Maternal high-fat diet during pregnancy and lactation reduces the appetitive behavioral component in female offspring tested in a brief-access taste procedure

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Maternal high-fat diet during pregnancy and lactation reduces the appetitive behavioral component in female offspring tested in a brief-access taste procedure. Am J Physiol Regul Integr Comp Physiol 306: R499–R509, 2014. First published February 5, 2014; doi:10.1152/ajpregu.00419.2013.—Maternal high-fat diet appears to disrupt several energy balance mechanisms in offspring. Here, female offspring from dams fed a high-fat diet (HF) did not significantly differ in body weight compared with those fed chow (CHOW), when weaned onto chow diet. Yet when presented with both a chow and a high-fat diet, high-fat intake was significantly higher in HF compared with CHOW offspring. To assess taste-based responsiveness, offspring (12 wk old) were tested in 30-min sessions (10-s trials) to a sucrose concentration series in a brief-access taste test. Compared with CHOW, the HF offspring initiated significantly fewer trials but did not significantly differ in the amount of concentration-dependent licking. Thus, rather than affect lick response (summatory), maternal diet affects spout approach (appetitive), which may be attributed to motivation-related mechanisms. Consistent with this possibility, naltrexone, an opioid receptor antagonist, further reduced trial initiation, but not licking in both groups. With naltrexone administration, the group difference in trial initiation was no longer evident after naltrexone administration. The animal’s approach to the spout to segregate the appetitive and consummatory components of the taste stimulus but may depend on alterations in satiety signals or absorptive mechanisms.

sweet; fat; maternal diet; appetitive; motivation

Evidence in the literature supports the hypothesis that consumption of a high-fat diet during pregnancy and lactation increases risk of obesity and disrupts other energy balance mechanisms in the offspring (e.g., 13, 22, 35, 46). Offspring from dams maintained on a high-fat or junk food diet displayed increased preference for 4% sucrose vs. water in a two-bottle intake test (51) and increased preference for fatty (34), sugary, and salty foods (2), compared with control offspring from dams maintained on standard chow during pregnancy and lactation, suggesting changes in taste preferences. Data from human studies have also identified taste preference alterations in offspring as a function of prenatal and postnatal environmental factors. For example, associations between emesis during pregnancy and salt preference in the offspring (5) and between garlic consumption by nursing mothers and breastfeeding behavior of infants (29) have been reported. Prenatal and early postnatal exposure to certain flavors (carrot juice) was shown to enhance the acceptance of those flavors in solid foods in infants (30).

It is possible that the changes in the taste preferences observed are at least partly driven by alterations in neural mechanisms underlying reward. Offspring from rodents fed a high-fat or junk food diet compared with offspring from dams maintained on standard chow during pregnancy and lactation show alterations in gene expression of dopamine and opioid signaling proteins (34, 51), decreased dopamine D2 receptor mRNA expression in the ventral tegmental area (33), and decreased performance in the acquisition of fixed-ratio procedures to work for sucrose (38), thus, collectively providing evidence for changes in reward-related mechanisms as a function of maternal diet.

Previously, our group has shown that offspring from dams fed a high-fat diet (HF) during pregnancy and lactation were significantly heavier than those from rats maintained on a standard chow diet (CHOW) across the lactation period. When these offspring were weaned onto standard chow at postnatal day 21, the males from high-fat dams continued to be significantly heavier, but in females, body weight difference as an effect of maternal diet was no longer evident after 8 wk of age (44). We have also demonstrated that when presented with a high-fat diet at the time of weaning, both male and female offspring of high-fat dams overconsume and become obese (46).

In the present study, we assessed food preference of female rat offspring from these two maternal groups when presented with both a standard chow and a high-fat diet at ~11 wk of age when there was no significant group body weight difference. Intake of the high-fat diet in the HF rats was significantly higher than that of the CHOW control group. Thus, although there does not appear to be a robust maternal diet effect when rats are maintained on a standard chow diet, group differences emerge when animals are presented a more palatable, calorie-dense, high-fat diet. This difference may be attributed to a number of factors, one of which is a difference in orosensory stimulation or other related ingestive behavioral components. To assess this, first, responses across a range of sucrose concentrations were measured in HF and CHOW animals in the brief-access taste test. The procedure allows for some segregation of the appetitive and consummatory components of ingestive behavior. The animal’s approach to the spout to
initiate a trial can be considered the appetitive component, as it involves bringing the animal to the stimulus, whereas the licking responses within a trial can be considered consummatory behavior, as it follows contact with the stimulus. Next, the effect of naltrexone, an opioid antagonist, which has been shown to reduce sucrose intake, on unconditioned concentration-dependent licking and approach to the spout was assessed.

