RESEARCH

Sucrose consumption alters steroid and dopamine signalling in the female rat brain

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Abstract

Sucrose consumption is associated with type 2 diabetes, cardiovascular disease, and cognitive deficits. Sucrose intake during pregnancy might have particularly prominent effects on metabolic, endocrine, and neural physiology. It remains unclear how consumption of sucrose affects parous females, especially in brain circuits that mediate food consumption and reward processing. Here, we examine whether a human-relevant level of sucrose before, during, and after pregnancy (17–18 weeks total) influences metabolic and neuroendocrine physiology in female rats. Females were fed either a control diet or a macronutrient-matched, isocaloric sucrose diet (25% of kcal from sucrose). Metabolically, sucrose impairs glucose tolerance, increases liver lipids, and increases a marker of adipose inflammation, but has no effect on body weight or overall visceral adiposity. Sucrose also decreases corticosterone levels in serum but not in the brain. Sucrose increases progesterone levels in serum and in the brain and increases the brain:serum ratio of progesterone in the mesocorticolimbic system and hypothalamus. These data suggest a dysregulation of systemic and local steroid signalling. Moreover, sucrose decreases tyrosine hydroxylase (TH), a catecholamine-synthetic enzyme, in the medial prefrontal cortex. Finally, sucrose consumption alters the expression pattern of FOSB, a marker of phasic dopamine signalling, in the nucleus accumbens. Overall, chronic consumption of sucrose at a human-relevant level alters metabolism, steroid levels, and brain dopamine signalling in a female rat model.

Key Words

- neurosteroids
- mass spectrometry
- liver steatosis
- glucose tolerance test
- inflammation
- sugar
- steroid profiling

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Introduction

Sucrose (table sugar) is abundant in the Western diet (Welsh *et al.* 2011, Newens & Walton 2016), and consumption of sucrose and other added sugars is three to five times higher (15–25% of kcal) than the recommended amount (5% of kcal; World Health Organization 2015) in the USA and other countries (Welsh *et al.* 2011). Excess sugar consumption is associated with type 2 diabetes, cardiovascular disease, non-alcoholic fatty liver disease (NAFLD), and certain cancers (Brown *et al.* 2008, Bray 2010, Tappy & Lê 2010, Friberg *et al.* 2011). Clinical and pre-clinical studies demonstrate that sucrose intake disrupts metabolic physiology, such as insulin insensitivity and hepatic steatosis (see Johnson *et al.* 2009 for review).

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In contrast to these well-known metabolic effects, little is known about how chronic sucrose consumption alters behaviour and the brain (Rada *et al.* 2005, Reichelt *et al.* 2016). Chronic sugar intake in rodents impairs short-term memory (Buyukata *et al.* 2018), spatial memory (Beilharz *et al.* 2016, Buyukata *et al.* 2018), and goal-directed behaviour (Sharpe *et al.* 2016). The underlying neural mechanisms are not clear but might include changes to the mesocorticolimbic system, a circuit comprised of dopamine (DA) synthesizing neurons in the ventral tegmental area (VTA) and DA-sensitive neurons in the nucleus accumbens (NAc), medial prefrontal cortex (mPFC), hippocampus (HPC), and other areas (Ikemoto 2007). This critical circuit regulates motivation, reward seeking, and executive functions (Floresco *et al.* 2008, Floresco 2013, Tobiansky *et al.* 2018*a*). Repeated sugar consumption affects DA release in the NAc (Rada *et al.* 2005, Avena *et al.* 2008) and DA receptors in the NAc (Sharpe *et al.* 2016). However, many rodent studies only examined males and far less is known about effects on females.

The mesocorticolimbic system and other neural circuits, as well as metabolism and immune function, are profoundly affected by steroid hormones. In particular, androgens and oestrogens modulate DA signalling in the mesocorticolimbic system (Mermelstein & Becker 1995, Tobiansky *et al.* 2016). Glucocorticoids also affect DA-dependent feeding behaviour (la Fleur 2006) and mobilize glucose (Kuo *et al.* 2015). Importantly, sugar intake affects steroid signalling. For example, in young men, sugar-sweetened beverage consumption is negatively associated with circulating testosterone levels (Chen *et al.* 2018). In rodents, consumption of sucrose or fructose, one of the monosaccharides that comprise sucrose, affects circulating corticosterone levels (Bursać *et al.* 2013, 2014) and decreases glucocorticoid receptors in the brain (Maniam *et al.* 2015). Steroidogenic enzymes in the brain are also altered by early-life fructose exposure (Mizuno *et al.* 2017). Thus, sugar consumption may influence steroid synthesis in the periphery and in the brain, which affect the function of steroid-sensitive neural circuits.

The current study examines whether sucrose consumption affects metabolism, steroids, and neurochemistry in female rat dams. Female rats were fed a diet that contained a human-relevant level of sucrose (i.e. similar to sucrose intake of many people in the USA and other developed countries (25% of kcal); Welsh *et al.* 2011, Ruff *et al.* 2013). Pregnancy and parturition involve major changes in metabolism, steroid hormones, and DA signalling, and thus it is of interest to examine the effects of sucrose intake during this time period. We examined glucose regulation, hepatic lipogenesis, adipose inflammation, serum and brain steroid levels, and markers of DA synthesis and signalling in the mesocorticolimbic system. Tyrosine hydroxylase (TH) was examined as a marker of DA synthesis, and FOSB was examined as a marker of phasic DA signalling, which is implicated in reward-seeking behaviours in rodent models (Muschamp *et al.* 2012). This integrative approach allowed us to examine the widespread effects of chronic, human-relevant sucrose consumption on multiple physiological systems in females.

Materials and methods

Animals and diets

Adult female Long–Evans rats (postnatal day 60–70, 232–286 g; n=20; Charles River Laboratories) were pair-housed in a temperature-controlled vivarium (22°C; 40 to 50% relative humidity) on a 12 h light:12 h darkness cycle (lights on at 07:00 h). Rats were given *ad libitum* access to a standard rat chow (Rat Diet 5012; LabDiet, Land O'Lakes Inc., St. Louis, MO, USA) for 1 week after arrival. Rats were given *ad libitum* access to standard tap water throughout the experiment.

During this week, subjects were gradually introduced to either a control diet (catalogue #: D12450k; Research Diets, Inc., New Brunswick, NJ, USA) or a custom diet containing sucrose (Research Diets Inc.; Table 1). Importantly, both diets were isocaloric and matched with respect to ingredients and macronutrient composition (~10% kcal from fat, ~20% kcal from protein, and ~70% kcal from carbohydrates). In the sucrose diet, 25% kcal came from sucrose and 45% from corn-starch, whereas, in the control diet, all carbohydrate calories came from corn-starch. Females were fed these diets for 10 weeks before mating (Fig. 1). Body mass and food consumption were recorded two times/week. Food consumption and body mass were not measured after mating to avoid disturbance during pregnancy and lactation.

For mating, each female was paired with a different adult male Long–Evans rat (postnatal day 80–90; n=20). One male and one female were paired together until the female became pregnant. Once pregnant, the males were removed from the cage. After parturition, the dams remained on their respective diet. If dams had a litter size of >8, then offspring were culled to eight pups. Dams were then left undisturbed until weaning of pups (25 days). In total, seven control females and nine sucrose

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Table 1 Composition of the control diet and sucrose diet from Research Diets. The sucrose diet with 25% kcal from sucrose is a custom diet. The macronutrient compositions are matched and the diets are isocaloric (3.8 kcal/g). Bold indicates difference in diet sucrose content.

