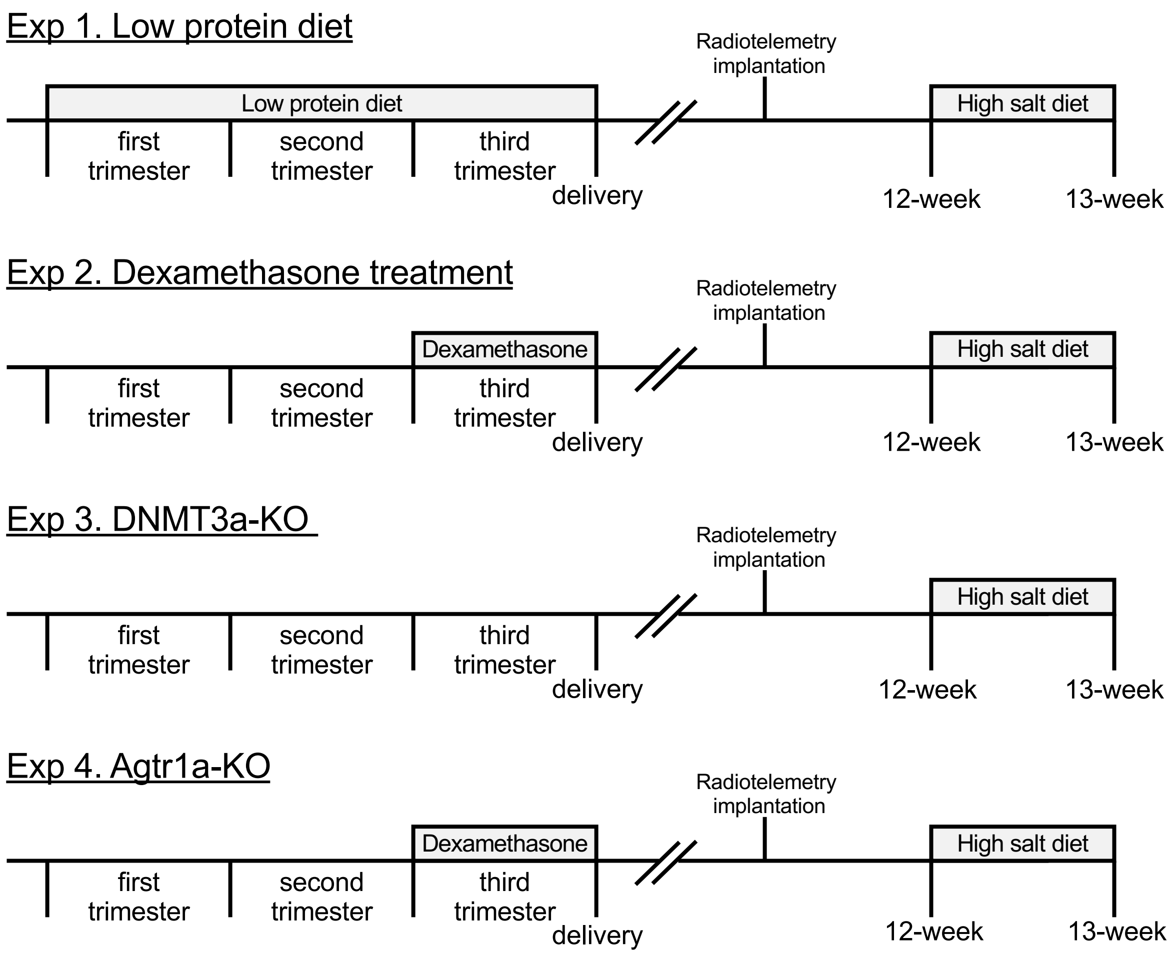
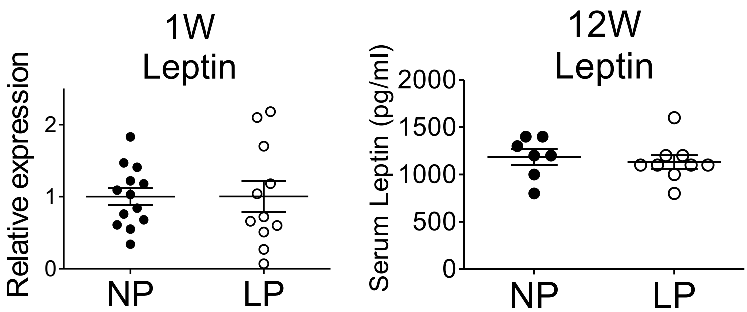
Supplemental Fig. 1



Supplemental Fig. 1

Experimental design.