In another cohort of animals, the relative expression of genes associated with the opioid system was compared across groups. Finally, short-term, one-bottle intake tests under conditions similar to those used in the brief-access taste test were also conducted to assess the effect of maternal diet on a behavioral measure that involves both appetitive and consummatory components.

MATERIALS AND METHODS

Subjects

Timed pregnant female Sprague-Dawley rats (Charles River, Kingston, NY) arrived on gestation day 2 and were individually housed in standard polycarbonate cages in a room where humidity, temperature, and a 12:12-h light-dark cycle were automatically controlled. Upon arrival, the pregnant rats were assigned to either a standard chow (CHOW; Lab Diet 5001, 14% kcal from fat) or high-fat diet (HF; Research Diets D12492, 60% kcal from fat). The day that a litter was found before the end of the light cycle was noted as postnatal day 0 (PN0). On the morning of PN1, pups were counted, sex was identified, and pups were weighed. Litter sizes were normalized to 10 (5 males and 5 females) per litter. On PN21, pups were weaned. One female offspring from each litter served as a behavioral subject in this study.

All offspring assigned to the behavioral study were given ad libitum access to standard chow (Lab Diet 5001; 14% kcal from fat) and water from PN21 onward, except where noted. These animals were group-housed (3 or 4 per polycarbonate cage) until 6–8 wk of age, after which they were individually housed in hanging-wire cages. All animals were habituated to the cages several weeks prior to testing, and all animals were treated similarly. When rats were transferred to the wire mesh cages after being housed in conventional plastic tub cages, we did not observe any negative effects (e.g., weight loss, failure to gain weight, or decreased food intake) that would indicate that the animals perceived the wire mesh housing as a significant stressor.

Behavioral testing began when animals were at least 11 wk old. Separate cohorts of animals were generated from different dams for each of the three behavioral experiments. During the behavioral studies, animals were given ad libitum access to water and chow, placed on a water restriction schedule, and partial food and water restriction schedule. For water restriction, water access was removed from the home cages no more than 23 h before testing, and water was available only during the daily test sessions. During the partial food and water restriction condition, rats were presented with ~10 g of chow and ~20 ml of water in their home cages for ~23 h before testing, as adapted from studies in mice (19) and since used to test rats (e.g., 28, 48). These amounts represent ~50% and ~80% of ad libitum chow and water intake, respectively. This testing condition was chosen to encourage responding without inducing a 24-h total deprivation that would result in maximal licking across the sucrose concentration range. Body weight was measured every day during water or partial food and water restriction conditions and did not fall below 85% of the ad libitum feeding and drinking weight. At least one repletion day (ad libitum access to chow and water) followed each testing day under food and water restriction. All procedures were approved by the Institutional Animal Care and Use Committee at The Johns Hopkins University School of Medicine.

**Experiment 1: Chow vs. High-Fat Preference Test**

**Behavioral procedure.** Daily intake measures were conducted in the home cages of offspring from dams assigned to either a standard chow CHOW (n = 4) or high-fat diet (HF) (n = 5). All animals were presented ad libitum access to water, standard chow, and a high-fat diet in the home cages for seven consecutive days.

**Data analysis.** Two-sample t-tests were used to compare the intake values by the two groups of standard chow diet, high-fat diet, total diet intake, and preference ratio. Preference ratio was calculated as follows:

\[
\text{Preference Ratio} = \frac{\text{Intake of high-fat diet}}{\text{Intake of high-fat diet} + \text{Intake of standard chow}}
\]

**Experiment 2: Brief-Access Taste Test**

**Behavioral procedure.** Behavioral training and testing of 15 animals (CHOW: n = 7; HF: n = 8) was conducted in a lickometer (Davis MS-160; DiLog Instruments, Tallahassee FL), as described elsewhere (e.g., 39). The rat was placed in the testing chamber and presented with a single spout positioned ~5 mm behind a slot. The spout was connected to a glass container holding a taste stimulus. A small fan was placed above the testing chamber wall to direct an air current past the drinking spout, so as to minimize potential olfactory cues from the stimulus. A trial was initiated when the rat licked the spout. A shutter closed at the end of each trial (10 s). During each 8-s intertrial interval, a motorized block moved to change the tube presentation, and the shutter reopened for the next trial. Concentrations were presented in randomized blocks without replacement. Animals were able to initiate as many trials as possible during the 30-min sessions.