Macronutrient	Control diet		Sucrose diet	
	g (%)	kcal (%)	g (%)	kcal (%)
Protein	19	20	19	20
Carbohydrate	67	70	67	70
Fat	4	10	4	10
Total		100		100
kcal/gm	3.8		3.8	
Ingredient	**g**	**kcal**	**g**	**kcal**
Casein	200	800	200	800
L-Cystine	3	12	3	12
Corn starch	700	2800	450	1800
Sucrose	**0**	**0**	**250**	**1000**
Cellulose, BW200	50	0	50	0
Soybean oil	25	225	25	225
Lard	20	180	20	180
Mineral mix S10026	10	0	10	0
Dicalcium phosphate	13	0	13	0
Calcium carbonate	5.5	0	5.5	0
Potassium citrate	16.5	0	16.5	0
Vitamin mix V10001	10	40	10	40
Choline bitartrate	2	0	2	0
Total	1055.05	4057	1055.05	4057

females were successfully impregnated and gave birth to a litter of ≥4 pups. The offspring of these dams will be described separately. All procedures were approved by the University of British Columbia Committee on Animal Care and were in accordance with the guidelines of the Canadian Council on Animal Care.

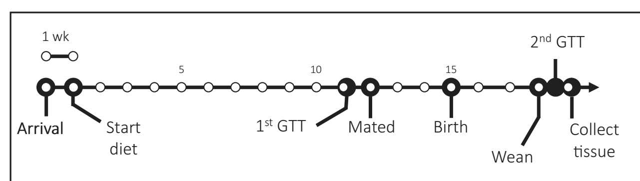

Figure 1
Study timeline. 'Start diet' is the point at which the females were taken off the standard lab chow and placed on their respective diet for the remainder of the study. The females were given two glucose tolerance tests (GTT): at week 11–12 and week 18–19 after the start of their respective diet. Females were paired with males at week 13 and, on average, gave birth at week 16. The pups were weaned at approximately week 18. Females were killed and tissue was collected at approximately week 19.

Glucose tolerance test (GTT)

A GTT was performed on the females 1 week prior to mating and 3–4 days after weaning of her pups. First, the female was transferred to a clean cage in a procedure room. The subject was fasted for 5 h (food was removed 1 h after lights on; 08:00 h), after which a blood sample was collected from the saphenous vein to measure fasting insulin and glucose. Blood glucose was measured using a handheld glucometer (Contour, Bayer AG; Leverkusen, Germany). Sixty minutes later, a second baseline glucose sample was collected via tail puncture. Next, 1.5 g/kg glucose was administered i.p. by a 50% (w/v) glucose solution. Blood glucose levels were measured 15, 30, 60, 90, and 120 min post-injection via tail puncture.

Insulin ELISA

After a 5-h fast, blood samples were collected in heparinized haematocrit tubes from a saphenous vein puncture. Blood was transferred to microcentrifuge tube and centrifuged at 4000 ***g*** for 10 min at 4°C. Plasma was stored at −80°C until analysis. Plasma insulin concentrations were performed according to the manufacturer's instructions using a rat insulin ELISA kit (Mercodia Inc., Uppsala, Sweden).

Tissue collection

Females were killed 3–4 days after the second GTT between 10:00 and 12:00 h (3 to 5 h after lights on). Food and water were available *ad libitum* on the day of killing. Subjects were rapidly and deeply anesthetized using isoflurane, weighed, and killed by rapid decapitation. Trunk blood was collected and immediately stored on wet ice until tissue collection was completed. The time between moving the cage and trunk blood collection was <3 min, to minimize the effects of stress on steroid levels (Taves *et al.* 2011).

The brain was then extracted and bisected along the midsagittal plane. One hemisphere was flash frozen on powdered dry ice and stored at −80°C for steroid analyses. The other hemisphere was immersion-fixed in 4% (w/v) formaldehyde for 4 h at 4°C, transferred to a 30% sucrose solution for 3–4 days at 4°C, flash frozen on dry ice, and stored at −80°C for immunohistochemistry. After collection of the brain, the liver was weighed. A portion (300–500 mg) of the liver was fixed in 4% (w/v) formaldehyde for 1 h at RT and then stored in 70% EtOH for histological analysis. The remainder of the liver (~3 g) was flash frozen on dry ice and stored at −80°C for total

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lipid and triglyceride analyses. Gonadal adipose tissue was weighed, flash frozen on dry ice, and stored at −80°C for mRNA analyses. Finally, the mesentery adipose depot was weighed, and the retroperitoneal and perirenal adipose depots were weighed together. Blood samples were centrifuged (3000 ***g*** for 5 min), and serum was stored at −80°C for steroid analyses.

Hepatic lipid quantification

Lipids were isolated from the liver utilizing a modified Folch extraction method and were quantified gravimetrically, as previously described (Enos *et al.* 2014, 2015, 2016).

Hepatic histology and qualification of pathology

Formaldehyde-fixed livers were embedded in paraffin blocks and sectioned. Livers were stained with haematoxylin and eosin. Histological examination of the liver specimens was performed by a trained pathologist blind to the treatment groups for the presence of NAFLD according to the scoring system designed by the Pathology Committee of the NASH Clinical Research Network, which addresses the full spectrum of lesions of NAFLD (Kleiner *et al.* 2005).

Real-time quantitative polymerase chain reaction

Total RNA was isolated from gonadal adipose tissue using a RNeasy Lipid Tissue Mini Kit (Qiagen) and quantified using a Nanodrop (Thermo Scientific). All samples had 260/280 and 260/230 ratios >1.8. cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit with an RNAse inhibitor (Applied Biosystems). Quantitative RT-PCR (Applied Biosystems 7300 Real-Time PCR) analysis ($2^{-\Delta\Delta CT}$) was carried out as per the manufacturer's instructions (Applied Biosystems) using TaqMan Gene Expression Assays for *Emr1* (EMR1; Rn01527631_m1), *Tnfa* (TNF-α; Rn99999017_m1), and *Il1b* (IL-1β; Rn00580432_m1) with 18S rRNA as the reference gene. *Emr1* is a marker for macrophages in the adipose tissue, and *Tnfa* and *Il1b* are proinflammatory cytokines. Diet did not influence reference gene (18S) expression in any of the analyses ($P>0.68$ in all cases).

Brain microdissection and steroid extraction

Fresh-frozen brain cryosectioning (at 300 µm) and microdissection for steroid analysis are described in detail elsewhere (Tobiansky *et al.* 2018*b*). The Palkovits punch technique was employed using an Integra Miltex biopsy punch (1 mm diameter; Fisher Scientific) to microdissect the mPFC (infralimbic/prelimbic border), NAc (shell/core border), medial VTA, medial preoptic area/medial hypothalamus (POA/HYP), dorsal HPC, and ventral HPC. Wet weight of tissue per punch is 0.245 mg (Taves *et al.* 2011) and, depending on the region, 5 to 15 punches were collected. Punch locations were confirmed following cresyl violet counterstain (Supplementary Fig. 1, see section on supplementary materials given at the end of this article).

The steroid extraction protocol has been described in detail (Mohr *et al.* 2019). Briefly, 1 mL of HPLC-grade acetonitrile and 50 µL of 50% methanol containing deuterated internal standards (corticosterone-d_8, dehydroepiandrosterone(DHEA)-d_6, 17β-oestradiol(E_2)-d_4, progesterone-d_9, pregnenolone-d_4, and testosterone-d_5; C/D/N Isotopes Inc., Pointe-Claire, QC, Canada) were added, to correct for matrix effects and sample losses (Tobiansky *et al.* 2018*b*). Certified reference standards were used to create calibration curves for a panel of eight steroids (androstenedione, corticosterone, DHEA, 5α-dihydrotestosterone (DHT), E_2, pregnenolone, progesterone, and testosterone). Blanks, standards, and quality controls were processed with samples.

