

Supplementary Material

**Maternal sucrose consumption
alters steroid levels in the mother, placenta, and fetus**

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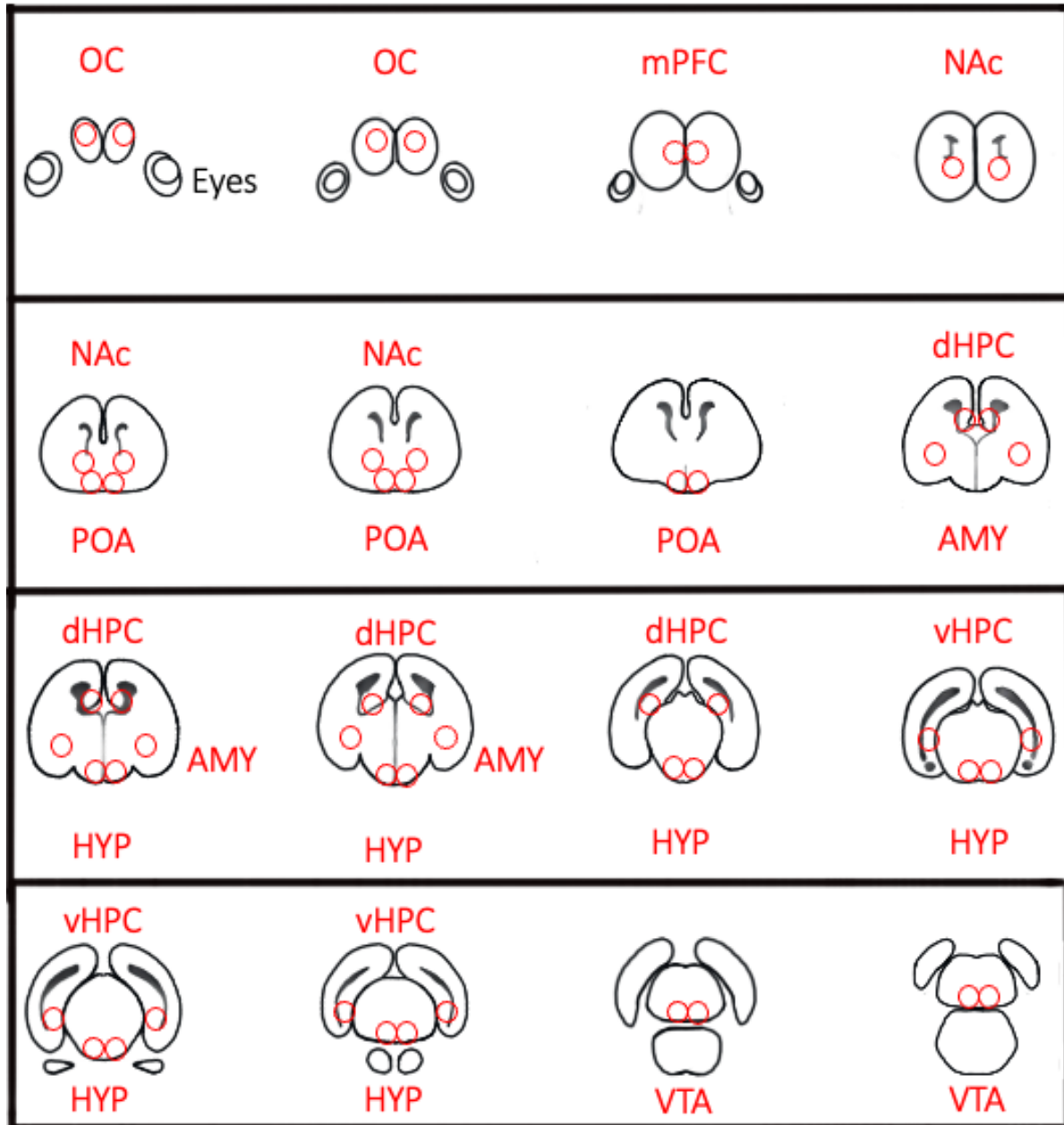


Figure S1. Schematic illustrating rostral to caudal locations for microdissection performed using 1 mm diameter Palkovits punch for LC-MS/MS steroid analysis. Section thickness was 300 μ m. OC, orbital cortex; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; POA, preoptic area; AMY, amygdala; dHPC, dorsal hippocampus; vHPC, ventral hippocampus; HYP, hypothalamus; VTA, ventral tegmental area.

