Original Article

MOLECULAR METABOLISM

Maternal overnutrition is associated with altered synaptic input to lateral hypothalamic area

Kuldeep Shrivastava [1], Thaarini Swaminathan [1], Alessandro Barlotta [1], Vikshar Athreya [1], Hassan Choudhry [1], Mark A. Rossi [1,2,3,*]

ABSTRACT

Objective: Maternal overnutrition is associated with adverse outcomes in offspring, including increased risk for obesity and diabetes. Here, we aim to test the effects of maternal obesity on lateral hypothalamic feeding circuit function and determine the relationship with body weight regulation.

Methods: Using a mouse model of maternal obesity, we assessed how perinatal overnutrition affected food intake and body weight regulation in adult offspring. We then used channelrhodopsin-assisted circuit mapping and electrophysiological recordings to assess the synaptic connectivity within an extended amygdala-lateral hypothalamic pathway.

Results: We show that maternal overnutrition during gestation and throughout lactation produces offspring that are heavier than controls prior to weaning. When weaned onto chow, the body weights of over-nourished offspring normalize to control levels. However, when presented with highly palatable food as adults, both male and female maternally over-nourished offspring are highly susceptible to diet-induced obesity. This is associated with altered synaptic strength in an extended amygdala-lateral hypothalamic pathway, which is predicted by developmental growth rate. Additionally, lateral hypothalamic neurons receiving synaptic input from the bed nucleus of the stria terminalis have enhanced excitatory input following maternal overnutrition which is predicted by early life growth rate.

Conclusions: Together, these results demonstrate one way in which maternal obesity rewires hypothalamic feeding circuits to predispose offspring to metabolic dysfunction.

© 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords Bed nucleus of the stria terminalis; Lateral hypothalamus; Maternal obesity; Overnutrition; Perinatal programming

1. INTRODUCTION

As the prevalence of obesity and overweight continue to rise throughout the world [1,2], the proportion of children born to overweight mothers also increases. In humans, elevated maternal weight is associated with increased risk of neuropsychiatric illness and metabolic dysfunction later in life [3–7]. This is recapitulated in animal models in which perinatal exposure to obesogenic diets leads to offspring that are predisposed to widespread phenotypic changes including those that mirror the neuropsychiatric and metabolic disorders seen in humans [8–12]. In rodent models, perinatal exposure to obesogenic diets causes pronounced metabolic changes later in life. Offspring born to mothers maintained on high fat diet (HFD) are more likely to develop obesity and type 2 diabetes as adults [8,13], which may be driven in part by a preference for highly palatable foods [14]. It has long been thought that behavioral and metabolic alterations associated with maternal overnutrition are caused by developmental programming within brain systems responsible for regulating energy balance [15–18]. Currently, the circuit- and cell-type specific mechanisms underlying these functional changes remain largely unexplored.

The lateral hypothalamic area (LHA) is a critical node in the neurocircuitry that regulates feeding, energy balance, and reward-related behavior across species [19–21]. LHA lesions disrupt food intake and body weight regulation, whereas stimulation induces feeding and weight gain [22–24]. The LHA contains molecularly distinct cell types [25,26] that are capable of bidirectionally influencing food intake, body weight, and reward-related behavior [27–29]. Moreover, LHA neurons have been shown to undergo transcriptional and functional remodeling in response to acute and long-term changes in energy balance in adulthood [26,30]. Similarly, the LHA has been shown to exhibit physiological changes in response to maternal overnutrition. Offspring born to overweight dams have increased expression of orexigenic peptides and increased proliferation of the peptide-producing cells within the LHA [31,32]. Maternal overnutrition is also associated with an increase in the density of synaptic inputs to the LHA [33]. Despite this, little is known about how maternal overnutrition impacts the function of hypothalamic feeding circuits. Thus, we sought to test whether maternal overnutrition predisposes offspring to diet induced obesity and determine the associated functional changes in an LHA pathway that regulates food intake.

[1]Child Health Institute of New Jersey, USA [2]Department of Psychiatry, Robert Wood Johnson Medical School, USA [3]Brain Health Institute, Rutgers University, New Brunswick, NJ, USA

*Corresponding author. Child Health Institute of NJ, 89 French St., New Brunswick, NJ 08901, USA. E-mail: Mark.Rossi@Rutgers.edu (M.A. Rossi).

Received January 4, 2023 • Revision received February 18, 2023 • Accepted March 2, 2023 • Available online 8 March 2023

https://doi.org/10.1016/j.molmet.2023.101702

 © 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Within the LHA, glutamatergic neurons are a potent negative regulator of feeding. Acute activation suppresses feeding [28,34], whereas selective ablation yields susceptibility to diet-induced obesity [28]. Their endogenous activity is inversely related to motivation for food, is sensitive to leptin, and is suppressed by HFD in adulthood [26,30]. LHA glutamatergic neurons are part of a larger circuitry that exerts control over feeding. They receive inhibitory synaptic input from the bed nucleus of the stria terminalis (BNST) [34]. In addition to its well characterized roles in fear and anxiety [35,36], the BNST mediates feeding behavior [34,37–40]. BNST neurons controlling food intake develop during early postnatal life and are influenced by maternal nutrition [41]. Importantly, GABAergic BNST neurons project to the LHA where they selectively synapse onto local glutamatergic neurons, and activation of this pathway promotes feeding by inhibiting the glutamatergic cells [34]. Thus, the BNST→LHA pathway critically mediates feeding behavior in adulthood. We therefore reasoned that perinatal overnutrition may influence feeding behavior through modifications of such pathways that exert bidirectional control over food intake.

Here, we tested the hypothesis that maternal overnutrition would influence food intake and energy balance through alterations in the BNST→LHA pathway. We show that maternal overnutrition during gestation and throughout lactation produces offspring that are heavier than controls prior to weaning. When weaned onto chow, the body weights of over-nourished offspring normalize to control levels. However, when presented with highly palatable food as adults, maternally over-nourished offspring are highly susceptible to diet-induced obesity. This is associated with altered synaptic strength in the BNST→LHA pathway, which is predicted by developmental growth rate. Together, these results demonstrate one way in which maternal obesity rewires hypothalamic feeding circuits to predispose offspring to metabolic dysfunction.

2. MATERIALS AND METHODS

2.1. Subjects

Male and female C57BL/6 J mice (Jackson Laboratory) were used for all experiments. Mice were maintained on a 12-hr light–dark cycle with ad libitum food and water in their home cages at all times. All experiments were conducted in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Rutgers University Institutional Animal Care and Use Committee.

2.2. Maternal overnutrition

Female C57BL/6 J mice aged 7 weeks (n = 6 littermates) were given ad libitum access to either high fat diet (HFD) (Bio-Serv #F3282: 5.49 kcal/g; 60% fat calories) and standard chow (Purina Mouse Diet 50,583: 3.56 kcal/g, 21% fat calories) or a taste matched low fat diet (LFD) (Bio-Serv F4031: 3.93 kcal/g, 16% fat calories) and chow for 6 weeks. Mice were then separated and bred with a male C57BL/6 J mouse that had been maintained on standard chow. HFD + chow or LFD + chow diets were maintained throughout breeding, gestation, and lactation. Offspring of HFD fed mothers (mHFD) and LFD fed mothers (mLFD) were weighed at postnatal days 10, 15, and 21 and were then weaned onto standard chow at p21. Food intake per cage and individual mouse body weights were monitored >3 times per week during the first 6 h of the lights on period of the light cycle for the duration of experiments. Growth rate was defined as the slope of the weight from p10 to p21. Litter size was not significantly different between mLFD (mean: 8.0 ± 0.71) and mHFD (mean: 6.8 ± 1.72) dams (t (9) = 1.16, p = 0.28). Nor was the time from the start of the diet to the birth of each litter different between mLFD (mean: 87.4 ± 13.2 days) and mHFD (mean: 88.33 ± 25.7 days) dams (t (9) = 0.06, p = 0.96). However, mHFD pups were less likely to survive (50%) than mLFD pups (90%) (Fisher's exact test, p = 9.9e-7).

Acute HFD intake: At ∼ p40, mice were given access to HFD for 24 h. At the start of the light cycle, mice were separated into individual cages with ad libitum access to chow, HFD, LFD, and water simultaneously. Food consumption for all diets was measured at 6-hr and 24-hr timepoints. After 24 h, mice were returned to their home cages with chow.

Chronic HFD intake: At ∼ p60, all mice were given ad libitum access to HFD and chow in their home cages for 3 weeks during which time body weight and food intake (per cage) were monitored at least 3 times per week. Data were averaged across 2-day increments to account for the possibility different litters of mice being measured on different days (e.g., the first data point after the start of HFD is an average of weights taken during the first 2 days of HFD access). Following 3 weeks of HFD, all mice were returned to chow.