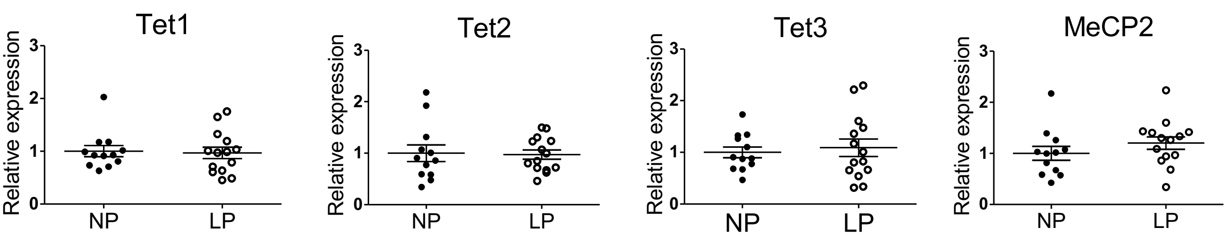
Supplemental Fig. 2



Supplemental Fig. 2

Lep mRNA expression in visceral adipose tissue at week 1 (left panel, NP, n=13; LP, n=11) and serum leptin concentration at week 12 (right panel, NP, n=7; LP, n=9) in NP- or LP-treated offspring.