Liquid chromatography tandem mass spectrometry

Samples were analysed as previously described (Tobiansky *et al.* 2018*b*) with minor modifications. Briefly, samples were injected into a Nexera X2 UHPLC system (Shimadzu Corp., Kyoto, Japan), passed through a KrudKatcher ULTRA HPLC In-Line Filter (Phenomenex, Torrance, CA, USA) and a Poroshell 120 HPH C18 guard column (2.1 mm; Agilent), and separated on a Poroshell 120 HPH C18 column (2.1 × 50 mm; 2.7 µm; at 40°C; Agilent) using 0.1 mM ammonium fluoride in MilliQ water as mobile phase A and HPLC-grade methanol as mobile phase B (MPB). The flow rate was 0.4 mL/min. As soon as the samples were loaded, MPB was kept at 10% for 0.5 min, then gradually increased to 42% MPB over 3.4 min where it remained for another 5.4 min. At 9.4 min, the gradient was increased to 60% MPB and gradually increased to 70% over 1 min where it remained for another 2.4 min. Finally, the column was washed at 98% MPB for 1 min. The MPB was then returned to starting conditions for 1.3 min. The injection needle was rinsed before and after each sample injection with 100% isopropanol. Total run time was 14.9 min.

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Steroids were analysed with scheduled multiple reaction monitoring (sMRM) with two MRM transitions for each steroid and one sMRM transition for each internal standard. A Sciex 6500 Qtrap triple quadrupole tandem mass spectrometer (Sciex LLC, Framingham, MA, USA) was used to quantify steroid concentrations. Androstenedione, corticosterone, DHEA, DHT, pregnenolone, progesterone, and testosterone were ionized using positive electrospray ionization mode, while E_2 was ionized using negative electrospray ionization mode. Progesterone in the dorsal and ventral HPC had low recovery (< 60%) and evidence of high matrix effects, so progesterone levels in these two regions were excluded from analyses. All water blanks were non-detectable for all steroids of interest. All quality controls were within 10% of expected values. Assay linearity, accuracy, precision, and matrix effects have been reported previously (Tobiansky *et al.* 2018*b*).

Immunohistochemistry and quantification for TH and FOSB

Fixed brain samples were cryosectioned at 40 µm and collected in five series and stored in cryoprotectant at −20°C until immunostaining. A complete series was utilized for each protein of interest, and all subjects were processed together. The immunostaining protocol for TH ((1:1000); clone LNC1, mouse MAB; Millipore; Cat# MAB318) is described previously (Heimovics *et al.* 2012, Tomm *et al.* 2018). Briefly, free-floating sections were incubated in 0.5% H_2O_2 for 30 min at room temperature (RT), followed by an incubation in blocking solution (2% normal goat serum (NGS) in PBS) for 2 h at RT. The tissue was then incubated in the blocking solution with the anti-TH antibody for 24 h at RT. Thereafter, sections were incubated in secondary antibody ((1:2000); Biotin-SP-conjugated AffiniPure Goat anti-mouse IgG; Jackson ImmunoResearch Laboratories; Cat# 115-065-116) in blocking solution for 16 h at RT. Next, sections were placed in an avidin-biotin complex ((1:2000); VectaStain ABC Kit; Vector Laboratories) for 1 h at RT. Finally, a chromogenic reaction (20 min at RT) was performed using Vector Labs Peroxidase Substrate Kit as per the manufacturer's instructions (Vector Laboratories; Cat# SK-4100). The reaction was terminated by several washes. All incubations were followed by washes (5 × 5 min). All washes were with 0.1% Triton-X 100 in PBS (PBS-T) and all solutions (except the blocking solution) were made with TBS with 0.1% Triton-X 100 (TBS-T).

The immunostaining protocol for FOSB follows the protocol for cFos immunostaining in Tobiansky *et al.* (2013) with slight modifications. All washes and solutions were in PBS-T with 0.3% Triton-X 100. All incubations and washes occurred at RT. Sections were washed in between each incubation step, but were washed once for 1 min and three times for 5 min. The blocking solution was 3% NGS in PBS-T. Incubation in the rabbit monoclonal anti-FOSB primary antibody (CST FosB (5G4) Rabbit MAB #2251; (1:1000); previously used in Zhang *et al.* 2014) was for 12 h. Tissue was then incubated in a biotinylated secondary antibody (Biotin-SP AffiniPure Donkey Anti-Rabbit IgG; Jackson ImmunoResearch Laboratories; cat #: 711-065-152) in the protein block solution for 3 h. Incubation in the avidin-biotin complex was done at a concentration of 1:500 for 1 h at RT. Finally, the chromogenic reaction was performed as previously described, but was only allowed to develop for 14 min.

Photomicrographs of TH-ir and FOSB-ir were captured in regions of the mesocorticolimbic system. We captured one rostral image, one central image, and one caudal image of the medial OFC (MOFC; anterior-posterior from bregma (AP): 4.68 to 4.20), lateral OFC (LOFC; bregma +4.68 to +3.00), prelimbic mPFC (PrL; bregma +4.20 to +2.76), and infralimbic mPFC (IL; bregma +3.72 to +2.76). Photomicrographs were also taken in the NAc shell (NAcS; bregma +2.76 to +1.20), NAc core (NAcC; bregma +2.52 to +1.28), and the parabrachial pigmented nucleus of the VTA (bregma −5.04 to −6.12; Paxinos & Watson 2007).

Slides were coded so that the researcher was blind to the group. TH-ir images were captured using a Nikon Digital Sight DS-U1 camera connected to a Nikon Eclipse 90i microscope (10× objective; resolution 2560 × 1920; Nikon). FOSB-ir images were captured using an Olympus CX41 microscope (10× objective; Olympus Corporation). Damaged sections were excluded from analysis. Following image acquisition, a region of interest (ROI) was identified for each image, cropped, and converted into 8 bit using Fiji/ImageJ (Schindelin *et al.* 2012). ROI areas for TH-ir and FOSB-ir were all 44.94 mm².

For TH-ir quantification, ROIs were processed to produce a mean greyscale value for each image, ranging from 1 (black) to 256 (white). Thus, each ROI had one value (1–256) that denoted the mean greyscale of the pixels. To analyse difference in TH staining, mean greyscale values of the ROIs were compared across groups, similar to previous studies (Mosedale *et al.* 1996, Matkowskyj *et al.* 2000). We also validated this method by comparing it to our previous method, in which we quantified the area of the ROI with colorimetric signal above a threshold based on multiple background measurements (Heimovics *et al.* 2012, Tomm *et al.* 2018). For FOSB-ir quantification,

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number of FOSB-ir nuclei were counted in the region of interest using TMARKER (Wild *et al.* 2013), where the images underwent colour deconvolution and detection parameters were adjusted (tolerance = 5; blur = 1).

Data analyses

For GTT, body mass, and adipose depot mass, a repeated measure mixed model ANOVA was used to examine the fixed factor effects and two-factor interactions, with individual as a random factor. Only the main effect of diet is reported due to the absence of significant two-factor interactions (the interactions terms remained in the model). For analysis of food consumption, the weekly average of daily food consumed was computed for all pair-housed individuals and used to represent a single value for those two females (e.g. $n = 5$ values/group). For analysis of insulin, we were only able to collect enough plasma for measurements from five control-fed animals and eight sucrose-fed animals. Moreover, fasting glucose levels were only able to be measured in five control-fed animals and six sucrose-fed animals due to sample loss. Therefore, the quantitative insulin sensitivity check index (QUICKI) had sample sizes of five and six for control and sucrose-fed animals, respectively.

The mRNA levels in the gonadal adipose tissue were examined using a two-way ANOVA with diet and gene as the explanatory variables. Effects of diet were assessed using Sidak's multiple comparison test, and no further corrections for multiple statistical testing were performed. Adjusted *P*-values are reported.