**Training and testing.** On days 1 and 2 of behavioral testing, water-restricted animals were placed in the testing chamber and presented with water via a stationary spout for 15 min by administration of 1 mg/kg ip naltrexone (Sigma Aldrich, St. Louis MO) were prepared daily with distilled water and presented at room temperature. Ad libitum access to water resumed after the last training session.

The following week, animals were presented with the same range of sucrose concentrations in a partially food- and water-restricted state across three testing sessions. After at least 2 days of ad libitum access to food and water, animals were injected with saline (0.9% NaCl, 1 ml/kg ip) in their home cages. Next, animals were tested during three additional sessions with the sucrose solutions with each of these test sessions preceded ~15 min by administration of 1 mg/kg ip naltrexone (Sigma Aldrich). This dose of naltrexone previously induced a significant reduction in food intake (27). Finally, after at least 2 days of ad libitum access to food and water repletion, three additional sucrose test sessions were conducted. Animals were injected with saline ~15 min before these test sessions (Table 1).

**Data Analysis**

Two-sample t-tests were used to compare total licks and interlick interval (ILI) values to stationary water. Only ILIs that fell between 50 and 250 ms were included for analysis because ILI values less than 50 ms were considered double licks, and values greater than 250 ms were considered pauses between licking bursts (see Refs. 1, 6, and 7). The mean number of licks at each concentration was calculated by collapsing all trials across the sucrose training session when animals were water-restricted and across the three sucrose test sessions for each testing phase. The mean number of licks to water was subtracted from the mean number of licks at each concentration, yielding a “licks
relative to water. This measure has been used in previous studies (23, 43, 48) to produce concentration-response curves that are adjusted to a water baseline. If an individual animal did not initiate at least two trials per concentration collapsed across the three sucrose testing sessions of each phase, data from that animal were not included in the licks relative to water value analysis. Data from all animals were included for analysis of number of trials.

The licks relative to water value for each concentration was compared using ANOVAs. Two-sample t-tests were used to compare the number of trials initiated by the two groups of animals. The statistical rejection criterion of 0.05 was used for all analyses.

Curves were fit to mean data for each group by using the following logistic function:

\[ f(x) = \frac{a}{1 + 10^{(x-c)/b}} \]

where \( x = \log_{10} \) stimulus concentration, \( a \) is the asymptotic lick response adjusted for water, \( b \) is the slope, and \( c \) is the \( \log_{10} \) concentration at the inflection point.

**Experiment 3: Intake Test**

*Behavioral procedure.* Intake measures to sucrose and corn oil were taken in the home cage of 13 animals (CHOW: \( n = 5 \); HF: \( n = 8 \)). Compounds were presented in 50-ml conical bottom centrifuge tubes (Nalgene) attached via rubber stoppers (Allentown Caging Equipment) to drinking tubes with orifice size ~2.7 mm. These were presented to animals by inserting the straight sipper tube between the metal bars of the hanging wire cage.

The rats were habituated to drinking from these straight sipper tubes, with ad libitum access to water for at least 2 days. Access to water was removed from the home cages no more than 23 h before testing, and water was available only during the daily 30-min sessions. Water intake was measured during two consecutive daily 30-min sessions during the light cycle. Water was returned ~1 h after the second water intake session.

To include a test condition similar to that used during experiment 2, during testing with sucrose and corn oil compounds, rats were presented ~10 g of chow and ~20 ml of water in the home cages for ~23 h before testing. At least one repletion day (ad libitum access to chow and water) followed each testing day under food and water restriction. Single concentrations of sucrose and corn oil were presented in ascending order in 30-min sessions. Six concentrations of sucrose (0.01, 0.03, 0.06, 0.1, 0.3, and 1.0 M; Sigma Aldrich, St. Louis MO) and six concentrations of corn oil (0.5, 1, 2, 4, 8, and 16% [vol/vol] corn oil; ACH Food Companies) were prepared daily with distilled water and presented at room temperature. The corn oil emulsions were prepared by blending corn oil with 5 ml of the emulsifier Tween 80 (Sigma Aldrich, St. Louis, MO) in 1,000-ml mixtures for at least 2 min before presentation. Although the use of the emulsifier caused the corn oil to go into solution, over time, there is some partial separation of the oil and water (40). Nevertheless, all of the animals were treated identically.