2.3. Surgery

Approximately 2 weeks after the chronic HFD test ended, a subset of mice (mLFD: 3 M/2 F; mHFD 3 M/3 M) were surgically injected with the light-gated cation channel, channelrhodopin-2 (ChR2) in the BNST. Mice were anesthetized with isoflurane (induction at 3%, maintenance at <1.5%) and were placed in a stereotaxic frame (Kopf Instruments). Ophthalmic ointment was placed on their eyes, and bupivacaine 2 mg/kg was injected subcutaneously at the incision site. Burr holes were drilled bilaterally above the BNST, and an adeno-associated virus coding for ChR2 (AAV5-hsyn-ChR2-eYFP, 400 nL at 100 nL/min; UNC Viral Vector Core, titer: 5.3×10^{12}) was injected into the BNST at the following coordinates relative to Bregma: AP +0.15, ML +0.9, DV -3.85 (from brain surface) via a custom steel injection cannula. Following surgery, carprofen (20 mg/kg) was injected subcutaneously daily for 3 days. Mice recovered for 4–6 weeks before electrophysiological recordings were made.

2.4. Electrophysiological recordings

mHFD and mLFD mice that had been surgically injected with ChR2 in the BNST were deeply anesthetized with pentobarbital and then transcardially perfused with ice cold sucrose solution containing in mM: 70 sucrose, 87 NaCl, 2.5 KCl, 1.25 NaH_2P0_4, 25 $NaHCO_3$, 7 $MgCl_2 * 6H_2O$, 0.5 $CaCl_2$, 306–312 mOsm. Brains were rapidly removed, and coronal sections were taken through the BNST and LHA at 300 μm. Sections were incubated in artificial cerebrospinal fluid at 32 °C containing in mM: 126 NaCl, 2.5 KCl, 1.2 NaH_2P0_4, 1.2 $MgCl_2 * 6H_2O$, 26 $NaHCO_3$, 15 glucose, and 2.4 $CaCl_2$; 299–303 mOsm. Cells were visualized with a 40*X* objective under differential interference contrast imaging on a BX51 (Olympus) microscope. Data were acquired with an EPC 10 recording system running Patchmaster software (Heka Elektronik) at 20 kHz and bandwidth filtered with a 10 kHz Bessel filter and gain 10.

2.4.1. Current clamp recordings

Whole-cell voltage clamp recordings were used to assess excitability in LHA neurons. Borosilicate pipettes were pulled to 4–8 MΩ and backfilled with solution containing in mM: 135 potassium gluconate, 10 HEPES, 4 KCl, 4 Mg-ATP, and 0.3, Na-GTP (pH 7.35, 285 mOsm). Current was injected to hold cells at −70 mV. A current ramp of +200 pA over 10 s was used to assess rheobase. Action potential characteristics were calculated from the first or second spike elicited during the rheobase recording. Next, current ranging from −100 pA to +300 pA in 20 pA steps was injected (500 ms duration). The

 © 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

number of action potentials elicited during positive current injections were quantified per sweep post hoc. Input resistance was calculated as the slope of the I–V curve from −100 to 0 pA current injections. Finally, cells were recorded at 0 pA holding current to determine basal firing rate and resting membrane potential. To validate ChR2 functionality, ChR2+ neurons were patched within the BNST. Trains of 10 5-ms blue light pulses were delivered to the field of view at frequencies of 1–40 Hz. Spike probability was calculated for each spike train.

2.4.2. Voltage clamp recordings

Whole-cell voltage clamp recordings were used to identify LHA neurons receiving monosynaptic input from the BNST ($^{\rightarrow BNST}$LHA neurons) and to assess spontaneous synaptic input. Borosilicate pipettes were pulled to 4–8 MΩ and backfilled with solution containing in mM: 117 Cs-Methanesulfonate, 20 HEPES, 0.4 EGTA, 2.8 NaCl, 5 TEA, 5 Mg-ATP, and 0.5 Na-GTP (pH 7.35, 285 mOsm). Cells were held at −70 mV to evaluate EPSCs and +10 mV to evaluate IPSCs. 5 ms pulses of 470 nm light was delivered to the field of view (2 5-ms pulses spaced 100 ms apart; 10–20 sweeps of 10 s each). Spontaneous and evoked EPSCs and IPSCs were recorded. To confirm that recorded LHA neurons receive monosynaptic GABAergic input from BNST, TTX (500 nM) and 4-AP (1 mM) were applied to the external solution while eliciting optically evoked post-synaptic currents. Once monosynaptic connectivity was confirmed, gabazine (10 μM) was applied, which abolished optically evoked IPSCs. In the rare instances in which evoked EPSCs were observed in the presence of TTX, 4-AP, and gabazine, the AMPA receptor blocker DNQX (10 μM) was applied to confirm glutamatergic transmission.

2.5. Data analysis

Electrophysiological data were exported from Patchmaster (Heka) for offline analysis. Excitability data were analyzed using custom Python code to identify action potential characteristics and count spikes. Spontaneous EPSCs and IPSCs were identified and quantified using Clampfit v11.0.3 (Molecular Devices). Statistical analysis was performed using GraphPad Prism (v9.4.1). All statistical tests were two-sided and corrected for multiple comparisons and unequal variance where appropriate. Outliers were identified by the Grubbs' or ROUT methods with alpha 0.05.

3. RESULTS

3.1. Maternal overnutrition causes hyperphagia that predisposes offspring to diet-induced obesity

Maternal overnutrition was induced by allowing dams to consume an obesogenic diet prior to mating, during gestation, and throughout lactation [9,42]. Female C57BL/6 J mice were given ad libitum access to either LFD or HFD and standard chow for 6 weeks, at which point males were introduced (Figure 1A). Diets were maintained throughout gestation and lactation. mHFD offspring had increased body weight compared to mLFD offspring during lactation at postnatal days 10, 14, and 21 (Figure 1B). At postnatal day 21, all mice were weaned onto standard chow. Body weights were comparable between mHFD and mLFD groups at postnatal day 50, suggesting metabolic normalization in a low-fat, non-obesogenic environment.

To test whether maternal overnutrition causes hyperphagia in adulthood, we assessed intake of chow, LFD, and HFD in mLFD and mHFD mice in an acute feeding assay (Figure 1C–J). At both 6-hr and 24-hr timepoints, all mice showed strong preference for HFD with mHFD mice showing pronounced increase in consumption compared with mLFD. When food intake was normalized by body weight, we observed no difference between mHFD and mLFD groups at 6 h (Figure 1C–F). At 24 h, there was no difference in chow (Figure 1G) or LFD (Figure 1H) intake. However, mHFD mice consumed more HFD calories per gram body weight (Figure 1I), which led to increased total caloric intake (Figure 1J). This suggests that despite similar body weights and chow consumption, mHFD mice become hyperphagic relative to mLFD controls and overconsume highly palatable foods.

We therefore reasoned that this hyperphagia in response to highly palatable foods may predispose mHFD mice to diet-induced obesity. To test this, we provided mHFD and mLFD mice with ad libitum HFD access in their home cages in addition to chow for 3 weeks while food intake and body weights were measured. As predicted, mHFD mice consumed significantly more calories in the obesogenic environment, which rapidly returned to mLFD control levels when HFD was removed (Figure 1K-L). Similarly, body weight of mHFD mice significantly increased relative to mLFD mice during the HFD access period (Figure 1M–N). Consistent with their reduced energy intake when returned to chow, both mHFD and mLFD mice lost weight when HFD access was removed (Figure 1N).

Interestingly, we found that both male and female mHFD mice were hyperphagic and obesity prone (Fig. S1). Male and female mHFD pups were heavier than their mLFD counterparts prior to weaning but normalized by p50 (Figs. S1A and B). Similarly, male and female mHFD mice showed increased HFD intake in both acute and chronic feeding assays (Fig. S1C-L). In the acute feeding assay, female mice consumed more calories per gram body weight than males in both mLFD and mHFD groups (Fig. S1J). Moreover, both mLFD and mHFD female mice consumed more LFD during the acute feeding test than did males (Fig. S1H). Intriguingly, female mHFD mice tended to show more pronounced weight gain during chronic HFD access than males when compared to mLFD groups (Fig. S1M,N). Together, these results suggest that maternal overnutrition predisposes offspring to obesity by promoting overconsumption of highly palatable foods.

3.2. Maternal overnutrition increases LHA neuronal excitability

Neurons within the LHA are known to influence food intake and body weight regulation and are sensitive to changes in metabolic state [19,43]. Importantly, maternal overnutrition increases orexigenic peptide expression, neurogenesis, and synaptic innervation within the LHA [31–33]; however, how this affects the electrophysiological characteristics of resident neurons remains unknown. We therefore sought to determine if maternal overnutrition alters the excitability of LHA neurons. We performed whole-cell current clamp recordings from LHA neurons in mHFD and mLFD mice ex vivo in brain slices. Food intake and body weight prior to surgery as well as body weight at the time of recording were similar between mLFD and mHFD mice (Figs. S2A–D). We observed no differences in the basic membrane properties or action potential characteristics of LHA neurons recorded from mHFD and mLFD mice. Maternal overnutrition did not affect the resting membrane potential of LHA neurons (Figure 2A). Action potential threshold (Figure 2B), amplitude (Figure 2C), width (Figure 2D), and afterhyperpolarization (Figure 2E) were also similar between mHFD and mLFD groups. Basal firing rate was recorded with zero input current and revealed similarly low levels of spontaneous spiking in mHFD (1.26 ± 0.57 Hz) and mLFD (1.14 ± 0.53 Hz) mice (Figure 2F). We next tested LHA neuron responsivity to injections of current. The minimum current required to elicit a single action potential (rheobase) was unchanged by maternal overnutrition (Figure 2G). Interestingly, LHA neurons recorded from mHFD mice were more sensitive to injections of large amplitude positive current (>200 pA), suggesting they can achieve higher firing rates in response to input (Figure 2H–I).