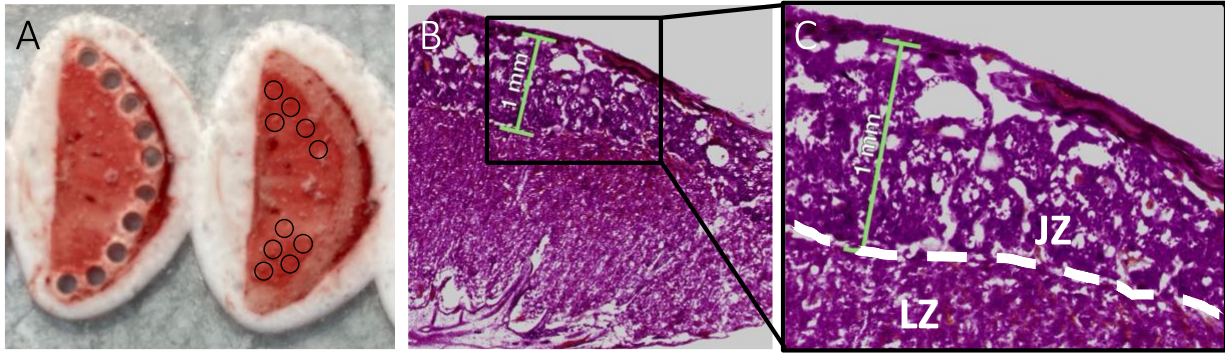


Figure S2. Punch locations for placenta. A, Representative 300µm-thick frozen placenta sections. In the section on the left, 1mm-diameter punches of the junctional zone have been collected. In the section on the right, black circles indicate punch locations for the labyrinth zone. B and C, 30µm-thick H&E stained placenta section, highlighting the junctional zone that is 1mm in height. LZ, labyrinth zone; JZ, junctional zone.

Table S1. Steroids (ng/g) in maternal serum that were not significantly affected by HSD

	CON	HSD
<i>Maternal serum</i>		
Pregnenolone	4.31 ± 0.62	5.89 ± 1.73
Progesterone	90.97 ± 9.30	102.03 ± 3.89
Allopregnanolone	11.48 ± 1.47	13.82 ± 1.06
Androstenedione	0.76 ± 0.09	0.77 ± 0.05
Estrone	0.02 ± 0.004	0.03 ± 0.005
17β-estradiol	nd	nd
Estriol	nd	nd

Data are shown as mean ± s.e.m. n=10-12. nd, non-detectable. All p-values > 0.05.

Table S2. Steroids (ng/g) in placenta that were not significantly affected by maternal HSD

	CON Female	HSD Female	CON Male	HSD Male
<i>Pregnenolone</i>				
Junctional zone	31.27 ± 8.75	29.55 ± 4.32	31.35 ± 5.51	23.15 ± 3.21
Labyrinth zone	24.22 ± 3.36	22.00 ± 3.27	23.11 ± 0.92	21.88 ± 2.19
<i>Progesterone</i>				
Junctional zone	29.43 ± 4.26	21.74 ± 3.06	25.58 ± 3.37	20.71 ± 1.75
Labyrinth zone	29.48 ± 4.24	22.63 ± 4.61	29.73 ± 4.40	27.81 ± 3.50
<i>Allopregnanolone</i>				
Junctional zone	65.61 ± 10.47	82.23 ± 15.43	88.67 ± 12.79	79.39 ± 12.16
Labyrinth zone	76.74 ± 15.34	89.27 ± 16.39	118.54 ± 19.27	74.76 ± 16.10
<i>Corticosterone</i>				
Junctional zone	36.02 ± 4.83	31.51 ± 4.68	44.34 ± 10.28	31.60 ± 6.79
Labyrinth zone	54.56 ± 6.86	53.58 ± 9.64	80.05 ± 18.34	52.06 ± 7.88
<i>11-Dehydrocorticosterone</i>				
Junctional zone	63.14 ± 5.66	75.95 ± 10.51	68.06 ± 12.02	72.19 ± 11.32
Labyrinth zone	102.91 ± 12.40	105.61 ± 15.19	114.18 ± 16.73	121.00 ± 13.47
<i>Estrone</i>				
Junctional zone	0.10 ± 0.04	0.09 ± 0.02	0.06 ± 0.03	0.10 ± 0.03
Labyrinth zone	0.30 ± 0.06	0.35 ± 0.07	0.37 ± 0.06	0.39 ± 0.06

Data are shown as mean ± s.e.m. n=6-10. Progesterone showed a trend to be reduced by maternal HSD in the junctional zone (p=0.07), shown in **bold**. All p-values > 0.05. No significant effects of sex. 17β-estradiol and estriol were non-detectable.

