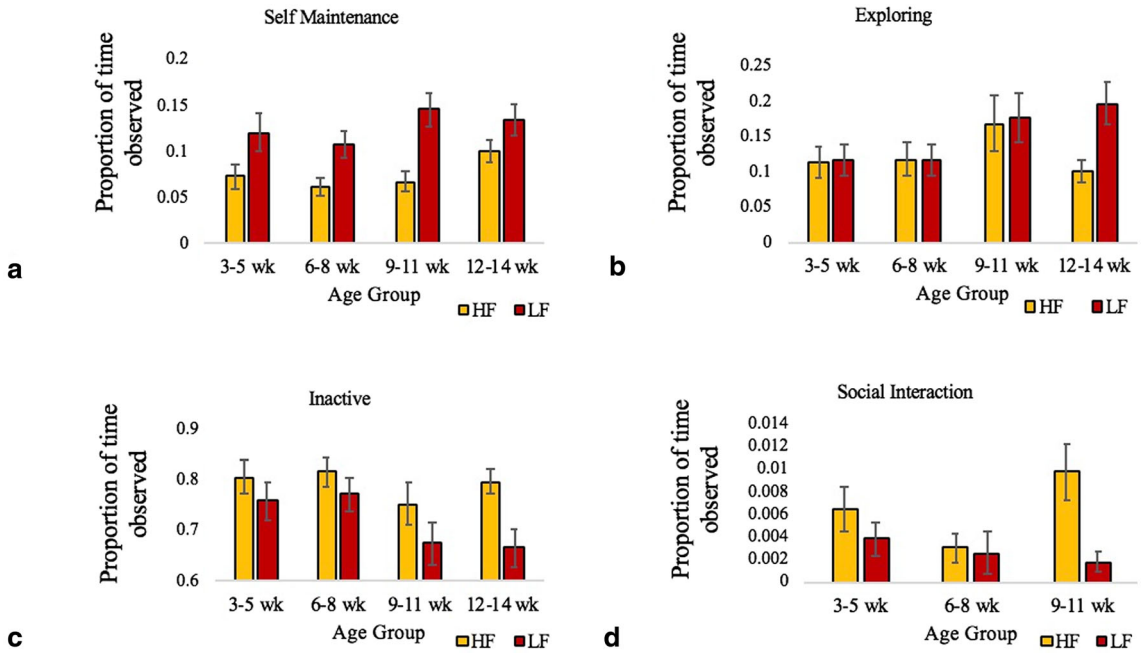

Fig. 4 **a** High-fat mice performed self-maintenance behaviors significantly less often than low-fat mice at every age group. **b** By 12–14 weeks of age, high-fat mice spent less time exploring than low-fat mice ($p = 0.0001$). **c** Low-fat mice became less inactive in adulthood, whereas high-fat mice never decreased their level of inactivity ($p = 0.00001$). **d** At 9–11 weeks of age, high-fat mice engaged in more social interaction behaviors than low-fat mice ($p = 0.0033$). Error bars represent ± a single standard error, HF = High-Fat diet, LF = Low-Fat diet

Nest quality

Mice on an HF diet built poorer quality nests than mice on an LF diet ($p = 0.040$) (Fig. 6a). The difference was driven by the males, where HF-fed males scored an average of 2.8 out of 5 on the Deacon scale, compared to 3.8 for LF-fed males. Although offspring diet did not significantly affect where the nests were built ($p = 0.075$), it is interesting to note that a t-test of just the sons shows that HF sons built their nests inside of the hut less often ($p = 0.021$) (Fig. 6b). Maternal diet did not affect the offspring's Deacon score, however it did affect where they built their nests. Regardless of their own diets, offspring of LF diet mothers were 2.5 to 3.5 times more likely to build their nests inside of the hut than offspring of HF diet mothers ($p = 0.020$) (Fig. 6c). In other words, having an HF-fed mother reduced the offspring's probability of building a nest inside of the hut (11% of offspring of HF mothers built their nests inside the hut, compared to 38% of offspring of LF mothers).