 © 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

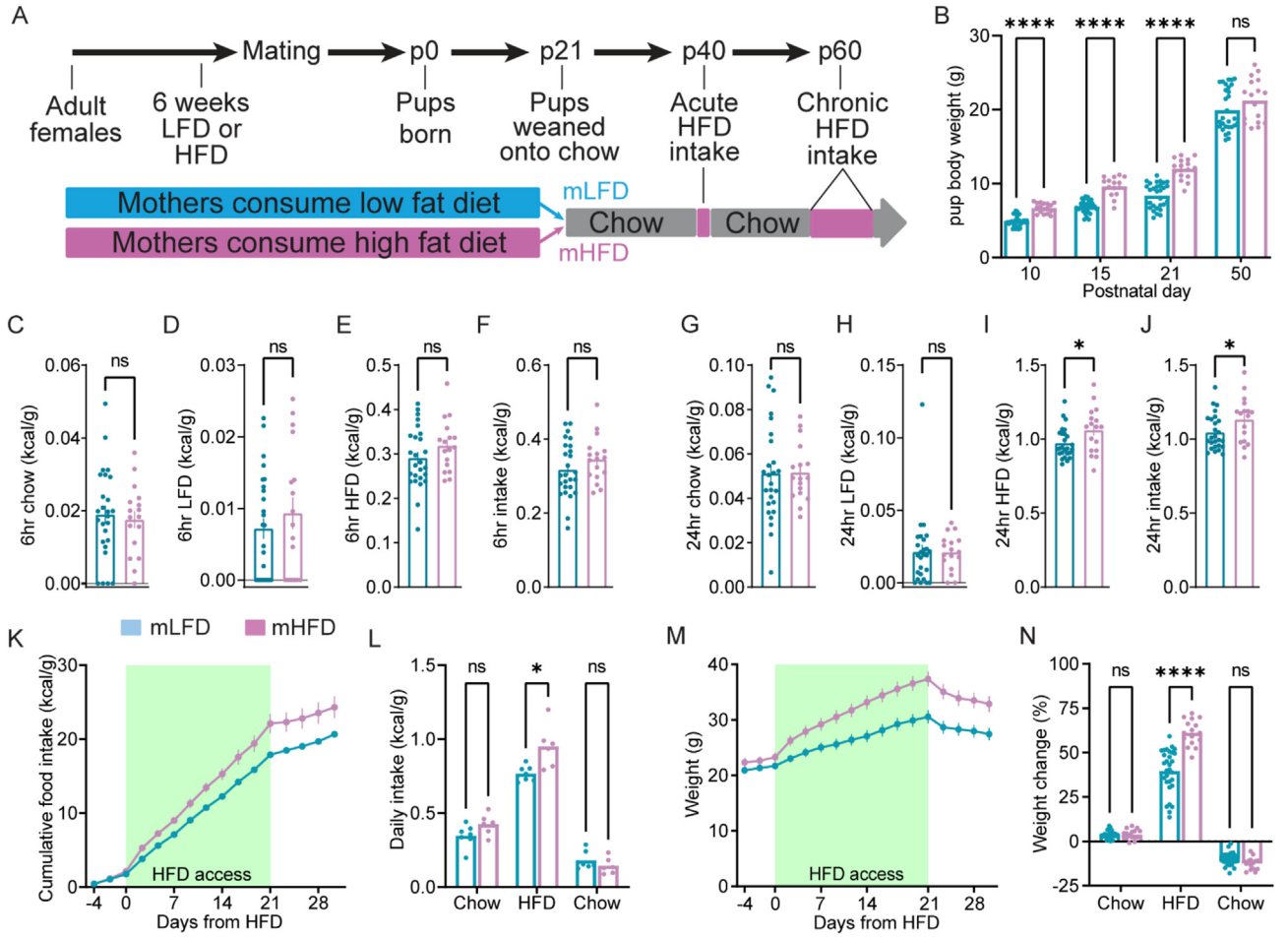

Figure 1: Maternal overnutrition causes hyperphagia and predisposes offspring to diet-induced obesity. A. Schematic of experimental timeline. Dams were maintained on HFD or LFD for 6 weeks prior to mating, and diet was maintained through weaning. Offspring of HFD (mHFD) and LFD (mLFD) dams were weaned onto chow. **B.** mHFD pups have increased body weight prior to weaning but normalize by p50 (two-way ANOVA, main effect of Group: $F(1,45) = 42.83$, $p = 4.8e\text{-}8$; main effect of Time: $F(1.39,62.58) = 817.06$, $p < 1.0e\text{-}15$; interaction between Group and Time: $F(3,135) = 5.02$, $p = 0.0025$. ****Sidak's multiple comparisons test $p < 0.0001$). n = 30/17 per group. **C**—**F.** 6hr food consumption. Chow intake (**C**. $t(41) = 0.41$, $p = 0.69$), LFD intake (**D**. $t(41) = 0.84$, $p = 0.40$), HFD intake (**E**. $t(41) = 1.37$, $p = 0.18$), and total intake (**F**. $t(41) = 2.31$, $p = 0.03$) were similar between groups. **G-J.** 24hr food consumption. **G.** Chow intake is similar between groups ($t(41) = 0.07$, $p = 0.95$). **H.** LFD intake is similar between groups ($t(41) = 0.05$, $p = 0.96$). **I.** HFD intake is increased in mHFD mice ($t(41) = 2.26$, $p = 0.029$). **J.** Total intake is increased in mHFD mice ($t(41) = 2.12$, $p = 0.04$). n = 26/16 per group. *$p < 0.05$. **K**—**N.** Extended HFD access. **K.** Cumulative food intake. **L.** Average daily calorie intake was similar on chow but increased in mHFD mice when given HFD access (two-way ANOVA, main effect of Group: $F(1,11) = 5.99$, $p = 0.03$; main effect of Diet: $F(1.95,21.80) = 295.25$, $p < 1.0e\text{-}15$; interaction between Group and Diet: $F(2,22) = 7.06$, $p = 0.004$. *Sidak's multiple comparisons test $p < 0.05$). n = 7/6 cages per group. **M.** Body weight. **N.** Average weight change (% body weight) during chow or HFD consumption (two-way ANOVA, main effect of Group: $F(1,44) = 37.48$, $p = 2.2e\text{-}7$; main effect of Diet: $F(1.15,50.42) = 870.79$, $p < 1.0e\text{-}15$; interaction between Group and Diet: $F(2,88) = 36.69$, $p = 2.6e\text{-}12$. ****Sidak's multiple comparisons test $p < 0.0001$). n = 30/16 per group. Values are mean ± sem.

Together, these results suggest a modest increase in excitability amongst mHFD LHA neurons.

3.3. BNST→LHA connectivity is primarily GABAergic and is negatively correlated with early life growth rate

LHA neurons are embedded within a broader neurocircuitry that coordinates feeding behavior and energy homeostasis. The BNST is an important upstream region that provides synaptic input to the LHA to mediate food intake [34,37]. Maternal nutrition is thought to influence the development of BNST neurons that regulate feeding and provide synaptic input to LHA cells that control energy intake [41]. Moreover, the BNST→LHA pathway orchestrates feeding and reward related behaviors through inhibition of LHA glutamatergic neurons [34]. In adulthood, diet-induced obesity (i.e., prolonged hyperphagia) suppresses the anorexigenic functions of those LHA glutamatergic neurons [26]. We therefore reasoned that functional remodeling of this circuitry may also underlie the hyperphagia and increased LHA excitability seen in mHFD mice.