Table S3. Steroids (ng/g) in fetal blood and amniotic fluid that were not significantly affected by maternal HSD

	CON Female	HSD Female	CON Male	HSD Male
<i>Fetal blood</i>				
Pregnenolone	5.20 ± 1.17	4.40 ± 1.25	4.28 ± 0.93	4.32 ± 0.85
Progesterone	9.60 ± 0.93	8.17 ± 0.74	7.92 ± 0.62	8.99 ± 0.80
Allopregnanolone	15.11 ± 3.37	18.85 ± 1.90	16.82 ± 2.48	15.25 ± 1.43
Corticosterone	192.65 ± 12.89	193.72 ± 9.31	177.42 ± 15.51	171.78 ± 16.00
Estrone	2.18 ± 0.27	2.53 ± 0.15	2.21 ± 0.17	2.48 ± 0.23
17β-estradiol	nd	nd	nd	nd
Estriol	nd	nd	nd	nd
<i>Amniotic fluid</i>				
Pregnenolone	0.50 ± 0.12	0.67 ± 0.16	0.83 ± 0.19	0.50 ± 0.07
Progesterone	0.93 ± 0.08	0.92 ± 0.14	1.14 ± 0.14	1.11 ± 0.18
Allopregnanolone	2.74 ± 0.22	2.94 ± 0.46	3.79 ± 0.80	3.12 ± 0.28
Corticosterone	49.86 ± 4.13	55.86 ± 7.96	60.10 ± 5.46	57.58 ± 9.11
Estrone	0.59 ± 0.05	0.68 ± 0.04	0.72 ± 0.06	0.73 ± 0.05
17β-estradiol	nd	nd	nd	nd
Estriol	nd	nd	nd	nd

Data are shown as mean ± s.e.m. n=9-11. nd, non-detectable. All p-values > 0.05. No significant effects of sex.

Table S4. Steroids (ng/g) in fetal brain regions

	CON Female	HSD Female	CON Male	HSD Male
<i>Pregnenolone</i>				
OC	13.60 ± 0.74	12.49 ± 0.94	12.24 ± 0.71	15.04 ± 1.56
mPFC	12.83 ± 1.38	14.60 ± 3.25	13.57 ± 1.46	15.30 ± 2.17
NAc	13.53 ± 0.84	13.61 ± 1.63	13.45 ± 1.01	15.07 ± 1.40
AMY	13.90 ± 1.26	12.58 ± 0.99	14.72 ± 1.60	15.72 ± 2.04
POA	13.96 ± 0.98	13.21 ± 0.76	13.10 ± 0.94	14.24 ± 1.37
dHPC	13.91 ± 1.00	15.60 ± 1.25	14.28 ± 1.25	15.85 ± 1.35
vHPC	16.10 ± 1.67	16.26 ± 1.28	14.75 ± 0.96	16.75 ± 1.92
HYP	18.68 ± 1.72	16.41 ± 1.06	16.34 ± 1.08	20.32 ± 1.93
VTA	21.71 ± 1.76	20.93 ± 1.30	20.87 ± 1.23	23.49 ± 2.58
<i>Progesterone</i>				
OC	27.98 ± 2.20	23.92 ± 4.58	24.51 ± 3.90	25.11 ± 2.87
mPFC	25.91 ± 2.68	25.93 ± 5.35	25.85 ± 2.59	26.10 ± 3.62
NAc	26.85 ± 2.42	30.29 ± 7.58	26.76 ± 3.88	27.47 ± 2.71
AMY	26.12 ± 1.69	23.72 ± 3.86	26.44 ± 4.60	24.67 ± 3.00
POA	26.59 ± 2.50	25.97 ± 2.93	26.80 ± 3.87	25.18 ± 2.79
dHPC	28.06 ± 1.98	29.22 ± 5.88	28.15 ± 4.71	24.57 ± 2.31
vHPC	27.04 ± 2.38	30.02 ± 5.60	26.98 ± 4.17	25.28 ± 2.48
HYP	25.68 ± 2.55	26.97 ± 6.08	26.29 ± 5.00	25.43 ± 3.25
VTA	27.51 ± 2.96	25.80 ± 4.43	28.28 ± 5.34	25.03 ± 3.98
<i>Allopregnanolone</i>				
OC	64.10 ± 16.41	75.68 ± 29.68	56.26 ± 6.77	50.05 ± 6.60
mPFC	67.43 ± 13.37	81.46 ± 20.46	81.53 ± 13.99	54.39 ± 10.46
NAc	51.06 ± 11.82	64.16 ± 16.28	56.44 ± 6.70	45.44 ± 5.40
AMY	45.68 ± 9.69	59.12 ± 18.36	52.88 ± 8.48	42.08 ± 5.18
POA	50.33 ± 8.27	67.91 ± 15.75	62.25 ± 7.82	47.51 ± 7.20
dHPC	40.55 ± 5.40	50.63 ± 10.52	48.64 ± 6.98	36.39 ± 4.06
vHPC	45.73 ± 7.50	56.88 ± 15.12	56.54 ± 9.71	43.88 ± 4.49
HYP	41.38 ± 4.05	49.53 ± 6.23	50.82 ± 6.84	44.37 ± 3.51
VTA	54.78 ± 8.54	71.65 ± 15.60	67.85 ± 7.39	60.15 ± 6.05