Discussion

We found that a high-fat (HF) diet in offspring increased urine and fecal boil production in the Open Field Test, reduced levels of activity and exploration, and reduced nest quality, indicating that diet impacts a wide range of behaviors in mice. This generally supports the findings of previous studies [3, 4, 6, 40]. Unlike previous studies, we did not detect an effect of a maternal HF diet in the Open Field Test. There is a dearth of knowledge in the scientific literature about the effect that a maternal HF diet has on activity levels and nest building in rodent offspring, and we report finding no effect. If there is an effect of

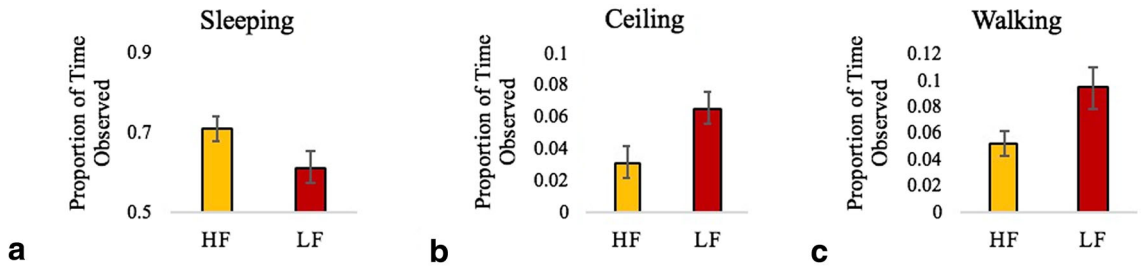

Fig. 5 These graphs contrast the behavior of the high-fat and low-fat diet mice at 12–14 weeks of age. **a** High-fat mice spent more time sleeping than low-fat mice ($p = 0.0017$). **b** High-fat mice spent less time climbing on the ceiling bars than low-fat mice ($p = 0.014$). **c** High-fat mice also spent less time walking around ($p = 0.0031$). Error bars represent ± a single standard error, HF = high-fat diet, LF = low-fat diet, sample size is high-fat ($n = 35$), low-fat ($n = 38$)