For the qualitative analysis of hepatic histology, a χ^2 test of goodness-of-fit was performed to assess differences in frequency of steatosis and a two-sided Fisher's exact test to assess cell ballooning. Portal inflammation was not present in any hepatic samples and therefore was not analysed.

For steroid analysis, a value was considered non-detectable if it was below the lowest point on the calibration curve (i.e. the limit of quantification; LOQ). If more than 20% of the values were above the LOQ in both groups, then the missing values were estimated via quantile regression imputation of left-censored missing data (Wei *et al.* 2018). If less than 20% of the values were above the LOQ in either group, then inferential statistics were not performed and only descriptive statistics were reported. In order to compare serum and brain steroid concentrations, we estimated that 1 mL of serum weighs 1 g (Schmidt & Soma 2008, Taves *et al.* 2011). For each steroid, a linear mixed model was used to determine the effect of diet. Here, diet and brain region were the fixed factors, and animal ID was a random factor to control for pseudo-replication of steroid measurements within an animal. There were no statistical adjustments made for examining multiple steroids, because corticosterone and progesterone were the only steroids that were present in all tissues in both diets and thus the only steroids assessed using a full-mixed model that included all interaction terms.

For all other endpoints, a two-tailed independent *t*-test was used to determine differences between diets. For data that did not exhibit homoscedasticity, an unpaired *t*-test with Welch's correction was used (e.g. liver mass as a percent of body mass). For all analyses, α was set at ≤ 0.05. All graphs are presented using non-transformed data. All statistics were conducted using IBM SPSS Statistics for Windows version 23 (IBM Corp), GraphPad Prism version 6.00 (GraphPad Software), and R version 3.6.0 (2019-04-26; 'Planting a Tree') (https://www.r-project.org/) using the 'nlme' package (Pinheiro *et al.* 2019).

Results

Chronic sucrose consumption did not affect body mass, offspring sex ratio, offspring mass, or litter size

The amount of food (and calories) consumed did not significantly differ between diet groups over the first 10 weeks (diet × time: $F_{9,36} = 0.858$, $P = 0.569$; Fig. 2A). Moreover, body mass did not significantly differ between diet groups throughout the entire study ($F_{1,14} = 0.69$, $P = 0.421$; Fig. 2B). Diet did not significantly influence offspring sex ratio (Welch-corrected $t_{10.1} = 1.280$, $P = 0.229$), offspring mass at birth ($t_{14} = 1.832$, $P = 0.088$), litter size ($t_{14} = 0.831$, $P = 0.420$), or postnatal mortality (Welch-corrected $t_{9.52} = 1.377$, $P = 0.200$; Supplementary Table 1).

Chronic sucrose consumption impaired glucose tolerance

The sucrose diet significantly impaired the ability to regulate glucose at the post-weaning timepoint (effect of diet: $F_{1,14} = 4.63$, $P = 0.049$) but not at the pre-conception timepoint (effect of diet: $F_{1,14} = 2.062$, $P = 0.173$; Fig. 3). At the pre-conception timepoint, fasting insulin levels did not significantly differ between the two groups ($t_{11} = 0.58$, $P = 0.574$), and the QUICKI was not significantly different between the two groups ($t_9 = 1.319$, $P = 0.220$; Supplementary Table 2).

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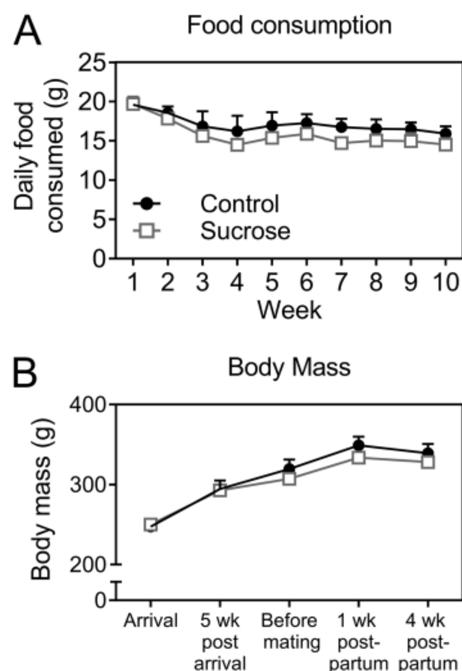

Figure 2
Food consumption and body mass. (A) The line graph represents food consumption as the daily amount (g) of food consumed from the start of their respective diet to mating (10 weeks). Females given the same diet were pair-housed during this period, so food consumption is an average of total amount of food consumed per week divided by two. (B) The line graph represents the body mass of each diet group throughout the study (arrival to killing) at each major point during the study. Data are presented as mean + s.e.m.

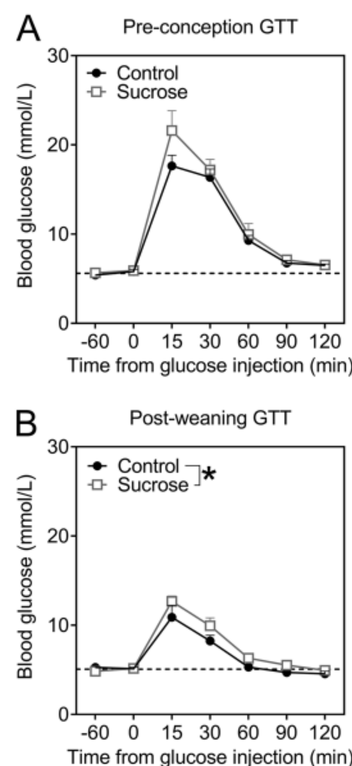

Figure 3
Sucrose consumption impairs glucose tolerance. (A and B) Line graphs representing blood glucose levels (mmol/L) during a glucose tolerance test (GTT) in control-fed and sucrose-fed females at (A) pre-conception and (B) post-weaning. Data are presented as mean + s.e.m. *$P<0.05$.

Chronic sucrose consumption increased liver lipids

The sucrose diet significantly increased relative liver mass (percentage of body mass; Welch-corrected $t_{8.89}=2.512$, $P=0.034$; Fig. 4A). In addition, sucrose-fed dams had significantly higher hepatic lipids (Welch-corrected $t_{11.49}=2.552$, $P=0.026$; Fig. 4B), hepatic steatosis (χ^2 (df=2, $n=16$)=9.808, $P=0.007$; Fig. 4C and D), and higher hepatic cell ballooning that approached significance (two-sided Fisher's exact test ($n=16$), $P=0.089$; Supplementary Table 3).

Chronic sucrose consumption affected visceral adipose depots

The relative mass of total visceral adipose (percentage of body mass) did not significantly differ between control and sucrose females ($t_{14}=1.685$, $P=0.114$). However, when analysed with a mixed model ANOVA, there was a significant diet × depot interaction ($F_{2,28}=9.062$, $P=0.0009$). Sidak's multiple comparison tests revealed that the perirenal/retroperitoneal adipose depot weighed significantly less in sucrose females ($P_{adj}=0.007$) (Fig. 5A).

In gonadal adipose tissue surrounding the uterine horns, we examined mRNA levels of the macrophage-specific *Emr1* and the proinflammatory cytokines *Tnf* (TNFα) and *Il1b* (IL-1β) via multiple *t*-tests with Holm–Sidak corrected *P*-values. Sucrose-fed dams had significantly higher levels of *Emr1* mRNA (t-ratio=2.635, df=42, $P_{adj}=0.012$) but not *Tnf* mRNA (t-ratio <0.01, df=42, $P_{adj}=0.999$) or *Il1b* mRNA (t-ratio=0.23, df=42, $P_{adj}>0.999$) (Fig. 5B).