	CON Female	HSD Female	CON Male	HSD Male
<i>Corticosterone</i>				
OC	30.51 ± 1.71	22.98 ± 2.72	29.20 ± 5.39	24.41 ± 2.05
mPFC	29.81 ± 3.01	30.52 ± 6.86	29.65 ± 4.05	22.57 ± 3.70
NAc	18.37 ± 1.28	17.07 ± 2.88	19.99 ± 2.95	15.58 ± 1.89
AMY	24.41 ± 2.12	19.50 ± 2.47	21.95 ± 3.01	19.73 ± 2.41
POA	22.35 ± 2.19	20.64 ± 4.30	27.44 ± 3.92	16.51 ± 2.63
dHPC	39.13 ± 3.31	35.67 ± 4.65	42.92 ± 4.90	34.05 ± 3.97
vHPC	27.61 ± 2.89	29.47 ± 4.00	30.60 ± 4.32	27.45 ± 2.30
HYP	32.83 ± 2.59	28.20 ± 3.55	30.83 ± 5.04	24.67 ± 3.48
VTA	27.48 ± 2.65	28.24 ± 4.00	37.43 ± 6.12	26.40 ± 2.95
<i>11-Dehydrocorticosterone</i>				
OC	25.67 ± 2.13	21.37 ± 2.55	21.75 ± 2.13	25.47 ± 1.97
mPFC	28.21 ± 2.63	30.97 ± 5.92	28.08 ± 2.30	27.90 ± 2.85
NAc	32.41 ± 3.17	30.55 ± 2.35	27.20 ± 2.70	32.42 ± 3.23
AMY	30.44 ± 2.96	29.18 ± 3.72	24.82 ± 1.85	29.99 ± 2.96
POA	38.42 ± 4.61	37.26 ± 4.89	35.69 ± 3.72	35.51 ± 3.73
dHPC	31.51 ± 3.08	33.51 ± 3.18	32.02 ± 3.15	31.37 ± 2.91
vHPC	26.82 ± 3.00	37.84 ± 5.11	28.62 ± 1.59	31.11 ± 2.15
HYP	31.42 ± 3.68	33.06 ± 4.17	28.02 ± 2.70	31.32 ± 3.14
VTA	29.23 ± 2.55	33.61 ± 4.24	29.06 ± 2.82	32.07 ± 3.75
<i>Aldosterone</i>				
OC	0.07 ± 0.01	0.12 ± 0.05	0.06 ± 0.01	0.21 ± 0.08
mPFC	0.06 ± 0.003	0.2 ± 0.04	0.06 ± 0.01	0.12 ± 0.03
NAc	0.08 ± 0.01	0.21 ± 0.08	0.09 ± 0.02	0.21 ± 0.08
AMY	0.08 ± 0.02	0.19 ± 0.05	0.12 ± 0.01	0.21 ± 0.09
POA	0.08 ± 0.01	0.17 ± 0.06	0.08 ± 0.01	0.23 ± 0.11
dHPC	0.11 ± 0.03	0.24 ± 0.09	0.14 ± 0.03	0.28 ± 0.11
vHPC	0.09 ± 0.02	0.25 ± 0.10	0.12 ± 0.03	0.26 ± 0.10
HYP	0.12 ± 0.02	0.22 ± 0.08	0.13 ± 0.02	0.24 ± 0.09
VTA	0.09 ± 0.02	0.24 ± 0.10	0.12 ± 0.03	0.29 ± 0.11