As a comparison, animals were next presented with the same six concentrations of sucrose in ascending order across six consecutive days in daily 30-min sessions and tested again under conditions with ad libitum access to chow and water. Following the sucrose series, animals were presented the same corn oil concentration series across six consecutive days.

**Data Analysis**

Intake under the partial food and water restriction and ad libitum schedules by the two groups was compared using ANOVA. Post hoc two-sample t-tests were used to compare the intake values at each concentration.

In addition, nonlinear regression was used to fit the following quadratic equation to the data:

\[ y = ax^2 + bx + c \]

where \( x \) is the stimulus concentration and \( a \), \( b \) and \( c \) are parameters. The coordinates for point of inflection were calculated using:

\[ x_{inf} = \frac{-b}{2a}, y_{inf} = ax_{inf}^2 + bx_{inf} + c \]

where \( x_{inf} \) is the concentration corresponding to maximum intake and \( y_{inf} \) is the maximal intake. The \( (x_{inf}, y_{inf}) \) values derived for each animal were then compared across groups using t-tests. The statistical rejection criterion of 0.05 was used for all analyses.

*mrNA expression.* After behavioral testing, all animals from the cohort tested in experiment 3 were given ad libitum access to chow and water for 6 days. Animals underwent partial food and water restriction (~10 g chow, ~20 ml water) for ~23 h and during the light cycle, were decapitated, brains were removed and frozen on dry ice. Anatomiocally appropriate punches of nucleus accumbens, ventral tegmental area, hypothalamus, and hippocampus were collected. Tissue was homogenized in QIAzol lysis reagent (Qiagen). For each sample, 500 ng of total RNA was used in reverse transcription (QuantiTec reverse transcription kit; Qiagen). The genes for pro dynorphin (Pdyn), proenkephalin (Penk), opioid receptor \( 1 \) (Oprl), opioid receptor \( 1 \) (Oprk1), and opioid receptor \( 1 \) (Oprm1) were selected for their associations with the opioid system. Expression of genes of interest was determined by RT-PCR using TaqMan gene-specific probes (ABI), TaqMan Universal PCR Master Mix on the ABI 7900HT fast real-time PCR system set for 40 PCR cycles. To determine relative expression values, the \( -\Delta \Delta C_t \) method (Applied Biosystems) was used, where triplicate \( C_t \) values for each sample were averaged and subtracted from those derived from the housekeeping gene \( Actb \).

**RESULTS**

*Experiment 1: Chow vs. High-Fat Preference Test*

The CHOW and HF offspring did not significantly differ in body weight measures on any of the testing days \( [t(7) \leq -1.346, P = 0.220] \). Both groups preferred the high-fat diet over the chow, as evident from the high preference ratio scores (Fig. 1A). A two-way ANOVA comparing preference ratio...
values revealed no main effect of group \( F(1,7) = 1.414, P = 0.273 \), a main effect of day \( F(6,42) = 8.613, P < 0.001 \), and no significant interaction \( F(6,42) = 1.327, P = 0.267 \). The HF group showed a significantly higher total intake compared with the CHOW controls: group \( F(1,7) = 9.630, P = 0.017 \), day \( F(6,42) = 1.986, P = 0.089 \), group \( \times \) day \( F(6,42) = 0.604, P = 0.725 \) (Fig. 1B). The higher total intake in the HF group compared with that in the CHOW group, appeared to be attributed to a significantly higher intake of the high-fat diet: group \( F(1,7) = 15.578, P = 0.006 \), day \( F(6,42) = 1.996, P = 0.088 \), group \( \times \) day \( F(6,42) = 0.807, P = 0.570 \) (Fig. 1C), rather than intake of the chow diet: group \( F(1,7) = 0.161, P = 0.700 \), day \( F(6,42) = 8.805, P < 0.001 \), group \( \times \) day \( F(6,42) = 0.766, P = 0.601 \) (Fig. 1D).

**Experiment 2: Brief-Access Taste Test**

**Body weight.** The two groups did not significantly differ in body weight measures on any of the training or testing days \( t(13) = 1.739, P \geq 0.106 \).