Chronic sucrose consumption altered corticosterone and progesterone levels

Using ultra-sensitive and specific LC-MS/MS, we measured a panel of eight steroids in the serum and multiple microdissected brain regions. Sucrose intake had the greatest effects on corticosterone and progesterone (Fig. 6 and Tables 2, 3). There was a significant diet × tissue interaction for corticosterone ($F_{6,80}=2.89$; $P=0.013$), and post-hoc analyses revealed that the sucrose diet significantly decreased corticosterone levels in the serum but not in the brain (Fig. 6A). The sucrose diet did not

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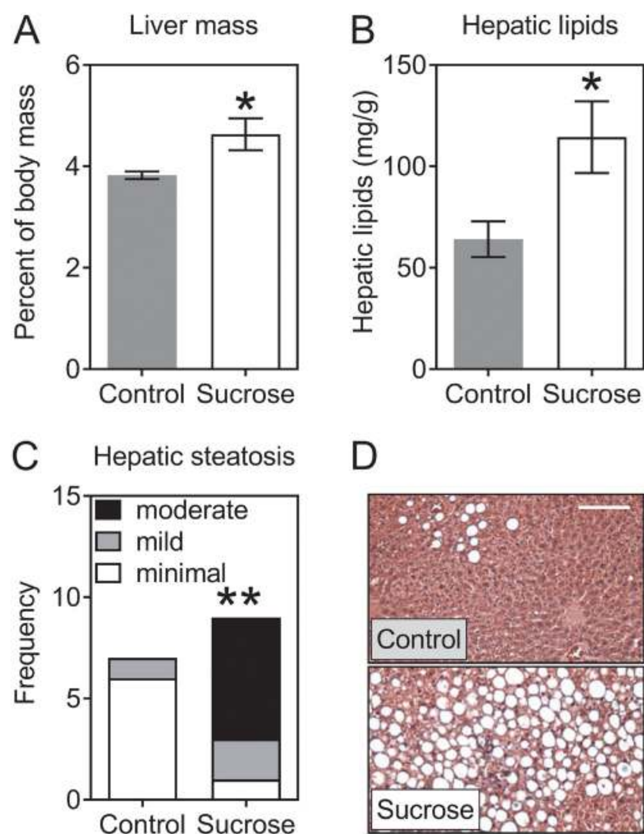

Figure 4
Sucrose consumption increases liver mass and liver lipids. (A) Bar graphs representing liver mass as percent of body mass. Dams fed with the sucrose diet had significantly higher relative liver mass than control dams. (B) Bar graph representing hepatic lipids (mg) per each g of tissue as measured by gravimetric quantification. (C) Stacked bar graph representing the frequency of minimal (score = 0 (<5%)), mild (score = 1 (5-33%)), or moderate (score = 2 (34-66%)) steatosis as determined by a pathologist blind to treatment using the NASH CRN scoring system. (D) Representative photomicrographs of H&E-stained liver sections. White, circular objects are abnormal hepatic lipid deposits, indicative of steatosis. Scale bar, 100 µm. (A and B) Data are presented as mean ± s.e.m. $^{**}P < 0.01$; $^{*}P < 0.05$.

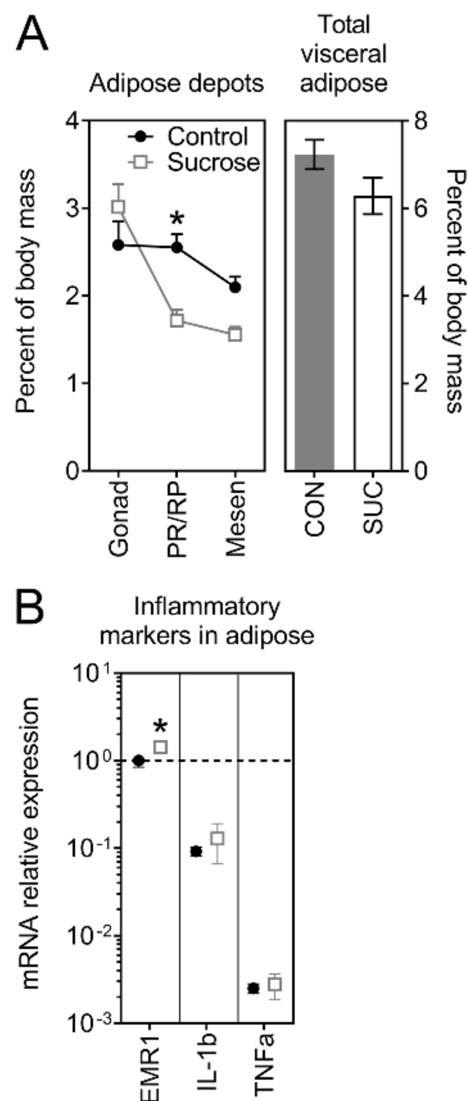

Figure 5
Sucrose consumption affects adipose depots. (A) Line graph represents relative mass of the gonadal adipose depot ('Gonad'), perirenal/retroperitoneal adipose depot ('PR/RP'), and mesenteric adipose depot ('Mesen'), while the adjacent bar graph represents the relative mass of total visceral adipose (the three depots combined). (B) Graphs represent the relative expression of EMR1, IL-1β, and TNFα transcripts in gonadal adipose tissue, as calculated by the $2^{-(\Delta\Delta Ct)}$ method using 18S rRNA as the reference gene. Note the log-scale on the y-axis. Data are presented as mean ± s.e.m. $^{*}P < 0.05$.

significantly affect the brain:serum ratio of corticosterone ($F_{1,59} = 1.16$; $P = 0.28$; Fig. 6C). In contrast, sucrose intake significantly increased progesterone levels in both serum and brain (main effect of diet: $F_{1,65} = 5.89$; $P = 0.046$; Fig. 6B) and increased the brain:serum ratio of progesterone (main effect of diet: $F_{1,39} = 10.75$; $P = 0.002$; Fig. 6D).

For androstenedione, pregnenolone, and testosterone (Tables 2 and 3), several interesting patterns emerged. First, androstenedione was detectable in the serum and all brain regions, except the VTA. Second, pregnenolone was detectable in all mPFC samples, but it was rarely detectable in serum or other brain regions. Third, testosterone was detectable in all serum samples, but it was rarely detectable in HPC and POA/HYP and was non-detectable in mPFC, NAc, and VTA. Finally, testosterone was detectable in sucrose-fed dams in the dHPC (33%) and vHPC (44%), but it was rarely detectable in control dams in both HPC subregions (14%). DHEA, DHT, and E_2 were not detectable in the serum or brain samples. Overall, there is some evidence of the possibility of an effect of chronic sucrose consumption on androstenedione, pregnenolone, and testosterone. However, we interpret these results cautiously due to

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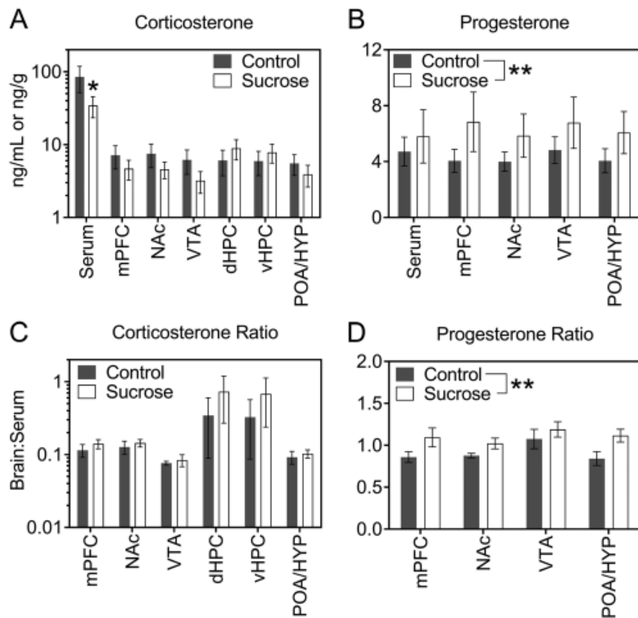

Figure 6
Sucrose consumption decreases serum corticosterone and increases serum and neural progesterone. (A and B). Graphs represent steroid concentrations of (A) corticosterone and (B) progesterone in the serum, mesocorticolimbic system, hippocampus (HPC), and preoptic area/hypothalamus (POA/HYP). (C and D) Graphs represent the ratio of brain-to-serum (brain:serum) (C) corticosterone and (D) progesterone. Note the log-scale on the y-axis in (A and C). Data are presented as mean ± S.E.M. **P* < 0.05, ***P* < 0.01. mPFC, medial prefrontal cortex; NAc, nucleus accumbens; dHPC, dorsal hippocampus; vHPC, ventral hippocampus; VTA, ventral tegmental area. Progesterone was not quantified in the HPC because of high matrix interference.

insufficient sample sizes to detect moderate differences in binary measures (i.e. presence/absence).