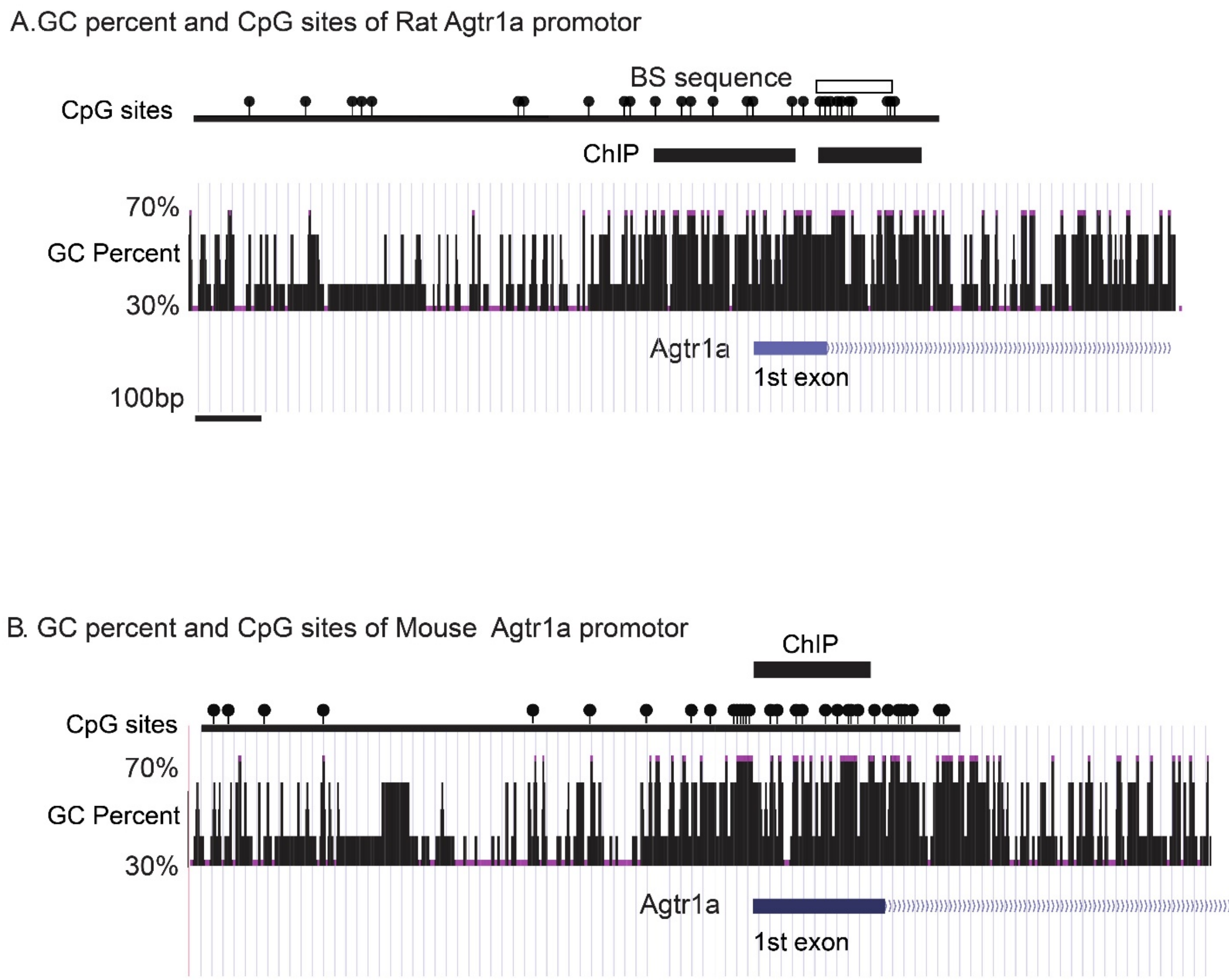
Supplemental Fig. 3



Supplemental Fig. 3

Real-time PCR of Tet 1,2,3 and MeCP2 mRNA in the paraventricular nucleus (PVN) of NP- (n=12) and LP-treated (n=14) offspring. Filled circles: NP group; open circles: LP group. Throughout, data represent means ± SEM. No significant differences were observed between NP and LP (Student's t-test).

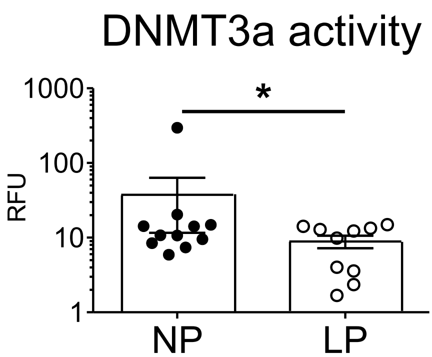
Supplemental Fig. 4



Supplemental Fig. 4

GC percentages and CpG sites in the *Agtr1a* locus (CpG sites were estimated using the PyroMark Assay Design 2.0 software (Qiagen, Valencia, CA, USA), and GC percentages were from the UCSC Genome Browser gateway; rat assembly: RGSC6.0/rno, mouse assembly: GRC m38/mm10). Upper panel, rat; lower panel, mouse. DNA segments analyzed by bisulfite sequencing (BS) and ChIP are indicated by white and black bars, respectively.