We injected the light-gated cation channel, channelrhodopsin-2, under control of the synapsin promotor (AAV-Syn-ChR2-eYFP) into the BNST of mHFD and mLFD mice (Figure 3A—B). We first validated ChR2 functionality by recording from ChR2-expressing cells in the BNST (Figure 3C). These cells reliably spiked in response to brief pulses of blue light (Figure 3D). To identify LHA neurons receiving monosynaptic input from the BNST cells, LHA neurons were patched near eYFP-expressing axons while ChR2 was activated with 5-ms pulses of blue light (Figure 3E). Light-evoked excitatory and inhibitory post-synaptic currents (EPSCs and IPSCs, respectively) were recorded. We found that 60% (42/70) of recorded LHA neurons received synaptic input from BNST. Synaptically connected cells were found across the anterior-posterior extent of the LHA but were most concentrated in middle and posterior regions and weakest in the anterior part

 © 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

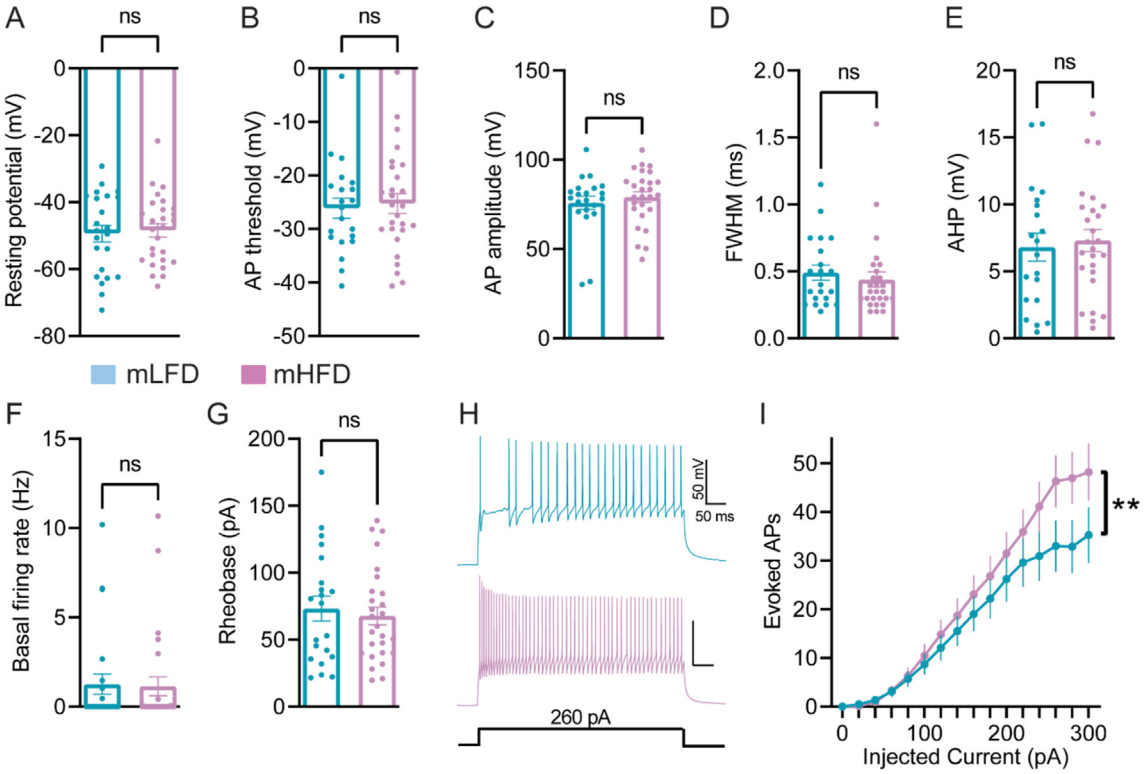

Figure 2: Maternal overnutrition increases LHA neuron excitability. A-G. LHA neurons recorded from mHFD and mLFD show similar basal electrophysiological properties. **A.** Resting membrane potential (t (46) = 0.49, p = 0.63). **B.** Action potential threshold (t (46) = 0.34, p = 0.74). **C.** Action potential amplitude (t (46) = 0.68, p = 0.50). **D.** Action potential width. Full-width half max (FWHM; t (46) = 0.64, p = 0.53). **E.** Afterhyperpolarization (AHP, t (44) = 0.38, p = 0.71). **F.** Basal firing rate (t (48) = 0.16, p = 0.88). **G.** Rheobase (t (46) = 0.49, p = 0.63). **H–I.** mHFD mice show increased firing in response to positive current injection. **H.** Example traces from +260 pA current injection. **I.** Population response. Two-way ANOVA, no main effect of Group: F (1,48) = 1.39, p = 0.24; main effect of Current: F (1.85,88.65) = 84.79, p < 1.0e-15; interaction between Group and Current: F (15,720) = 2.32, p = 0.003). n = 23/27 cells from 5/6 mice per group. Values are individual data points with mean ± sem overlaid.

(Figs. S2E–G). Consistent with previous findings that the BNST→LHA pathway is largely GABAergic [34,44], 86% of connected LHA neurons (36/42) exhibited light-evoked IPSCs, whereas 14% (6/42) exhibited exclusively light-evoked EPSCs (Figure 3F,S3). Monosynaptic connections were confirmed by the presence of optically evoked currents after the addition of tetrodotoxin (500 nM) and 4-Aminopyridine (1 mM) to the extracellular recording solution. To confirm that the IPSCs were indeed mediated through GABA transmission, we applied the $GABA_A$ receptor antagonist, gabazine (10 μM), and the amplitude of light-evoked IPSCs was reduced to negligible levels (Figure 3G–H). We observed no group difference in the amplitude of light evoked IPSCs between mHFD and mLFD cells (Figure 3I). However, the rate of growth prior to weaning (Figure 1B) significantly predicted the evoked IPSC amplitude within the BNST→LHA pathway (Figure 3J). That is, mice that gained more weight during lactation had weaker BNST→LHA connectivity. Together, these results confirm the GABAergic composition of the BNST→LHA pathway and demonstrate that this connectivity is negatively correlated with early life growth rate.

3.4. Maternal overnutrition increases excitatory input onto $^{\rightarrow BNST}$LHA neurons

We next sought to understand how synaptic strength onto LHA neurons receiving BNST input ($^{\rightarrow BNST}$LHA) is modified by maternal overnutrition. We recorded spontaneous EPSCs and IPSCs from $^{\rightarrow BNST}$LHA neurons of mLFD and mHFD mice with whole-cell voltage clamp recordings from neurons that were determined to receive monosynaptic BNST input. Using a cesium-based internal solution, we recorded spontaneous EPSCs at −70 mV and IPSCs at +10 mV (Figure 4A,B). We observed no differences in inhibitory input; neither the rate nor amplitude of IPSCs was altered by maternal overnutrition (Figure 4C,D and Figure S4 A,B). While mHFD cells showed no change in the rate of EPSCs relative to mLFD cells (Figure 4E, Figure S4 C), there was a marked increase in EPSC amplitude onto mHFD cells (Figure 4F, S4D). This was associated with an increase in the excitation/inhibition balance onto these cells (Figure 4G,S4E), which is consistent with our observation of generally increased excitability within the LHA of mHFD mice (Figure 2). Interestingly, we found that EPSC amplitude could also be predicted by perinatal growth rate (Figure 4H). Faster weight gain during lactation (Figure 1B) was associated with increased excitatory input onto $^{\rightarrow BNST}$LHA neurons. These results suggest that maternal overnutrition remodels the synaptic strengths onto LHA neurons.

To further evaluate the specificity of the observed changes in synaptic strength in maternally over-nourished mice, we assessed spontaneous synaptic input onto LHA cells that were not identified as direct downstream targets of BNST neurons (Figure 5). We found no differences in IPSC rate (Figure 5A,S4F) or amplitude (Figure 5B,S4G) between mHFD and mLFD mice. Similarly, maternal overnutrition did not alter EPSC rate (Figure 5C,S4H) or amplitude (Figure 5D,S4I), and the excitatory/inhibitory balance was unaffected (Figure 5E,S4J). In contrast to $^{\rightarrow BNST}$LHA neurons, EPSC amplitude onto LHA neurons that do not receive direct BNST input was not predicted by juvenile growth rate (Figure 5F). Collectively, this suggests that maternal overnutrition