**Water.** The two groups did not significantly differ in the total number of licks to water during the 30-min access to a stationary water spout \( t(13) = 1.300, P = 0.216 \). Nor did the groups significantly differ in ILI values \( t(13) = -0.639, P = 0.534 \), which is an indication of local lick rate thought to be governed by a central pattern generator (see Ref. 47). No significant difference was revealed between the groups in the number of trials initiated to water \( t(13) = -0.620, P = 0.546 \) (Fig. 2, right). These measures did not significantly differ between the groups on any of the training days but days 1 and 3 were considered to be acclimation days to the apparatus and the 10-s trials respectively; thus, data from days 2 and 4 are presented.

Sucrose training. Unconditioned licking responses across the sucrose concentrations did not significantly differ across the two groups. Two-way ANOVAs comparing lick values to sucrose of the two groups when water-restricted did not reveal a significant main effect of group \( F(1,14) = 0.029, P = 0.867 \), did reveal a main effect of concentration \( F(6,84) = 11.258, P < 0.001 \) and did not reveal a significant interaction \( F(6,84) = 1.840, P = 0.101 \). Furthermore, the number of trials initiated when water-restricted did not significantly differ between the two groups \( t(13) = 0.242, P = 0.813 \) (Fig. 2).

Sucrose testing. Both groups responded to sucrose in a concentration-dependent manner in the no-drug condition (phase 1), when tested 15 min after naltrexone administration (phase 2) and 15 min after saline injection (phase 3). A three-way ANOVA comparing the licks relative to water values did not reveal a significant main effect of phase \( F(2,37) = 2.261, P = 0.119 \), main effect of group \( F(1,37) = 0.942, P = 0.335 \), significant phase \( \times \) group interaction \( F(2,37) = 0.558, P = 0.577 \), concentration \( \times \) phase interaction \( F(10,185) = 0.441, P = 0.925 \), concentration \( \times \) group interaction \( F(5,185) = 0.405, P = 0.845 \) nor a significant concentration \( \times \) phase \( \times \) group interaction \( F(10,185) = 0.441, P = 0.925 \) but did reveal a significant main effect of concentration \( F(5,185) = 168.326, P < 0.001 \). Two-way ANOVAs comparing the licks relative to water values did not reveal significant main effects of group or significant interaction effects in any of the sucrose-testing phases (Table 2). Furthermore, two-way ANOVAs comparing licks relative to water values of the CHOW animals across the three phases did not reveal a main effect of phase \( F(2,21) = 2.826, P = 0.082 \), did reveal a main effect of concentration \( F(5,105) = 98.341, P < 0.001 \), and did not reveal a significant interaction \( F(10,105) = 1.154, P = ...
Phase 3

0.330. Similarly, for the HF group, there was no main effect of phase \( F(2,16) = 0.348, P = 0.711 \), a main effect of concentration was observed \( F(5,80) = 74.140, P < 0.001 \), and there was no significant interaction \( F(10,80) = 0.828, P = 0.603 \) (Fig. 3).

In contrast, during phase 1, the HF group initiated significantly fewer trials compared with the CHOW animals \( t(13) = 3.222, P = 0.007 \). Naltrexone injection significantly decreased the number of trials initiated by both groups, but in this condition, a significant group difference was not observed \( t(13) = 1.040, P = 0.317 \). When animals were tested 15 min after saline injection during the last phase, the group difference was again apparent \( t(13) = 2.818, P = 0.015 \) (Fig. 4).

In both groups, compared with the number of trials initiated during phase 1, the number of trials significantly decreased during phase 2 with naltrexone administration [CHOW \( t(7) = 10.058, P < 0.001 \); HF \( t(6) = 4.104, P = 0.006 \)]. After experience with testing in the naltrexone condition, a number of trials during phase 3 (saline injection) remained significantly lower than the number of trials initiated during phase 1 [CHOW \( t(7) = 7.643, P < 0.001 \); HF \( t(6) = 6.643, P = 0.001 \)]. When animals were tested for an additional phase (phase 4), both groups showed an increase in the number of trials initiated (Fig. 5). For the CHOW group, the number of trials during phase 4 was lower than that initiated during phase 1 \( t(7) = 2.686, P = 0.031 \), but this difference did not survive Bonferroni correction. For the HF rats, the number of trials initiated during phases 1 and 4 were not significantly different \( t(6) = 1.439, P = 0.200 \).