Chronic sucrose consumption decreased TH-ir and altered the pattern of FOSB-ir in the mesocorticolimbic system

The sucrose diet significantly reduced TH-ir in both subregions of the mPFC (effect of diet: $F_{1,28}=7.79$; $P=0.009$; Fig. 7). Furthermore, there was a trend for a reduction of TH-ir in both subregions of the NAc (effect of diet: $F_{1,28}=3.829$; $P=0.060$). There were no significant effects of diet on TH-ir in the VTA ($t_{15}=0.007$; $P=0.994$) or OFC ($F_{1,28}=1.751$; $P=0.196$). TH-ir was significantly higher in the medial OFC than the lateral OFC (effect of subregion: $F_{1,28}=20.90$; $P<0.0001$), regardless of diet.

For FOSB-ir, sucrose intake did not have a significant main effect in any of the brain regions (Fig. 8). Specifically, there was no effect of diet in the OFC (medial OFC (control: 92.93±19.19; Sucrose: 89.63±12.97); lateral OFC (control: 106.1±27.86; sucrose: 108.4±17.36);

Table 2 Serum and mesocorticolimbic steroid concentrations (ng/µL or ng/g) as analyzed by LC-MS/MS in control-fed (*n* = 7) and sucrose-fed (*n* = 9) rat dams.

	Control	Sucrose
Serum[a]		
Androstenedione	0.08 ± 0.04	0.11 ± 0.04
Corticosterone	85.0 ± 33.4	34.4 ± 11.1
Pregnenolone	n.d.	(11.9)
Progesterone	4.72 ± 1.03	5.81 ± 1.91
Testosterone	0.06 ± 0.01	0.07 ± 0.02
Medial prefrontal cortex		
Androstenedione	0.58 ± 0.35	n.d.
Corticosterone	7.15 ± 2.52	4.68 ± 1.43
Pregnenolone	47.6 ± 5.33	51.4 ± 5.41
Progesterone	4.05 ± 0.82	6.85 ± 2.13
Testosterone	n.d.	n.d.
Nucleus accumbens		
Androstenedione	0.27 ± 0.15	0.08 ± 0.04
Corticosterone	7.50 ± 2.68	4.58 ± 1.17
Pregnenolone	n.d.	(83.00)
Progesterone	4.00 ± 0.69	5.86 ± 1.55
Testosterone	n.d.	n.d.
Ventral tegmental area		
Androstenedione	n.d.	n.d.
Corticosterone	5.18 ± 2.28	3.20 ± 1.06
Pregnenolone	(47.9)	67.8 ± 20.9
Progesterone	4.82 ± 0.95	6.78 ± 1.83
Testosterone	n.d.	n.d.

Data are presented as mean ± S.E.M.
[a]Note that four serum samples were lost during processing (*n* = 6/group). Values in parentheses are shown when the group only had one detectable sample.
n.d., not detectable; 17β-estradiol, DHEA, and 5α-dihydrotestosterone were not detected in any of the samples.

effect of diet ($F_{1,13}<0.001$, $P=0.98$)), mPFC ($F_{1,14}=0.0163$, $P=0.90$), or NAc ($F_{1,14}=0.2273$, $P=0.64$). Note that one animal from the control group in the OFC ($n=6$) was missing completely at random due to experimenter error. Interestingly, there was a significant diet × subregion (core vs shell) interaction in the NAc ($F_{1,14}=7.607$, $P=0.015$). Control-fed dams had higher FOSB-ir in the core than the shell, while sucrose-fed dams exhibited the opposite pattern and had higher FOSB-ir in the shell than the core.

Discussion

Overall, chronic sucrose consumption had widespread and marked significant effects on metabolism, steroid levels in blood and brain, and markers of DA synthesis and signalling in the mesocorticolimbic system of parous female rats. We used a human-relevant level of sucrose intake (25% kcal) and compared it to an isocaloric and macronutrient-matched control diet over a long period (17–18 weeks). In contrast, many previous studies have

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Table 3 Hippocampal and preoptic area/hypothalamus steroid concentrations (ng/μL or ng/g) as analyzed by LC-MS/MS in control-fed (n = 7) and sucrose-fed (n = 9) rat dams.

	Control	Sucrose
Hippocampus – dorsal		
Androstenedione	0.11 ± 0.03	0.22 ± 0.06
Corticosterone	6.06 ± 2.32	8.91 ± 2.74
Pregnenolone	(19.1)	11.7 ± 2.39
Progesterone	6.17 ± 1.26	7.28 ± 2.11
Testosterone	(0.07)	0.13 ± 0.03
Hippocampus – ventral		
Androstenedione	0.07 ± 0.03	0.18 ± 0.08
Corticosterone	5.93 ± 2.17	7.82 ± 2.27
Pregnenolone	(14.8)	n.d.
Progesterone	6.99 ± 1.31	8.42 ± 2.45
Testosterone	(0.08)	2.79 ± 2.64
Preoptic area/ hypothalamus		
Androstenedione	0.22 ± 0.13	0.13 ± 0.05
Corticosterone	5.54 ± 1.75	3.91 ± 1.30
Pregnenolone	n.d.	24.9 ± 3.56
Progesterone	4.06 ± 0.87	6.07 ± 1.51
Testosterone	0.22 ± 0.10	n.d.

Data are presented as mean ± s.e.m. Values in parentheses are shown when the group only had one detectable sample.
n.d., not detectable; 17β-estradiol, DHEA, and 5α-dihydrotestosterone were not detected in any of the samples.

used very high levels of sucrose or fructose (>50% kcal) for short periods (1–2 weeks) (Bursać *et al.* 2014, Kjaergaard *et al.* 2014, Vasiljević *et al.* 2014, Lesser *et al.* 2017). We gave the sucrose in the diet instead of in the water, because animals with sucrose or fructose in the water eat less and thus consume less protein, fat, vitamins, and minerals (Ohashi *et al.* 2015). The present approach avoids group differences in the consumption of protein, fat, vitamins, and minerals. Here, there were also no significant group differences in food (energy) intake, body mass, or total visceral adipose, and thus the effects of sucrose seen here are independent of caloric consumption and obesity. In this study, we examined the effects of sucrose on parous females only, and future work will examine nulliparous females as well as males.

Little is known about how chronic sucrose intake influences steroids and neurotransmitters. Here, sucrose significantly decreases corticosterone levels in serum, but increases progesterone levels in serum and brain and increases the brain:serum progesterone ratio in all regions. To our knowledge, this is the first time that steroid profiling has been performed in microdissected regions of the female rodent brain. Sucrose consumption significantly decreases TH-ir in the mPFC, with a similar trend in the NAc, suggesting a decrease in DA synthesis in the mesocorticolimbic system, which is critical for reward seeking and decision making. Lastly, sucrose consumption significantly alters the pattern of FOSB-ir within the NAc. Overall, these results show that chronic intake of a human-relevant level of dietary sucrose has wide-ranging effects on physiology, including the endocrine and nervous systems.