 © 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

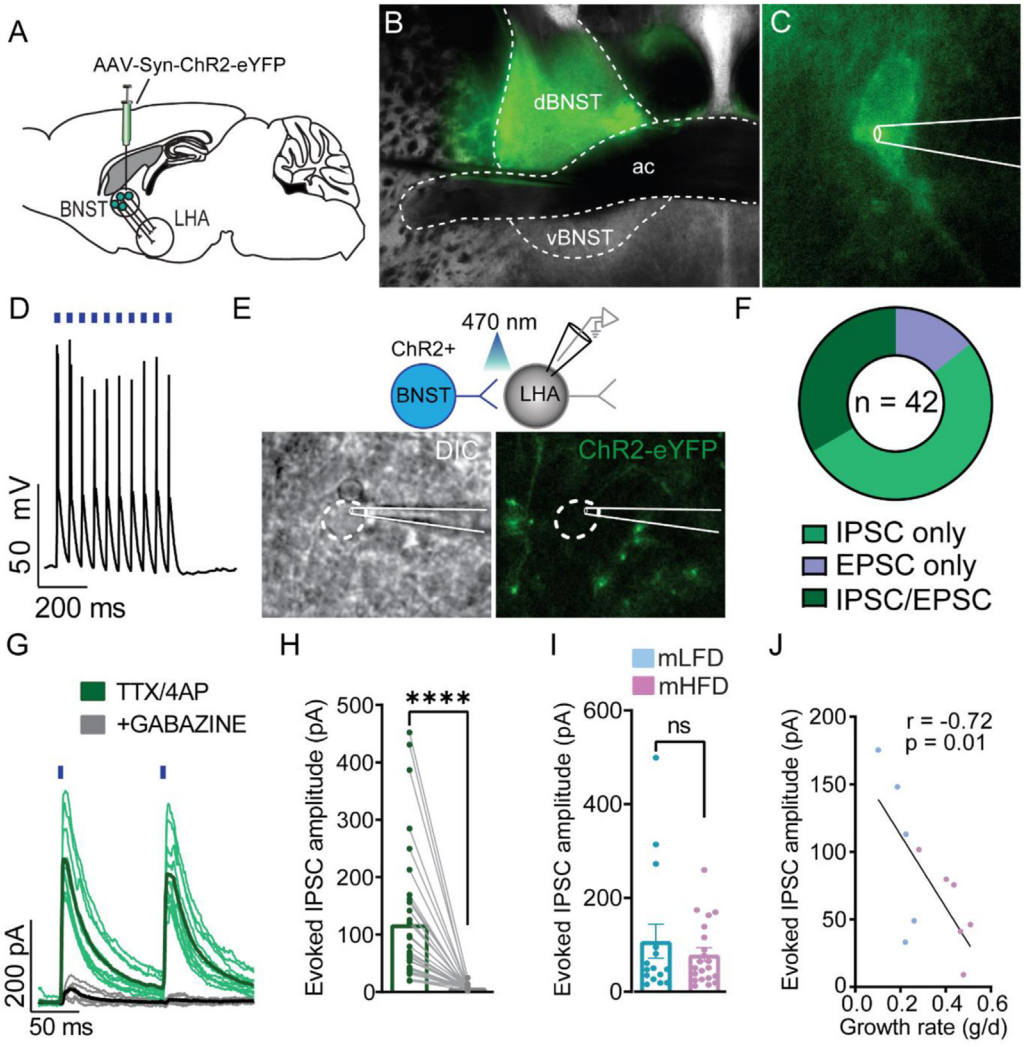

Figure 3: BNST→LHA connectivity is primarily GABAergic and is negatively correlated with early life growth rate. A. Experimental schematic. AAV-Syn-ChR2-eYFP was injected in BNST. **B.** Example of ChR2-eYFP expression in BNST. **C.** Whole-cell current clamp recordings were made from BNST:ChR2+ neurons to validate ChR2 functionality. **D.** Example BNST:ChR2+ neuron spiking in response to 5-ms blue light pulses. **E-J.** LHA cells were patched and evaluated for synaptic connectivity with BNST. **F.** Proportion of cells exhibiting IPSCs, EPSCs, or both. **G.** Example trace showing light evoked IPSCs that are blocked by the addition of the $GABA_A$ receptor antagonist, gabazine. **H.** The amplitude of evoked IPSCs was reduced by gabazine (paired t-test, t (33) = 5.61, p = 3.0e-6). **I.** Evoked IPSC amplitudes were similar between mHFD and mLFD mice (Welch's t-test: t (18.53) = 0.74, p = 0.47). **J.** Growth rate of juvenile mice significantly predicted the amplitude of evoked IPSCs onto $^{\rightarrow BNST}$LHA neurons (r = −0.72, p = 0.01). n = 15/21 cells from 5/6 mice per group. Values are individual data points with mean ± sem overlaid.

alters hypothalamic circuitry in the BNST→LHA pathway, but not broadly across all LHA cells.

4. DISCUSSION

Here, we demonstrated that maternal HFD overnutrition results in heavier offspring, but this body weight normalizes if returned to a low-fat diet upon weaning. However, despite similar body weights in adulthood, perinatally over-nourished mice remain primed for obesity. When given access to highly palatable food in adulthood, mHFD mice were hyperphagic and gained more weight than mLFD controls. When mice were returned to an exclusively chow diet after extended HFD access, both mHFD and mLFD groups exhibited pronounced weight loss and reduced caloric intake (Figure 1K-N). This is consistent with previous reports in which mice reduce chow intake following access to highly palatable foods [45,46], suggesting mHFD mice retain sensitivity to chow devaluation. Such weight loss may be driven by a reduction in basal metabolic rate and energy expenditure [47,48]. Electrophysiological recordings revealed that LHA neurons were more excitable in mHFD mice. We then investigated how maternal overnutrition effects the BNST→LHA pathway, which critically mediates food-seeking behavior. In LHA neurons that receive input from the BNST, the increased excitatory drive was especially evident. Increased perinatal growth rate was associated with weaker inhibitory input from the BNST and an overall increase in the excitatory input onto the same neurons. This was not the case for LHA neurons that were not synaptically connected with the BNST. Together, these results demonstrate that perinatal environment rewires the brain circuits critical for orchestrating feeding behavior in adulthood and predispose offspring to diet-induced obesity and type 2 diabetes.

The BNST provides inhibitory input to the LHA that is critical for controlling food intake [34,37]. The target of BNST afferents within the LHA

 © 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

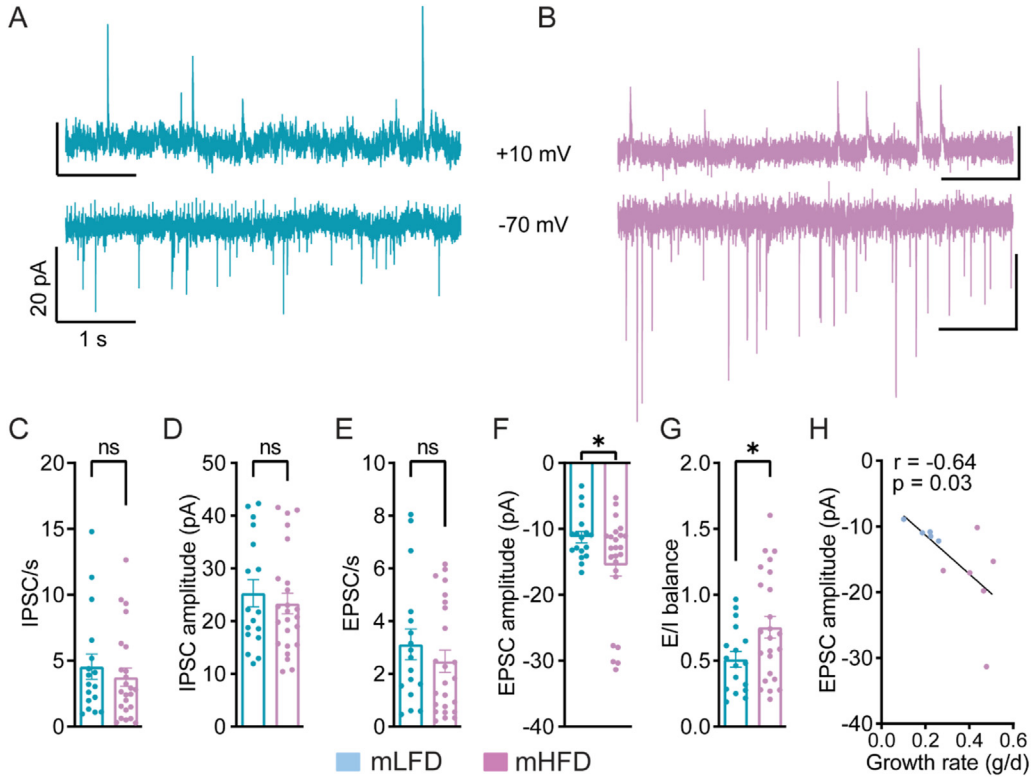

Figure 4: Maternal overnutrition increases excitatory input onto $^{\rightarrow BNST}$LHA neurons. LHA neurons receiving monosynaptic input from BNST were identified and spontaneous IPSCs and EPSCs were recorded. **A-B.** Example IPSC (top) and EPSC (bottom) recordings from mLFD (**A**) and mHFD (**B**) mice. All scale bars are 20 pA (vertical) and 1 s (horizontal). **C.** IPSC rate ($t(39) = 0.70$, $p = 0.49$). **D.** IPSC amplitude ($t(39) = 0.61$, $p = 0.54$). **E.** EPSC rate ($t(39) = 0.91$, $p = 0.37$). **F.** EPSC amplitude ($t(39) = 2.35$, $p = 0.02$). **G.** Ratio of excitatory to inhibitory input (E/I balance, t test with Welch's correction: $t(38.33) = 2.37$, $p = 0.02$). **H.** Growth rate of juvenile mice significantly predicted the amplitude of EPSCs onto $^{\rightarrow BNST}$LHA neurons ($r = -0.64$, $p = 0.03$). n = 17/24 cells from 5/6 mice per group. Values are individual data points with mean ± sem overlaid.