Experiment 3: Intake Test

Body weight. In the cohort of animals used for the intake tests, as observed in experiments 1 and 2, the two groups did not significantly differ in body weight measures on any of the behavioral days tested \( t(11) \leq -0.717, P \geq 0.488 \).

Water. Consistent with the licks to water values observed in experiment 1, the two groups did not significantly differ in 30-min water intake under water restriction \( t(11) = 0.935, P = 0.370 \) (Fig. 6A).

Sucrose. In both the partial food- and water-restricted and ad libitum access to chow and water states, intake of sucrose increased as a function of concentration and then decreased at the higher concentration in an inverted U-shaped manner, as described previously (e.g., 14, 36). There was no main effect of maternal diet for sucrose intake under either of the two conditions tested. Comparing intake values under the partial food and water restriction schedule, two-way ANOVA (group \( \times \) concentration) did not reveal a main effect of group \( F(1,11) = 2.519, P = 0.141 \), revealed a significant main effect of concentration \( F(5,55) = 38.070, P < 0.001 \), and no significant interaction effect \( F(5,55) = 0.800, P = 0.555 \) (Fig. 6B). Similarly, there was no significant main effect of group \( F(1,11) = 0.152, P = 0.704 \) for sucrose intake values measured when animals that had ad libitum access to chow and water were compared across the two groups. Two-way ANOVA revealed a significant main effect of concentration \( F(5,55) = 30.613, P < 0.001 \) and no significant interaction effect \( F(5,55) = 1.043, P = 0.402 \) (Fig. 6C).

Reasonable curve fits to the data across animals are indicated by the mean \( R^2 \) values of 0.84 \( \pm 0.03 \) (partially food and water-restricted) and 0.76 \( \pm 0.05 \) (nondeprived condition). Consistent with the intake value data, two sample t-tests did not reveal group differences for maximal intake \( (y_{max}) \) or concentration values corresponding to maximal intake \( (x_{max}) \) under partial food and water-restricted or nondeprived conditions (Table 3).

Corn oil. Similarly, there was no main effect of maternal diet on corn oil intake when the animals were tested in either a partial food- and water-restricted condition or nondeprived. Two-way ANOVAs comparing corn oil intake across the two

Table 2. Two-way ANOVA values comparing licks relative to water values

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration</th>
<th>Group ( \times ) Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>( F(1,13) = 0.731, P = 0.408 )</td>
<td>( F(5,65) = 101.841, P &lt; 0.001 )</td>
</tr>
<tr>
<td>Phase 2 (naltrexone)</td>
<td>( F(1,13) = 0.015, P = 0.905 )</td>
<td>( F(5,65) = 46.983, P &lt; 0.001 )</td>
</tr>
<tr>
<td>Phase 3</td>
<td>( F(1,11) = 0.347, P = 0.568 )</td>
<td>( F(5,55) = 41.920, P &lt; 0.001 )</td>
</tr>
</tbody>
</table>

\( F \)-tests were conducted using SigmaStat.
groups did not reveal a main effect of group \( F(1,11) = 2.071, \ P = 0.178 \), revealed a main effect of concentration \( F(5,55) = 7.247, \ P < 0.001 \), and no significant interaction effect \( F(5,55) = 1.099, \ P = 0.421 \) (Fig. 6D). Similarly, two-way ANOVAs comparing corn oil intake when animals had ad libitum access to chow and water did not reveal a significant main effect of group \( F(1,11) = 0.045, \ P = 0.834 \), revealed a main effect of concentration \( F(5,55) = 3.632, \ P = 0.007 \), and no significant interaction effect \( F(5,55) = 0.473, \ P = 0.795 \) (Fig. 6E).

The mean \( R^2 \) value of 0.65 ± 0.08 indicated moderate curve fits to the data under partial food and water-restricted conditions. Two sample t-tests did not reveal significant group differences between maximal intake \( (y_{\text{max}}) \) or corn oil concentration corresponding to maximal intake \( (x_{\text{max}}) \) (Table 3). The mean \( R^2 \) value of 0.47 ± 0.10 indicated poor curve fits to the data under nondeprived conditions for corn oil; thus, inflection coordinates were not calculated.

**mRNA expression.** The relative expression of OPRM1 in the ventral tegmental area was significantly lower in the HF compared with the CHOW group \( n(11) = 2.861, \ P = 0.015 \). There were no significant group differences in relative expression in the other brain areas or genes of interest examined (Fig. 7).