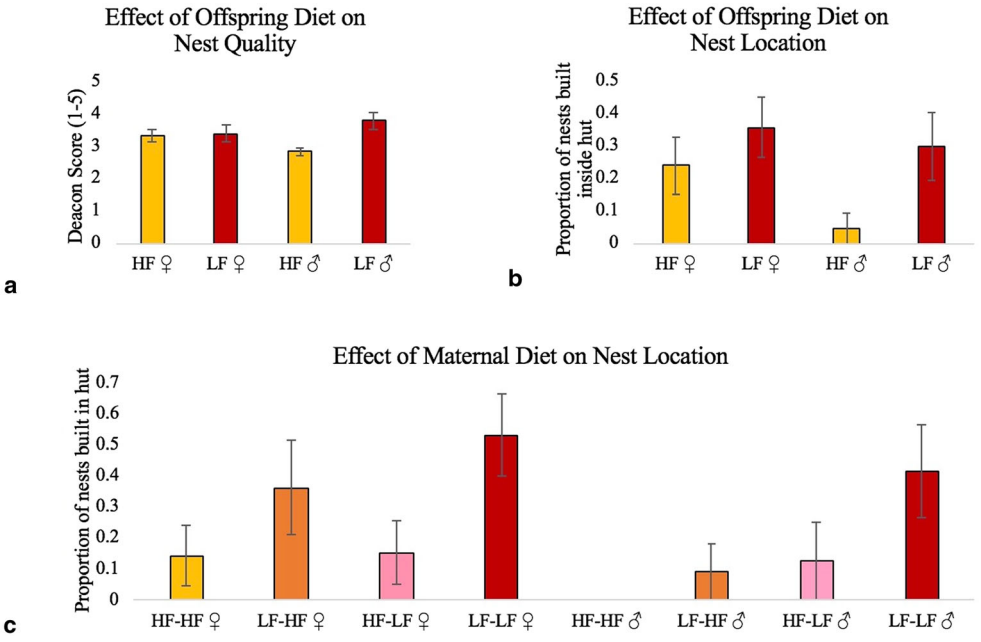

Fig. 6 **a** Offspring diet had a significant effect on nest quality, with this difference being driven by the sons. High-fat diet sons built lower-quality nests than low-fat diet sons. **b** High-fat offspring diet showed a nonsignificant trend ($p = 0.075$) of building nests in the hut less often, although a paired t-test of just the sons showed a statistically significant difference ($p = 0.021$). (C) Offspring of high-fat mothers built their nests inside of a hut less often. Error bars represent the standard error, HF = high-fat diet, LF = low-fat diet, sample size: $n = 10$ HF-HF♀, 10 LF-HF♀, 10 HF-LF♀, 10 LF-LF♀, 10 HF-HF♂, 10 LF-HF♂, 8 HF-LF♂, 10 LF-LF♂)

maternal HF diet on activity levels and nest building, it is possible that the effect size is very small and would require a larger sample size or different methods to detect, such as measuring nest size, nest temperature, or locomotive activity. Alternatively, it is possible that maternal HF diet does not affect these traits.

In the Open Field Test, the higher levels of urination and fecal boli production in both sexes of HF-fed mice support the hypothesis that obesity increases anxiety. However, there was no difference in rearing or the center:total squares ratio between the diet treatment groups. Open Field Test results can be difficult to compare across studies, since rodents may show a significant difference in only one or two of the anxiety measures, and there is not a standardized way of interpreting the collective findings. In the present study, the mice showed increased anxiety for two of the five measures. Although we found no effect of an HF diet on the number of squares crossed in the center of the arena, Bruce-Keller et al. [40] found that HF-fed male mice ($n = 10$) spent less time in the inner zone of the Open Field Test than chow-fed mice (n = 10), with the total distance traveled unchanged. Similarly, Sharma and Fulton [3] found that HF-fed mice ($n = 8$) entered the inner zone less often and spent less time in it than LF-fed mice (n = 8). Both of those studies were conducted with C57BL/6 J mice, whereas we used SM/J mice, so it is possible that the manifestation of anxiety is dependent on genetic background. Nevertheless, although different aspects of the Open Field Test came out as significant in these studies, all of them detected increased anxiety due to an HF diet. Since maternal diet had a p value just below the $p = 0.05$ significance threshold in the sons, we used G*Power [41] to perform an a priori power analysis with our data to provide researchers in the future with an idea of the sample size needed to detect maternal effects in the Open Field Test in a study like ours, which we may have just missed. We found that a sample size of 17 for each group would give 80% power to detect significance at the 0.05 level.

In addition to being more anxious, HF mice performed fewer self-maintenance behaviors at all ages, and by 11 weeks of age they explored the cage half as often and were far less active than low-fat (LF) mice. In fact, while LF mice became more active as they aged, HF mice became less active. HF mice slept significantly more and spent less time walking and climbing as adults. The lower activity levels in HF mice developed several weeks after they began to weigh more than the LF mice. This suggests that weight gain can lead to inactivity, and not just the other way around. The reduced activity levels in the HF-fed mice may compound the effect of the diet to lead to further weight gain. Compared to the LF mice, the HF mice had significantly higher serum leptin levels [37]. A diminished response to leptin may be linked to the reduced activity levels, since it known that treating leptin-deficient *ob/ob* mice with leptin increases their ambulatory activity, wheel running, and total energy expenditure [42].

The mice on an HF diet also built poorer quality nests. This could potentially be influenced by several factors, such as thermoregulatory changes due to obesity, the observed reduction in activity levels, hormonal changes, or alterations in brain regions known to both affect nesting behavior and be impaired by obesity, such as the hippocampus. Favoring a possible connection to a thermoregulatory mechanism, the HF mice had significantly more brown fat than the LF mice [37]. If the HF mice give off more heat, a lower quality nest may in fact be more optimal for them—although not necessarily for their pups.