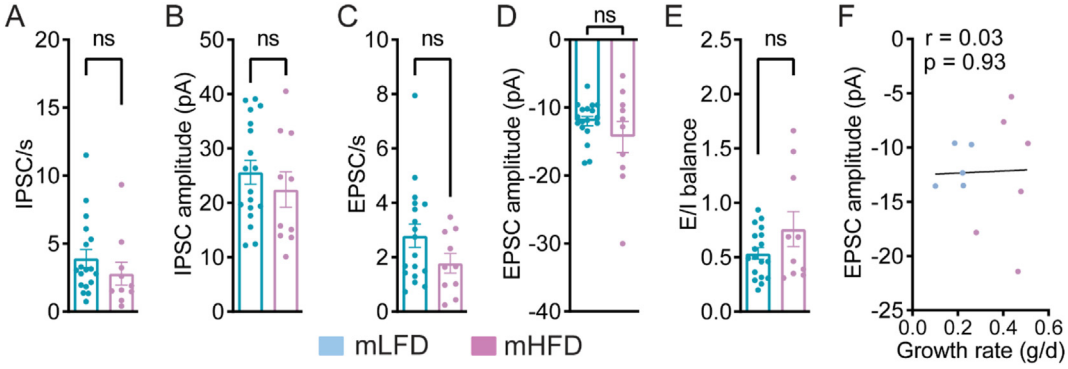

Figure 5: Maternal overnutrition does not alter synaptic input onto LHA neurons that do not receive BNST input. LHA neurons that did not exhibit post-synaptic responses to BNST afferent stimulation were identified and spontaneous IPSCs and EPSCs were recorded. **A.** IPSC rate ($t(26) = 1.03$, $p = 0.31$). **D.** IPSC amplitude ($t(26) = 0.84$, $p = 0.41$). **E.** EPSC rate ($t(26) = 1.58$, $p = 0.13$). **F.** EPSC amplitude ($t(26) = 1.21$, $p = 0.24$). **G.** E/I balance (t test with Welch's correction: $t(11.0) = 1.33$, $p = 0.21$). **H.** Growth rate of juvenile mice did not predict the amplitude of EPSCs onto LHA neurons that do not receive input ($r = 0.03$, $p = 0.93$). n = 18/10 cells from 5/6 mice per group. Values are individual data points with mean ± sem overlaid.

are glutamatergic neurons [34], which suppress food intake and become dysfunctional during diet-induced obesity [26]. Moreover, maternal nutrition influences BNST neuron development [41], and BNST dysfunction is implicated in neuropsychiatric conditions that are associated with early life overnutrition [35,36,44]. Thus, we hypothesized that the BNST→LHA pathway is a target of perinatal programming that underlies the susceptibility to obesity in mHFD mice. Indeed, we found an increase in the excitatory-inhibitory balance of $^{\rightarrow BNST}$LHA neurons after maternal overnutrition. Optically evoked inhibitory transmission as well as spontaneous excitatory inputs were negatively correlated with early life growth, suggesting perinatal programming of LHA circuitry. These results are consistent with previous findings demonstrating increased density of afferents from the dorsomedial and ventromedial hypothalamic nuclei in LHA following maternal overnutrition [33]. Furthermore, maternal overnutrition increases orexin/hypocretin expression within LHA as well as neurogenesis of orexin containing

 © 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

neurons [31], which release glutamate [49,50] and can increase local excitatory drive within the LHA [51].

Thus, our results are in accord with previous work implicating increased glutamatergic signaling within the LHA following maternal overnutrition. We show here for the first time that the BNST→LHA pathway is selectively modified by early life overnutrition. While the source of the increased glutamatergic inputs to $^{\rightarrow BNST}$LHA neurons remain to be determined, we speculate that intra-hypothalamic signaling is a likely candidate, specifically from the dorsomedial and ventromedial nuclei [33]. Distal glutamatergic inputs from regions such as the parabrachial nucleus [52] may also be involved. This suggests there may be widespread rewiring of reward circuitry within the brain following maternal overnutrition that is not limited to the BNST→LHA pathway.

Importantly, inhibitory BNST inputs to the LHA preferentially target the local glutamatergic neurons [34]. Therefore, the synaptically connected cells recorded here are putatively considered to be glutamatergic, which are known to negatively regulate food intake and goal-directed actions [26,28,30,34,53,54]. Thus, changes to the excitatory-inhibitory balance of LHA glutamate neurons may underlie the increased weight, hyperphagia, and predisposition to diet-induced obesity seen in maternally over-nourished offspring. Interestingly, maternal overnutrition was associated with increased excitatory drive onto putative LHA glutamatergic neurons, but increased activity in these cells has been shown to suppress feeding [26,34]. This paradoxical result may reflect compensatory changes that occur in response to early life overnutrition in an attempt to reign in food intake. Indeed, we found that the extent of changes in both evoked IPSCs and spontaneous EPSCs could be predicted by the early life growth rate. This is in agreement with previous observations that increased growth rate early in life is associated with enhanced risk for obesity and diabetes [17,55,56]. However, given the observation that mHFD mice are still obesity prone, the increased excitatory drive onto $^{\rightarrow BNST}$LHA neurons is not sufficient to suppress hyperphagia and subsequent weight gain. We speculate that increased excitatory input in this pathway is one locus of compensation, but is overshadowed by alterations in distributed parallel circuits regulating food intake [27,57,58]. Although we found a strong relationship between synaptic input in $^{\rightarrow BNST}$LHA neurons and early life growth rate, we did not observe such a relationship in LHA neurons that did not receive input from BNST. While we do not know the neurochemical identity of these unconnected neurons, they may be GABAergic or represent functionally distinct subsets of glutamatergic cells (e.g., orexin/hypocretin neurons). This suggests that the functional alterations in the BNST→LHA pathway induced by maternal overnutrition are not a general phenomenon but may be specific to feeding and reward circuitry.

Although inhibitory BNST projections to the LHA synapse preferentially onto glutamatergic neurons, there is evidence for sparse connections with other known LHA cell types [34,44]. Moreover, LHA glutamatergic neurons are anatomically, molecularly, and functionally heterogeneous with subsets exhibiting unique input–output organization [25,26,28,30,34,53]. While increased excitatory input onto $^{\rightarrow BNST}$LHA neurons is unlikely to be the only neurocircuit rewiring following maternal obesity, we have identified this pathway is an important entry point in the neurocircuitry relevant for predisposing maternally over-nourished offspring to weight gain and obesity that may be leveraged in future investigations.

Here, we show that maternal overnutrition before conception and through weaning causes rewiring of hypothalamic circuitry that persists into adulthood. This is consistent with the proposed critical windows for such developmental programming [59]. The exact timing of these windows is an area of active research. For example, limiting overnutrition to the prenatal period still results in obesity and persistent changes in hypothalamic function but to a lesser extent than if overnutrition occurs through lactation [31]. In contrast, limiting overnutrition to the early postnatal period still promotes obesity [60]. Lactation is generally thought to be a critical period for the programming of brain systems that control energy homeostasis, likely because many hypothalamic and hindbrain connections do not fully develop until after birth [41,61]. Future studies are needed to further refine the critical window for changes within BNST→LHA circuitry. In addition to the direct effects of maternal nutrition on pup brains, other factors may also influence developmental programming of offspring behavior and metabolism. One such factor is maternal behavior. Dams fed HFD have been reported to have reduced reproductive success, litter size, and impaired pup care as well as increased plasma glucocorticoid levels [60,62]. Although we did not observe differences in litter size, HFD-fed dams did show reduced reproductive success. This may be driven by impaired pup care, which may contribute to changes in brain reward circuitry independently of those related to overnutrition per se [63]. Moreover, the present study has focused specifically on maternal diet. However, evidence suggests that paternal overnutrition can also influence offspring health [64–66] even though maternal obesity may be a stronger predictor of offspring body mass index [67]. Much evidence also indicates that developmental programming is highly dependent on the sex of the offspring [68]. Our results (Fig. S1) are consistent with reports in which both males and females are at increased risk of developing diet-induced obesity following perinatal overnutrition [69,70]. Interestingly, female maternally over-nourished offspring develop increased adiposity and impaired glucose regulation [69], whereas over-nourished males may be at greater risk of developing broader hypothalamic changes and impaired reward seeking behavior [9,71]. Our results demonstrating pronounced HFD induced weight gain in females are consistent with this. Much work is yet needed to disentangle the effects of these influences on offspring behavior and physiology.

The present results add to the growing body of literature indicating that perinatal environment exerts profound influence over adult physiology and metabolism. Consistent with previous reports [6,8], we found that offspring of mothers who were maintained on HFD were hyperphagic and predisposed to diet-induced obesity. Previous studies have also found that maternal overnutrition is associated with increased risk for psychiatric illness in adulthood and adolescence, including eating disorders, anxiety, and depression [3,4]. In addition to their well-characterized role in feeding, connections between the BNST and LHA have been implicated in the control of anxiety-like and reward-related behaviors [34,44]. Thus, synaptic modifications within this pathway and associated circuitry have the potential to alter wide ranging motivated behaviors in response to maternal overnutrition. Future studies will be needed to determine if early life changes to the BNST→LHA circuitry are also associated with altered anxiety-like or reward-related behaviors.

Overall, the present results indicate that early life overnutrition predisposes offspring to diet-induced obesity later in life. This predisposition is associated with persisting enhancement of excitatory drive within lateral hypothalamic circuitry. Together, these results suggest that targeted manipulations of the BNST→LHA pathway or glutamatergic signaling within the LHA may be sufficient to mitigate the effects of perinatal overnutrition.

 © 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

AUTHOR CONTRIBUTIONS

M.A.R. designed and oversaw experiments, performed patch-clamp recordings, and analyzed data. T.S. performed maternal obesity breeding. K.S., A.B., V.A., and H.C. collected food intake and body weight data and carried out HFD tests. K.S. and M.A.R. wrote the manuscript with input from all authors.

DATA AVAILABILITY

Data will be made available on request.

ACKNOWLEDGEMENTS

This work was supported by NIH grant DK121883 to M.A.R. We are grateful for the support of the Robert Wood Johnson Foundation to the Child Health Institute of New Jersey (grant #74260). We thank Dr. Zhiping Pang for helpful comments on an earlier draft of this manuscript.

CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2023.101702.