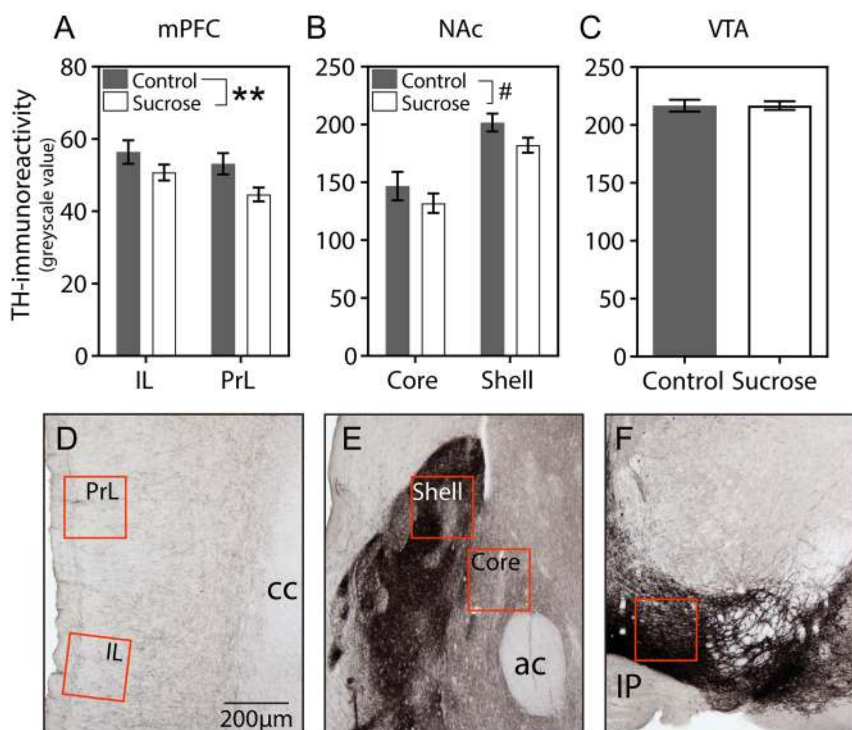

Figure 7
Sucrose consumption decreases tyrosine hydroxylase-immunoreactivity (TH-ir) in the mesocorticolimbic system. (A, B, and C) Bar graphs represent overall photomicrograph pixel intensity that corresponds to TH-ir in the (A and D) infralimbic (IL) and prelimbic (PrL) medial prefrontal cortex (mPFC), (B and E) the core and shell of the nucleus accumbens (NAc), and (C and F) the ventral tegmental area (VTA) in control- and sucrose-fed subjects. (D, E, and F) Representative photomicrographs of the mesocorticolimbic nodes with regions of interest outlined in red, used in the quantification of (D) mPFC, (E) NAc, and (F) the VTA. Data are presented as mean ± s.e.m. **Significant effect of diet (P < 0.01). #Effect of diet (P = 0.06). cc, corpus callosum; ac, anterior commissure; IP, interpeduncular nucleus.

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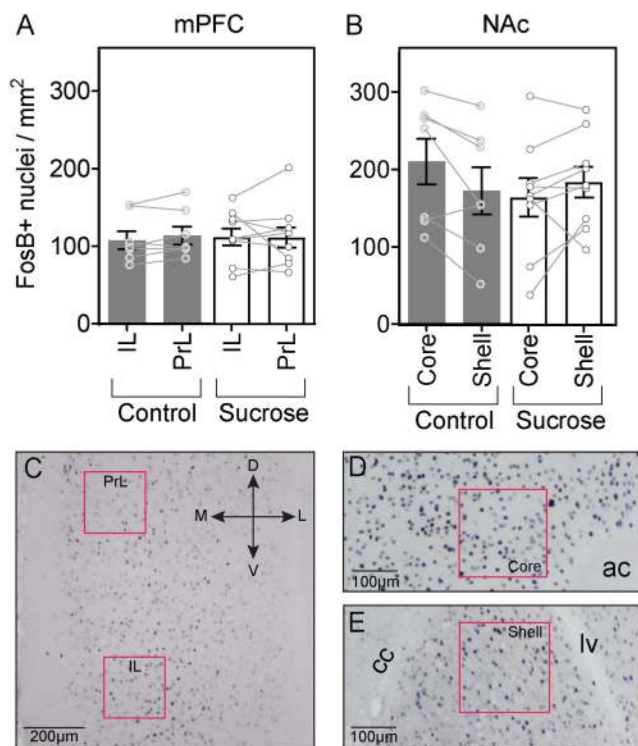

Figure 8
Sucrose consumption alters the pattern of FOSB-immunoreactivity in the nucleus accumbens. Bar graphs represent FOSB-ir nuclei in the (A) infralimbic (IL) and prelimbic (PrL) medial prefrontal cortex (mPFC), (B) core and shell of the nucleus accumbens (NAc) in control- and sucrose-fed subjects. (C and D) Representative photomicrographs of the mesocorticolimbic nodes with regions of interest outlined in red, used in the quantification of (C) mPFC and (D) NAc. Note that FOSB-ir in the VTA was sparse and thus not quantified. Data are presented as mean ± s.e.m.

Effects of sucrose consumption on metabolism

The sucrose diet significantly reduced the ability to regulate blood glucose at the post-weaning timepoint (after 17–18 weeks) but not at the pre-conception timepoint (after 10 weeks). These data are consistent with previous studies showing that high sucrose or fructose consumption impairs glucose tolerance (Asghar *et al.* 2016, Saben *et al.* 2016). In the present study, the difference between the two timepoints might be attributable to the different durations of sucrose exposure or perhaps to endocrine differences in the dams. Interestingly, the sucrose-fed dams did not significantly gain more weight than control dams and did not show significant evidence of fasting hyperinsulinemia or hyperglycaemia, similar to previous studies (Lomba *et al.* 2009, Oliveira *et al.* 2014).

Hepatic lipids were significantly increased in sucrose-fed dams. This is consistent with previous studies (Oliveira *et al.* 2014). Sucrose consumption significantly increased hepatic lipids, steatosis, relative liver mass, triglycerides, and showed a trend to increase hepatocellular ballooning. These data provide further evidence that a human-relevant level of sucrose consumption can contribute to the development of non-alcoholic steatohepatitis (see Michelotti *et al.* 2013 for review).

We also examined visceral fat mass relative to body size. Total visceral fat mass relative to body size did not significantly differ between the control- and sucrose-fed groups. These findings differ from some previous studies in rodents (Lomba *et al.* 2009). In rodents, sucrose consumption does not increase visceral adiposity in all studies. For example, Oliveira *et al.* (2014) reported that in male mice fed a high-sucrose diet, there is no effect on visceral adiposity. Many variables (e.g. sex, parity, species, dietary fat, sucrose content, duration, etc.) might affect how sucrose impacts visceral adiposity. Pregnancy and lactation affect visceral adipose distribution (Steingrimsdottir *et al.* 1980), and in this study, diet and pregnancy might have interacted to change the distribution of visceral adipose. Nonetheless, total visceral adiposity did not significantly differ between the groups. This is important because an increase in adiposity can have effects on metabolism, steroids, and the brain (Bray 1997, Bruce-Keller *et al.* 2009). With this model, we can examine how sucrose intake, independent of caloric intake and total visceral adiposity, affects metabolism, steroids, and DA signalling.