### DISCUSSION

These findings show that offspring from dams maintained on a high-fat diet during pregnancy and lactation overconsume a high-fat diet when presented with a choice between a chow and high-fat diet in adulthood. Adult offspring from high-fat-fed dams also initiate significantly fewer trials to sucrrose when partially food- and water-deprived, compared with offspring from dams maintained on a standard chow diet. There was no significant main effect of maternal diet on concentration-dependent unconditioned licking responses to sucrrose as measured in a brief-access taste test. The animal’s approach to the spout can be considered appetitive behavior, while licking during a trial reflects behavior elicited by contact with the stimulus and thus is primarily consummatory. The current findings show that under a partial food and water restriction condition, a high-fat maternal diet results in a reduction in appetitive behavior in female rat offspring with no significant effect on taste-induced consummatory components of ingestion, at least when tested in a brief-access taste procedure.

The brief-access taste test relies on the hedonic component of the stimulus to drive a behavioral response (see Ref. 19). The effect of maternal diet on approach to the spout but not on...
unconditioned licking responses raises the possibility that high-fat maternal diet reduces the spout approach behavior via motivation-related mechanisms in the offspring. Several neurotransmitter systems have been implicated in motivation-related behavior; here, we focused on the opioid system. To assess whether spout approach in this procedure is, indeed, mediated by mechanisms associated with reward, naltrexone, an opioid receptor antagonist was administered before testing in the brief-access taste procedure. As predicted, naltrexone decreased the number of trials initiated in both groups but had no significant effect on unconditioned licking responses across the sucrose concentration array. That naltrexone reduced the appetitive behavior with little effect on the consummatory component is also consistent with data from analysis of microstructure licking to a sucrose and saccharin mixture which revealed naltrexone reduced the number of lick clusters rather than lick cluster size (16).

Unconditioned licking responses to sucrose in the brief-access taste test did not significantly differ between the two groups. Furthermore, in 30-min one-bottle intake tests during which initiation of 10-s trials was not required, there was no main effect of maternal diet across the sucrose and corn oil concentration arrays whether tested in a partially food- and water-restricted or nondeprived condition. Thus, it appears that there is minimal effect of maternal diet on the orosensory features of the stimulus. In other paradigms, rodents exposed to sucrose pellets earlier in life showed similar preference compared with control animals when presented chow and sucrose pellets later in life (15, 52) compared with solutions in the partial reinforcement (49). In contrast, rats fed a high-fat high-sucrose diet increased responding in a progressive ratio schedule of reinforcement compared with control animals (26). Thus, the findings of the current study lend support to the hypothesis that a high-fat diet during pregnancy and lactation elicits a reduction in motivation-related behaviors with minimal effect on hedonic taste responses.

If maternal diet influences changes in reward-related mechanisms, it would follow that differential gene expression may be observed in offspring from chow-fed or HF-fed dams and that naltrexone would differentially reduce responses to sucrose in these two groups. Whereas it has been reported in mice that offspring from dams maintained on a high-fat diet during pregnancy and lactation show up-regulation of the expression of genes associated with reward processing (51), here, in rats, expression of the µ-opioid receptor was significantly down-regulated in the ventral tegmental area in the HF offspring. These findings are more in line with reports of the decrease in mRNA expression of µ-opioid receptor in the VTA in rats offspring of junk food-fed dams (34). Similarly, decreased mRNA expression of µ-opioid receptor was observed in diet-induced obese mice (50). Here, behaviorally during the naltrexone testing condition, the effect of maternal diet on number of trials initiated was no longer apparent. Furthermore, naltrexone appeared to reduce trial initiation more so in the CHOW rats than in the HF group. Several other studies in the literature have compared the degree to which naltrexone or naloxone (structurally similar to naltrexone) decreases behavioral measures across various groups. Naltrexone has been shown to reduce responses similarly (4, 21) or differentially (10–11, 31) across groups. These approaches have provided evidence to make inferences about endogenous opioid activity in the groups tested. Similarly here, the group difference in trial initiation between the two groups that was not apparent with naltrexone, reappeared under subsequent saline control injection testing conditions. Taken together, these findings suggest differential endogenous opioid activity between the two groups as a function of maternal diet. It appears that maternal high-fat diet decreases the sensitivity of the opioid system.