REFERENCES

[1] Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic. Obesity 2008;16(10):2323–30. https://doi.org/10.1038/oby.2008.351.

[2] Malik VS, Willet WC, Hu FB. Nearly a decade on — trends, risk factors and policy implications in global obesity. Nat Rev Endocrinol 2020;16(11):615–6. https://doi.org/10.1038/s41574-020-00411-y.

[3] Edlow AG. Maternal obesity and neurodevelopmental and psychiatric disorders in offspring. Prenat Diagn 2017;37(1):95–110. https://doi.org/10.1002/pd.4932.

[4] Rivera H, Christiansen K, Sullivan E. The role of maternal obesity in the risk of neuropsychiatric disorders. Front Neurosci 2015;9.

[5] Heslehurst N, Vieira R, Akhter Z, Bailey H, Slack E, Ngongalah L, et al. The association between maternal body mass index and child obesity: a systematic review and meta-analysis. PLoS Med 2019;16(6):e1002817. https://doi.org/10.1371/journal.pmed.1002817.

[6] Eriksson JG, Sandboge S, Salonen M, Kajantie E, Osmond C. Maternal weight in pregnancy and offspring body composition in late adulthood: findings from the Helsinki Birth Cohort Study (HBCS). Ann Med 2015;47(2):94–9. https://doi.org/10.3109/07853890.2015.1004360.

[7] Godfrey KM, Reynolds RM, Prescott SL, Nyirenda M, Jaddoe VWV, Eriksson JG, et al. Influence of maternal obesity on the long-term health of offspring. Lancet Diabetes Endocrinol 2017;5(1):53–64. https://doi.org/10.1016/S2213-8587(16)30107-3.

[8] Bouret S, Levin BE, Ozanne SE. Gene-environment interactions controlling energy and glucose homeostasis and the developmental origins of obesity. Physiol Rev 2015;95(1):47–82. https://doi.org/10.1152/physrev.00007.2014.

[9] Ceasrine AM, Devlin BA, Bolton JL, Green LA, Jo YC, Huynh C, et al. Maternal diet disrupts the placenta–brain axis in a sex-specific manner. Nature Metabolism 2022:1–14. https://doi.org/10.1038/s42255-022-00693-8.

[10] Peleg-Raibstein D, Luca E, Wolfrum C. Maternal high-fat diet in mice programs emotional behavior in adulthood. Behav Brain Res 2012;233(2):398–404. https://doi.org/10.1016/j.bbr.2012.05.027.

[11] Bilbo SD, Tsang V. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. Faseb J 2010;24(6):2104–15. https://doi.org/10.1096/fj.09-144014.

[12] Bale TL. Epigenetic and transgenerational reprogramming of brain development. Nat Rev Neurosci 2015;16(6):332–44. https://doi.org/10.1038/nrn3818.

[13] McMillen IC, Adam CL, Mühlhäusler BS. Early origins of obesity: programming the appetite regulatory system. J Physiol 2005;565(1):9–17. https://doi.org/10.1113/jphysiol.2004.081992.

[14] Bayol SA, Farrington SJ, Stickland NC. A maternal 'junk food' diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring. Br J Nutr 2007;98(4):843–51. https://doi.org/10.1017/S0007114507812037.

[15] Cottrell EC, Ozanne SE. Early life programming of obesity and metabolic disease. Physiol Behav 2008;94(1):17–28. https://doi.org/10.1016/j.physbeh.2007.11.017.

[16] Breton C. The hypothalamus–adipose axis is a key target of developmental programming by maternal nutritional manipulation. J Endocrinol 2013;216(2):R19–31. https://doi.org/10.1530/JOE-12-0157.

[17] Mühlhäusler BS. Programming of the appetite-regulating neural network: a link between maternal overnutrition and the programming of obesity? J Neuroendocrinol 2007;19(1):67–72. https://doi.org/10.1111/j.1365-2826.2006.01505.x.

[18] Nathanielsz PW, Poston L, Taylor PD. In utero exposure to maternal obesity and diabetes: animal models that identify and characterize implications for future health. Clin Perinatol 2007;34(4):515–26. https://doi.org/10.1016/j.clp.2007.09.005.

[19] Rossi MA, Stuber GD. Overlapping brain circuits for homeostatic and hedonic feeding. Cell Metabol 2018;27(1):42–56. https://doi.org/10.1016/j.cmet.2017.09.021.

[20] Stuber GD, Wise RA. Lateral hypothalamic circuits for feeding and reward. Nat Neurosci 2016;19(2):198–205. https://doi.org/10.1038/nn.4220.

[21] Berthoud H-R, Münzberg H. The lateral hypothalamus as integrator of metabolic and environmental needs: from electrical self-stimulation to opto--genetics. Physiol Behav 2011;104(1):29–39. https://doi.org/10.1016/j.physbeh.2011.04.051.

[22] Anand BK, Brobeck JR. Localization of a "feeding center" in the hypothalamus of the rat. PSEBM (Proc Soc Exp Biol Med) 1951;77(2):323–5. https://doi.org/10.3181/00379727-77-18766.

[23] Margules DL, Olds J. Identical "feeding" and "rewarding" systems in the lateral hypothalamus of rats. Science 1962;135(3501):374–5. https://doi.org/10.1126/science.135.3501.374.

[24] Hoebel BG, Teitelbaum P. Hypothalamic control of feeding and self-stimulation. Science 1962;135(3501):375–7. https://doi.org/10.1126/science.135.3501.375.

[25] Mickelsen LE, Bolisetty M, Chimileski BR, Fujita A, Beltrami EJ, Costanzo JT, et al. Single-cell transcriptomic analysis of the lateral hypothalamic area reveals molecularly distinct populations of inhibitory and excitatory neurons. Nat Neurosci 2019;22(4):642–56. https://doi.org/10.1038/s41593-019-0349-8.

[26] Rossi MA, Basiri ML, McHenry JA, Kosyk O, Otis JM, van den Munkhof HE, et al. Obesity remodels activity and transcriptional state of a lateral hypothalamic brake on feeding. Science 2019;364(6447):1271–4. https://doi.org/10.1126/science.aax1184.

[27] Jennings JH, Ung RL, Resendez SL, Stamatakis AM, Taylor JG, Huang J, et al. Visualizing hypothalamic network dynamics for appetitive and consummatory behaviors. Cell 2015;160(3):516–27. https://doi.org/10.1016/j.cell.2014.12.026.

[28] Stamatakis AM, Van Swieten M, Basiri ML, Blair GA, Kantak P, Stuber GD. Lateral hypothalamic area glutamatergic neurons and their projections to the

 © 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

lateral habenula regulate feeding and reward. J Neurosci 2016;36(2):302–11. https://doi.org/10.1523/JNEUROSCI.1202-15.2016.

[29] Leinninger GM, Jo Y-H, Leshan RL, Louis GW, Yang H, Barrera JG, et al. Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. Cell Metabol 2009;10(2):89–98. https://doi.org/10.1016/j.cmet.2009.06.011.

[30] Rossi MA, Basiri ML, Liu Y, Hashikawa Y, Hashikawa K, Fenno LE, et al. Transcriptional and functional divergence in lateral hypothalamic glutamate neurons projecting to the lateral habenula and ventral tegmental area. Neuron 2021;109(23):3823–3837.e6. https://doi.org/10.1016/j.neuron.2021.09.020.

[31] Chang G-Q, Gaysinskaya V, Karatayev O, Leibowitz SF. Maternal high-fat diet and fetal programming: increased proliferation of hypothalamic peptide-producing neurons that increase risk for overeating and obesity. J Neurosci 2008;28(46):12107–19. https://doi.org/10.1523/JNEUROSCI.2642-08.2008.

[32] Beck B, Kozak R, Moar KM, Mercer JG. Hypothalamic orexigenic peptides are overexpressed in young Long–Evans rats after early life exposure to fat-rich diets. Biochem Biophys Res Commun 2006;342(2):452–8. https://doi.org/10.1016/j.bbrc.2006.01.158.

[33] Kelley L, Verlezza S, Long H, Loka M, Walker C-D. Increased hypothalamic projections to the lateral hypothalamus and responses to leptin in rat neonates from high fat fed mothers. Front Neurosci 2020;13. https://doi.org/10.3389/fnins.2019.01454.

[34] Jennings JH, Rizzi G, Stamatakis AM, Ung RL, Stuber GD. The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding. Science 2013;341(6153):1517–21. https://doi.org/10.1126/science.1241812.

[35] Tovote P, Fadok JP, Lüthi A. Neuronal circuits for fear and anxiety. Nat Rev Neurosci 2015;16(6):317–31. https://doi.org/10.1038/nrn3945.

[36] Avery SN, Clauss JA, Blackford JU. The human BNST: functional role in anxiety and addiction. Neuropsychopharmacology 2016;41(1):126–41. https://doi.org/10.1038/npp.2015.185.

[37] Wang Y, Kim J, Schmit MB, Cho TS, Fang C, Cai H. A bed nucleus of stria terminalis microcircuit regulating inflammation-associated modulation of feeding. Nat Commun 2019;10(1):2769. https://doi.org/10.1038/s41467-019-10715-x.