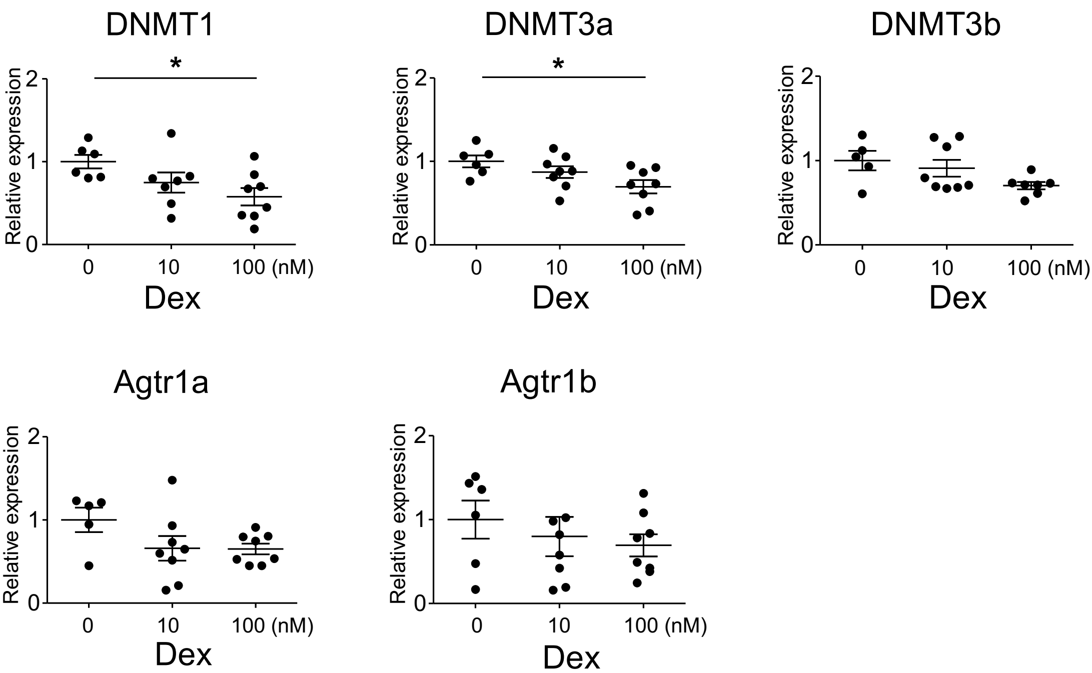
Supplemental Fig. 5



Supplemental Fig. 5

Activity of DNMT3a in the PVN of NP-treated (n=11) and LP-treated offspring (n=10). Filled circles: NP-treated offspring; open circles: LP-treated offspring (P<0.05, Mann-Whitney U test).

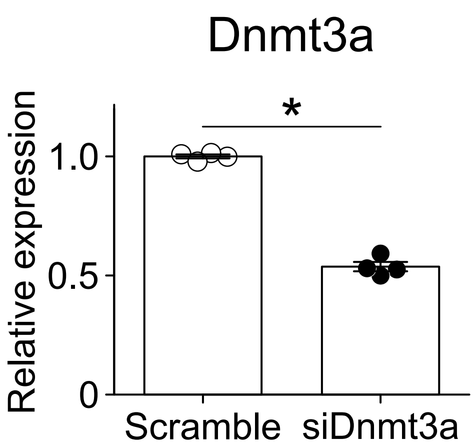
Supplemental Fig. 6



Supplemental Fig. 6

Twenty-four hour treatment with dexamethasone decreased the levels of *Dnmt1* and *Dnmt3a* mRNA in PVN cells. qRT-PCR of *Dnmt1*, *Dnmt3a*, *Agtr1a*, and *Agtr1b* mRNA was performed following 24 h treatment with vehicle, 10 nM Dex, or 100 nM Dex (n=5–8, *P<0.05, ANOVA post hoc by linear trend test).

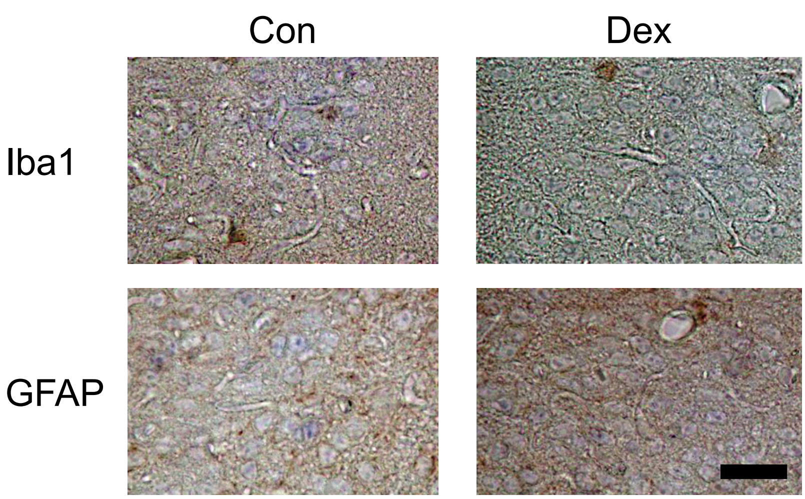
Supplemental Fig. 7



Supplemental Fig. 7

Inhibition of DNMT3a expression by transfection of siRNA against *Dnmt3a* into a mouse PVN cell line. (n=4, *P<0.05, t-test)

Supplemental Fig. 8



Supplemental Fig. 8

In situ hybridization of *Agtr1a* mRNA in the PVN of C57BL6/J, control (left), and Dex-treated offspring (right). Hybridization with an antisense probe indicates expression of *Agtr1a* mRNA (blue). Upper panels show staining for Iba1, a marker of glia cells (brown), and lower panels show GFAP, a marker of astrocytes (brown). *Agtr1a* co-localized with neither Iba1 nor GFAP, suggesting that it is expressed mainly in neuronal cells. Dex treatment did not affect the expression pattern in glia or astrocytes. Black bars, 50 μm.