	CON Female	HSD Female	CON Male	HSD Male
<i>Androstenedione</i>				
OC	1.29 ± 0.21	0.96 ± 0.17	1.83 ± 0.29	1.96 ± 0.16
mPFC	1.40 ± 0.32	0.97 ± 0.12	2.22 ± 0.37	2.20 ± 0.19
NAc	1.21 ± 0.19	0.92 ± 0.16	1.88 ± 0.34	1.95 ± 0.16
AMY	0.89 ± 0.14	0.72 ± 0.13	1.38 ± 0.32	1.60 ± 0.19
POA	1.01 ± 0.21	0.72 ± 0.15	1.50 ± 0.22	1.35 ± 0.19
dHPC	1.20 ± 0.14	0.88 ± 0.13	1.85 ± 0.28	1.80 ± 0.18
vHPC	1.27 ± 0.20	1.02 ± 0.13	2.04 ± 0.31	1.99 ± 0.18
HYP	1.21 ± 0.23	0.80 ± 0.12	1.64 ± 0.21	1.89 ± 0.20
VTA	1.41 ± 0.25	1.04 ± 0.11	2.05 ± 0.32	2.27 ± 0.24
<i>Testosterone</i>				
OC	0.06 ± 0.01	0.04 ± 0.01	1.2 ± 0.32	0.97 ± 0.11
mPFC	0.05 ± 0.003	0.05 ± 0.002	1.2 ± 0.23	1.0 ± 0.15
NAc	0.08 ± 0.02	0.03 ± 0.01	1.2 ± 0.28	1.0 ± 0.10
AMY	0.05 ± 0.01	0.03 ± 0.01	1.1 ± 0.35	0.92 ± 0.11
POA	0.04 ± 0.01	0.03 ± 0.01	1.3 ± 0.28	1.0 ± 0.13
dHPC	0.06 ± 0.01	0.04 ± 0.01	1.3 ± 0.37	1.0 ± 0.10
vHPC	0.05 ± 0.01	0.05 ± 0.01	1.3 ± 0.36	1.1 ± 1.10
HYP	0.06 ± 0.01	0.04 ± 0.01	1.3 ± 0.30	1.1 ± 0.13
VTA	0.05 ± 0.01	0.03 ± 0.01	1.4 ± 0.39	1.3 ± 0.18
<i>Estrone</i>				
OC	0.27 ± 0.03	0.27 ± 0.03	0.27 ± 0.06	0.30 ± 0.03
mPFC	0.27 ± 0.04	0.27 ± 0.06	0.29 ± 0.09	0.29 ± 0.03
NAc	0.35 ± 0.04	0.31 ± 0.03	0.33 ± 0.08	0.37 ± 0.04
AMY	0.60 ± 0.06	0.55 ± 0.09	0.60 ± 0.08	0.72 ± 0.10
POA	0.89 ± 0.11	0.84 ± 0.09	1.12 ± 0.08	1.27 ± 0.10
dHPC	0.43 ± 0.04	0.42 ± 0.06	0.47 ± 0.05	0.47 ± 0.04
vHPC	0.28 ± 0.03	0.36 ± 0.04	0.34 ± 0.04	0.37 ± 0.04
HYP	0.55 ± 0.07	0.52 ± 0.07	0.52 ± 0.03	0.64 ± 0.04
VTA	0.25 ± 0.03	0.29 ± 0.04	0.37 ± 0.03	0.36 ± 0.04

Data are shown as mean ± s.e.m. n=6-10. For 17 β -estradiol and estriol, less than 20% of samples had detectable levels. Two-way ANOVA was used for statistical analysis to examine the effects of diet and sex on steroids in different brain regions. No diet x sex interactions were observed. Androstenedione and testosterone were significantly higher in male fetuses compared to female fetuses across all brain regions. Main effect of diet: data shown in **bold**, p \leq 0.05. OC, orbital cortex; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; AMY, amygdala; POA, preoptic area; dHPC, dorsal hippocampus; vHPC, ventral hippocampus; HYP, hypothalamus; VTA, ventral tegmental area; CON, control; HSD, high-sucrose diet.