Naltrexone and naloxone (structurally similar to naltrexone) reduces intake (see Refs. 3 and 20) when administered peripherally or centrally (18). Naltrexone reduces food intake or selectively reduces intake of preferred stimuli depending on the brain sites to which it is administered (17). Previously, it has been shown that naloxone reduced the intake of a 10% sucrose solution in both deprived and nondeprived sham-drinking rats.
(37), indicating the naloxone effects were not attributed by postabsorptive feedback signals. Furthermore, naloxone reduced sham intake of sucrose solutions in rats with gastric fistulas, such that the intake of 10% sucrose with naloxone was similar to that of 5% sucrose without naloxone (24, 25), suggesting naloxone reduces sham intake of sucrose as if by decreasing the reward value of a concentration. In the current study, naltrexone did not affect responding to sucrose as measured by concentration-dependent licking. Although sensory thresholds and affective responding tap into different functional domains of taste and can be experimentally dissociated (see Refs. 41 and 42), these measures are not necessarily mutually exclusive; thus, it can be inferred that naltrexone had little effect on the concentration-dependent sensory component of sucrose in the current study. Complementary to these findings, whereas β-endorphin levels have been shown to increase after intake of palatable foods (12) or fluids (53), sucrose-induced increases in β-endorphin levels are not as robust in animals conditioned to avoid sucrose or when peripheral taste.

Table 3. Comparisons of maximal intake ($x_{\text{inf}}$) and concentration corresponding to maximal intake ($y_{\text{inf}}$)

<table>
<thead>
<tr>
<th></th>
<th>Nondeprived</th>
<th>Partial Food- and Water-Restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHOW</td>
<td>HF</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$x_{\text{inf}}$</td>
<td>0.68 ± 0.11</td>
<td>0.61 ± 0.01</td>
</tr>
<tr>
<td>$y_{\text{inf}}$</td>
<td>16.45 ± 1.81</td>
<td>19.00 ± 3.06</td>
</tr>
<tr>
<td>Corn Oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$x_{\text{inf}}$</td>
<td>11.56 ± 2.71</td>
<td>6.85 ± 4.73</td>
</tr>
<tr>
<td>$y_{\text{inf}}$</td>
<td>10.67 ± 1.48</td>
<td>7.53 ± 1.56</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE.
nerves have been transected (53). Furthermore, naltrexone has been shown to selectively suppress sucrose intake of the more valuable concentration in a contrast paradigm in rats (45). Collectively, these findings suggest that rather than “dilute” sucrose concentrations, naltrexone appears to decrease behavioral responsiveness to sucrose by decreasing the motivational value of the stimulus.

Compared to the number of trials initiated before the naltrexone phase, trial initiation after the naltrexone phase was lower for both groups. It is plausible that after three test sessions following naltrexone administration, the animals associated the naltrexone-induced state with the task, thereby, resulting in a lower number of trials. This type of association has been described in the literature as nonresponse extinction (e.g., 9, 32), in which there is a reduction in the effectiveness in which the external cues induce behavior, in this case, trial initiation. In favor of this explanation, the number of trials increased with subsequent nonnaltrexone sessions. Regardless, during both nonnaltrexone phases, the HF group initiated significantly fewer trials than their CHOW counterparts.

Across the lactation period, offspring from dams maintained on a high-fat diet during pregnancy and lactation are significantly heavier than offspring from dams maintained on a standard chow diet. In females, the body weight difference as an effect of maternal diet is no longer apparent after age if the animals are weaned onto a standard chow diet. Yet when these animals are presented chow and a high-fat diet in a preference test, offspring from high-fat dams show a significantly higher intake of the high-fat diet. On the basis of the current findings, this difference is not likely due to differences in the sensory orosensory component of the taste stimulus.

**Perspectives and Significance**

Development during gestation and lactation is a vulnerable period for the offspring. Maternal high-fat diet has been shown to have long-term effects on the offspring. The current findings show that first, offspring from high-fat diet-fed dams that are weaned and maintained on a standard chow have similar body weights to controls when they are adults. Yet certain ingestive behavioral components toward palatable stimuli are changed. These earlier experiences appear to influence changes that drive increased intake of high-fat diet, which may be attributed to changes in mechanisms underlying satiety or overall energy homeostasis. Second, it has been previously shown that naltrexone decreases intake of sucrose, and here, we show that rather than “diluting” the concentration-dependent response to sucrose, this is primarily via a decrease in the appetitive component of the behavior.

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References


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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