Supplemental Table 1

Selected Gene Ontology (GO) pathways differentially expressed in the PVN of Dex-treated rats

Pathway	P-value	Benjamini
Calcium signaling pathway	9.4×10^{-9}	2.0×10^{-6}
Amphetamine addiction	1.10×10^{-6}	1.10×10^{-4}
Neuroactive ligand-receptor interaction	1.30×10^{-6}	9.60×10^{-5}
Retrograde endocannabinoid signaling	3.50×10^{-5}	1.90×10^{-3}
Circadian entrainment	8.60×10^{-5}	3.70×10^{-3}
Long-term potentiation	2.80×10^{-4}	1.00×10^{-2}
Nicotine addiction	3.10×10^{-4}	9.50×10^{-3}
Dopaminergic synapse	3.60×10^{-4}	9.50×10^{-3}
Serotonergic synapse	8.60×10^{-4}	1.80×10^{-2}
Oxytocin signaling pathway	9.00×10^{-4}	1.80×10^{-2}
Glutamatergic synapse	1.50×10^{-3}	2.70×10^{-2}
Aldosterone synthesis and secretion	1.90×10^{-3}	3.00×10^{-2}
Rap1 signaling pathway	2.30×10^{-3}	3.50×10^{-2}
GABAergic synapse	2.40×10^{-3}	3.30×10^{-2}
MicroRNAs in cancer	2.90×10^{-3}	3.80×10^{-2}
Cholinergic synapse	2.90×10^{-3}	3.60×10^{-2}
MAPK signaling pathway	4.10×10^{-3}	4.80×10^{-2}
Pathways in cancer	5.90×10^{-3}	6.50×10^{-2}
African trypanosomiasis	7.10×10^{-3}	7.30×10^{-2}
Ras signaling pathway	1.00×10^{-2}	9.6×10^{-2}

These results were obtained using DAVID 6.8.

P-values were determined by modified Fisher’s exact test.

Benjamini: False discovery rate calculated by the Benjamini-Hochberg method.

Supplemental Table 2

a.

Specific primers for amplification of regions flanking the *Agtr1a* TSS, and primers for ChIP assays
Rat *Agtr1a*
<u>TSS -97/+107:</u>
Forward: 5´-GAACAGGACTCAAGCCACGA-3´
Reverse: 5´-AACTAGAGAGGGAGCG GGTT-3´
<u>TSS +174/+336:</u>
Forward: 5´-CTTCAATTGTTGGCCCCGC-3´
Reverse: 5´-CTCCGGATCAGGTGG GAGTA-3´
Mouse *Agtr1a*
<u>TSS +15/+251:</u>
Forward: 5´-CTTAGGGTTGGAACCTGCGG-3´
Reverse: 5´-CTGGGCTAGGACTCAC CCG-3´

b.

TaqMan primers used for RT-PCR

Dnmt1	Rat; Rn00109664_m1	Mouse; Mm01151063_m1
Dnmt3a	Rat; Rn01027162_g1	Mouse; Mm00432881_m1
Dnmt3b	Rat; Rn01536418_g1	Mouse; Mm01240113_m1
Agtr1a	Rn02758722_s1	Mouse; Mm01957722_s1
Agtr1b	Rat; Rn02132799_s1	Mouse; Mm02620758_s1
Hsd11b2	Rat; Rn04341420_g1	————————
Mc4r	————————	Mouse; Mm0457483_s1
Mecp2	Rat; Rn01529606_g1	Mouse; Mm01193537_g1
Tet1	Rat; Rn01428192_m1	Mouse; Mm01169087_m1
Tet2	Rat; Rn01522037_m1	Mouse; Mm00524395_m1
Tet3	Rat; Rn01425643_m1	Mouse; Mm00805756_m1
Actb	Rat; Rn00667869_m1	Mouse; Mm02619580_g1