[38] Luskin AT, Bhatti DL, Mulvey B, Pedersen CE, Girven KS, Oden-Brunson H, et al. Extended amygdala-parabrachial circuits alter threat assessment and regulate feeding. Sci Adv 2021;7(9):eabd3666. https://doi.org/10.1126/sciadv.abd3666.

[39] Hao S, Yang H, Wang X, He Y, Xu H, Wu X, et al. The lateral hypothalamic and BNST GABAergic projections to the anterior ventrolateral periaqueductal gray regulate feeding. Cell Rep 2019;28(3):616–624.e5. https://doi.org/10.1016/j.celrep.2019.06.051.

[40] Li HE, Rossi MA, Watson GDR, Moore HG, Cai MT, Kim N, et al. Hypothalamic-extended amygdala circuit regulates temporal discounting. J Neurosci 2021;41(9):1928–40. https://doi.org/10.1523/JNEUROSCI.1836-20.2020.

[41] Zeltser LM. Feeding circuit development and early-life influences on future feeding behaviour. Nat Rev Neurosci 2018;19(5):302–16. https://doi.org/10.1038/nrn.2018.23.

[42] Vucetic Z, Kimmel J, Totoki K, Hollenbeck E, Reyes TM. Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. Endocrinology 2010;151(10):4756–64. https://doi.org/10.1210/en.2010-0505.

[43] Bernardis LL, Bellinger LL. The lateral hypothalamic area revisited: neuroanatomy, body weight regulation, neuroendocrinology and metabolism. Neurosci Biobehav Rev 1993;17(2):141–93. https://doi.org/10.1016/S0149-7634(05)80149-6.

[44] Giardino WJ, Eban-Rothschild A, Christoffel DJ, Li S-B, Malenka RC, de Lecea L. Parallel circuits from the bed nuclei of stria terminalis to the lateral hypothalamus drive opposing emotional states. Nat Neurosci 2018;21(8):1084–95. https://doi.org/10.1038/s41593-018-0198-x.

[45] Mazzone CM, Liang-Guallpa J, Li C, Wolcott NS, Boone MH, Southern M, et al. High-fat food biases hypothalamic and mesolimbic expression of consummatory drives. Nat Neurosci 2020;23(10):1253–66. https://doi.org/10.1038/s41593-020-0684-9.

[46] Altherr E, Rainwater A, Kaviani D, Tang Q, Güler AD. Long-term high fat diet consumption reversibly alters feeding behavior via a dopamine-associated mechanism in mice. Behav Brain Res 2021;414:113470. https://doi.org/10.1016/j.bbr.2021.113470.

[47] Matikainen-Ankney BA, Kravitz AV. Persistent effects of obesity: a neuroplasticity hypothesis. Ann N Y Acad Sci 2018;1428(1):221–39. https://doi.org/10.1111/nyas.13665.

[48] Hall KD. Predicting metabolic adaptation, body weight change, and energy intake in humans. Am J Physiol Endocrinol Metab 2010;298(3):E449–66. https://doi.org/10.1152/ajpendo.00559.2009.

[49] Rosin DL, Weston MC, Sevigny CP, Stornetta RL, Guyenet PG. Hypothalamic orexin (hypocretin) neurons express vesicular glutamate transporters VGLUT1 or VGLUT2. J Comp Neurol 2003;465(4):593–603. https://doi.org/10.1002/cne.10860.

[50] Burt J, Alberto CO, Parsons MP, Hirasawa M. Local network regulation of orexin neurons in the lateral hypothalamus. Am J Physiol Regul Integr Comp Physiol 2011;301(3):R572–80. https://doi.org/10.1152/ajpregu.00674.2010.

[51] Li Y, Gao X-B, Sakurai T, van den Pol AN. Hypocretin/orexin excites hypocretin neurons via a local glutamate neuron—a potential mechanism for orchestrating the hypothalamic arousal system. Neuron 2002;36(6):1169–81. https://doi.org/10.1016/S0896-6273(02)01132-7.

[52] Phua SC, Tan YL, Kok AMY, Senol E, Chiam CJH, Lee C-Y, et al. A distinct parabrachial–to–lateral hypothalamus circuit for motivational suppression of feeding by nociception. Sci Adv 2021;7(19):eabe4323. https://doi.org/10.1126/sciadv.abe4323.

[53] Lazaridis I, Tzortzi O, Weglage M, Märtin A, Xuan Y, Parent M, et al. A hypothalamus-habenula circuit controls aversion. Mol Psychiatr 2019;24(9):1351–68. https://doi.org/10.1038/s41380-019-0369-5.

[54] Nieh EH, Vander Weele CM, Matthews GA, Presbrey KN, Wichmann R, Leppla CA, et al. Inhibitory input from the lateral hypothalamus to the ventral tegmental area disinhibits dopamine neurons and promotes behavioral activation. Neuron 2016;90(6):1286–98. https://doi.org/10.1016/j.neuron.2016.04.035.

[55] Ong KK. Size at birth, postnatal growth and risk of obesity. Hormone Research in Paediatrics 2006;65(Suppl. 3):65–9. https://doi.org/10.1159/000091508.

[56] Ong KKL, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ 2000;320(7240):967–71. https://doi.org/10.1136/bmj.320.7240.967.

[57] Betley JN, Cao ZFH, Ritola KD, Sternson SM. Parallel, redundant circuit organization for homeostatic control of feeding behavior. Cell 2013;155(6):1337–50. https://doi.org/10.1016/j.cell.2013.11.002.

[58] Li MM, Madara JC, Steger JS, Krashes MJ, Balthasar N, Campbell JN, et al. The paraventricular hypothalamus regulates satiety and prevents obesity via two genetically distinct circuits. Neuron 2019;102(3):653–667.e6. https://doi.org/10.1016/j.neuron.2019.02.028.

[59] Schoonejans JM, Ozanne SE. Developmental programming by maternal obesity: lessons from animal models. Diabet Med 2021;38(12):e14694. https://doi.org/10.1111/dme.14694.

[60] Chen H, Simar D, Lambert K, Mercier J, Morris MJ. Maternal and postnatal overnutrition differentially impact appetite regulators and fuel metabolism. Endocrinology 2008;149(11):5348–56. https://doi.org/10.1210/en.2008-0582.

[61] Alfaradhi M, Ozanne S. Developmental programming in response to maternal overnutrition. Front Genet 2011;2:27. https://doi.org/10.3389/fgene.2011.00027.

[62] Bellisario V, Panetta P, Balsevich G, Baumann V, Noble J, Raggi C, et al. Maternal high-fat diet acts as a stressor increasing maternal glucocorticoids' signaling to the fetus and disrupting maternal behavior and brain activation in C57BL/6J mice. Psychoneuroendocrinology 2015;60:138–50. https://doi.org/10.1016/j.psyneuen.2015.06.012.

 © 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

[63] Shin S, You I-J, Jeong M, Bae Y, Wang X-Y, Cawley ML, et al. Early adversity promotes binge-like eating habits by remodeling a leptin-responsive lateral hypothalamus—brainstem pathway. Nat Neurosci 2023;26(1):79—91. https://doi.org/10.1038/s41593-022-01208-0.

[64] Billah MM, Khatiwada S, Morris MJ, Maloney CA. Effects of paternal overnutrition and interventions on future generations. Int J Obes 2022;46(5):901—17. https://doi.org/10.1038/s41366-021-01042-7.

[65] Slyvka Y, Zhang Y, Nowak FV. Epigenetic effects of paternal diet on offspring: emphasis on obesity. Endocrine 2015;48(1):36—46. https://doi.org/10.1007/s12020-014-0328-5.

[66] Portha B, Grandjean V, Movassat J. Mother or father: who is in the front line? Mechanisms underlying the non-genomic transmission of obesity/diabetes via the maternal or the paternal line. Nutrients 2019;11(2):233. https://doi.org/10.3390/nu11020233.

[67] Patro B, Liber A, Zalewski B, Poston L, Szajewska H, Koletzko B. Maternal and paternal body mass index and offspring obesity: a systematic review. Ann Nutr Metabol 2013;63(1—2):32—41. https://doi.org/10.1159/000350313.

[68] Dearden L, Bouret SG, Ozanne SE. Sex and gender differences in developmental programming of metabolism. Mol Metabol 2018;15:8—19. https://doi.org/10.1016/j.molmet.2018.04.007.

[69] Dearden L, Balthasar N. Sexual dimorphism in offspring glucose-sensitive hypothalamic gene expression and physiological responses to maternal high-fat diet feeding. Endocrinology 2014;155(6):2144—54. https://doi.org/10.1210/en.2014-1131.

[70] Samuelsson A-M, Matthews PA, Argenton M, Christie MR, McConnell JM, Jansen EHJM, et al. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance. Hypertension 2008;51(2):383—92. https://doi.org/10.1161/HYPERTENSIONAHA.107.101477.

[71] Edlow AG, Guedj F, Pennings JLA, Sverdlov D, Neri C, Bianchi DW. Males are from Mars, and females are from Venus: sex-specific fetal brain gene expression signatures in a mouse model of maternal diet-induced obesity. Am J Obstet Gynecol 2016;214(5):623.e1—623.e10. https://doi.org/10.1016/j.ajog.2016.02.054.

 © 2023 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).