Sucrose-fed dams showed some evidence of inflammation in the gonadal adipose tissue. Sucrose-fed dams had a significant increase in the macrophage-specific transcript *Emr1* in gonadal adipose. However, transcripts for two proinflammatory cytokines released by macrophages, TNF-α and IL-1β, did not significantly differ between groups. These data suggest that sucrose consumption increases macrophage number or activity in the adipose tissue surrounding the uterine horns. Overall, these results provide partial evidence that sucrose induces latent inflammation in adipose tissue (Stafeev *et al.* 2017).

Effects of sucrose consumption on systemic and neural steroid levels

Pregnancy and lactation are marked by profound changes in steroids, including glucocorticoids, progestins, and oestrogens (Kovacs 2016). Here, sucrose consumption during pregnancy and lactation significantly alters both corticosterone and progesterone levels. Notably, we used state-of-the-art LC-MS/MS to measure a panel of steroids with great specificity and sensitivity in blood and microdissected brain regions.

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Chronic sucrose consumption significantly reduces baseline levels of serum corticosterone. This effect might be a regulatory mechanism to prevent even higher levels of circulating glucose resulting from corticosterone-induced glycogenolysis or gluconeogenesis. In contrast, some rodent studies have reported that sucrose or fructose increases circulating corticosterone (Hart *et al.* 1980, Gaysinskaya *et al.* 2011, Choi *et al.* 2017). However, these studies examined the effects of acute or short-term sucrose consumption and all focused on male rodents. A longer-term study by Corona-Pérez *et al.* (2017) used 8 weeks of sucrose exposure in male rats and found that sucrose lowered corticosterone levels. The long-term exposure and/or female subjects used here might explain the differences across studies. Moreover, sucrose consumption tended to decrease corticosterone levels in the brain, except the HPC. Interestingly, consumption of highly palatable foods, which includes sucrose, reduces the corticosterone response in stressed animals, thereby reducing stress-induced behaviours (Pecoraro *et al.* 2004).

The sucrose-induced decrease in circulating corticosterone may also partly explain the metabolic and immune effects observed. First, corticosterone increases circulating glucose levels (McMahon *et al.* 1988), yet we report significantly lower serum corticosterone and higher blood glucose in sucrose dams during the post-weaning GTT. Contrary to our findings, an acute increase in corticosterone improves glucose regulation during a GTT (Tirosh *et al.* 2010). However, many previous studies used acute corticosterone manipulations and do not give much insight into how basal corticosterone levels after chronic sucrose consumption may influence glucose regulation (Rafacho *et al.* 2014). Second, the sucrose-induced decrease in corticosterone might explain the increase in the macrophage-specific marker (*Emr1*) in the gonadal adipose tissue. Chronic increases in corticosterone are immunosuppressive, whereas lower levels of corticosterone can promote immune system reactivity (Taves *et al.* 2017). Thus, lower levels of corticosterone in the sucrose-fed dams may lead to increased macrophages in adipose.

In contrast to corticosterone, progesterone is significantly increased in the serum and brain of sucrose-fed dams. Progesterone can increase hepatic lipogenesis and triglycerides (Dahm *et al.* 1977). Thus, the increase in hepatic steatosis in sucrose-fed dams may be due, in part, to an increase in progesterone along with a decrease in corticosterone. The brain-to-serum progesterone ratio is also elevated in sucrose-fed dams. This suggests that sucrose intake increases neural progesterone synthesis or accumulation. Increased progesterone synthesis is likely for several reasons. First, regions with elevated progesterone ratio express the enzymes for progesterone synthesis (Kimoto *et al.* 2010, Tobiansky *et al.* 2018*b*). Second, brain slice cultures actively synthesize progesterone *de novo* (Hojo *et al.* 2009). Third, pregnenolone, the precursor to progesterone, was detectable in the mPFC of nearly all rat dams. Of the brain regions examined, the mPFC had one of the highest group differences in progesterone ratio. The high levels of pregnenolone suggest that the increase in progesterone ratio is due to synthesis. Taken together, these results suggest that sucrose consumption alters progesterone levels in regions that regulate reward seeking and motivation (Ducharme *et al.* 2010).

Finally, androstenedione and testosterone were not significantly affected by sucrose intake. However, future studies should have larger sample sizes to assess effects of sucrose intake on androstenedione and testosterone, due to low circulating levels of these steroids in females. DHEA, DHT, and E_2 were non-detectable in all samples. Circulating E_2 in female rats are quite low during lactation (Kovacs 2016). We are currently developing more sensitive LC-MS/MS methods for DHEA and E_2 measurement.

Sucrose alters DA signalling in the mesocorticolimbic system

Surprisingly, there is little known about how chronic sucrose intake affects DA signalling. Here, chronic sucrose consumption significantly decreased TH, the rate-limiting enzyme for DA synthesis, in the mPFC and there was a trend towards a decrease in the NAc. Similarly, long-term access to sucrose in the drinking water (10%) decreases TH-ir in the striatum (Ahmed *et al.* 2014). One way in which chronic sucrose consumption can decrease TH in the striatum and mPFC is via an increase in progesterone (as seen here); progesterone decreases TH mRNA in the brain (Arbogast & Voogt 1994). Lower TH in the NAc and mPFC of sucrose-fed dams can lead to important behavioural differences. DA signalling in the mPFC is particularly sensitive to sucrose and fat consumption. Prefrontal DA signalling is dysregulated by intermittent access to sucrose, causing deficits in instrumental learning, which are rescued by administration of a DA D2 receptor (D_2R) antagonist (Sharpe *et al.* 2016). Decreases in DA synthesis in the rat mPFC and NAc impact goal-directed behaviours (Cacciapaglia *et al.* 2011) and maternal behaviours (Keer & Stern 1999). To our knowledge, no studies have examined the possible effects of sucrose consumption on maternal behaviour, and this is an important question for future studies to address.

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Sucrose consumption might also impact DA signalling in the NAc, as suggested by the FOSB-ir results. Controls had significantly higher FOSB-ir in the NAc core than shell, whereas sucrose-fed dams had significantly higher FOSB-ir in the NAc shell than core. This is important because sustained changes in phasic DA signalling in the NAc due to repeated exposure to rewarding stimuli (e.g. sugar) upregulates the stable form of the transcription factor FOSB, ΔFOSB, which is implicated in addictive-like behaviours (e.g. binge eating (Robison & Nestler 2011)). Therefore, these data suggest that there is higher phasic DA release in the NAc shell (vs core) in sucrose-fed individuals. Indeed, daily binging on sucrose increases DA release in the NAc shell (Rada *et al.* 2005). Daily access to sucrose also increases D_1R and decreases D_2R in the NAc (Avena *et al.* 2008). These changes in DA signalling are associated with alterations in DA-dependent behaviours (Arbogast & Voogt 2002, Kendig *et al.* 2014).

Conclusions

The results from this study are particularly compelling because we used a human-relevant level of sucrose intake over a long period of time and we examined multiple physiological systems. Moreover, these results provide insight into the effects of sucrose on female physiology, which is lacking in the literature. Overall, the data are consistent with previous metabolic studies showing that sucrose consumption significantly impairs glucose tolerance, increases liver lipids, and increases adipose inflammation. Further, our steroid analyses via LC-MS/MS reveal that chronic sucrose intake significantly decreases circulating corticosterone levels. On the other hand, progesterone and the progesterone ratio are significantly increased by the sucrose diet, suggesting a change in brain steroidogenesis. Finally, sucrose intake significantly reduces TH-ir and alters FOSB-ir in important mesocorticolimbic nodes associated with reward seeking and motivated behaviour. Overall, these results show that a human-relevant level of dietary sucrose has wide-ranging effects on the whole body, including the endocrine and nervous systems.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/JOE-19-0386.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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