An HF diet affected a wide range of behaviors in SM/J mice, whereas we did not detect such an effect of maternal prenatal HF diet on the offspring behaviors that we measured.

However, we only used one test of anxiety and did not perform any cognitive tests, so it is possible there were maternal effects we did not detect. Our results do support the findings of Hiramatsu et al. [43], though, who recently found that a maternal Western diet did not have a major effect on the behavior of adult offspring, including no effect on anxiety as measured by an Elevated Plus Maze.

Although we did not find an effect of maternal diet, there was in fact a significant effect of nurse ID on offspring behavior, despite the nurses all being genetically identical and LF-fed. This environmental maternal effect persisted through adulthood, indicating that the rearing and lactation environment has a lasting effect on murine anxiety and activity levels, more so than the prenatal maternal diet. We did not measure maternal behavior in this experiment, but the significant effect of nurse ID indicates that this would be interesting to pursue in the future.

Findings on the effect that maternal obesity has on offspring anxiety are varied in rodents, ranging from decreasing to increasing anxiety [11–20]. The present study found no effect of maternal diet on offspring anxiety in the Open Field Test, other than a borderline increase in urinations in HF sons. If there is an effect of maternal diet, the effect size must be small, as our sample size gave us 80% power to detect differences of 0.4 residual standard deviation units. By not detecting an effect of maternal obesity on anxiety, our findings suggest that the effect of maternal diet found in other rodent studies may principally be due to postnatal maternal diet, since we only varied prenatal diet. This is supported by an experiment by Kang et al. [12], who found that the increased anxiety in mice with HF mothers was reduced in those whose mothers were transferred to a control diet during lactation. Postnatal maternal diet in rodents may thus have a stronger effect on offspring behavior than prenatal diet.

Maternal diet did not affect the offspring's behavior patterns as measured by instantaneous scan sampling, consistent with the outcome of the Open Field Test. It did, however, have an unexpected effect on offspring nesting behavior. Mice with mothers on an HF diet were less likely to build their nests inside of huts (not a single HF male with a HF mother built a nest inside of the hut). The connection between maternal HF diet and building nests outside of huts is unclear. Perhaps thermoregulation plays a role, although maternal diet only affected the weight of HF daughters and not of LF daughters or sons. Alternatively, anxiety could play a role if having a nest separate from the hut provides a second hiding place. This has yet to be established, however.

Limitations to our study include the sample size of 10 per treatment (a sample size of 17 would have been better) and our use of only the Open Field Test to measure anxiety (an additional test such as the Elevated Plus Maze could have been more informative). Our results suggest that it would be interesting in future studies to include measurements of maternal behavior.

Although this study did not investigate the effects of an HF diet on parenting behavior, the lasting effect of nurse ID as well as the observed changes in behavior due to dietary fat give reason to predict that parental HF diet could have major effects on the pups. For instance, pup survival could be reduced if the parents' poorer nest building failed to keep the pups warm and hidden from predators. Higher levels of inactivity in the mothers could lead to a reduction in arch-backed nursing, which could negatively affect offspring fitness. Higher levels of maternal anxiety could increase offspring stress response into adulthood, as seen in rats [44]. It will be important in the future to study the effect of a postnatal maternal HF diet and determine its underlying mechanisms.

Conclusions

Our results support the conclusion that an individual mouse's own high-fat diet affects anxiety, nest building, and activity patterns, while prenatal maternal high-fat diet does not. Other rodent studies have found an effect of maternal diet on offspring behavior when the high-fat diet continued through lactation; thus, it is possible that a postnatal maternal high-fat diet has a larger effect size on behavior than prenatal maternal high-fat diet.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Abbreviations *HF*, high-fat; *LF*, low-fat

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