



Supplementary Figure S1. Effects of prenatal conditions on adult male brain methylation. Nine loci (*Bdnf* promoter region 1, *Bdnf* promoter region 2, *Crhr1*, *Fkbp5*, *Htr1a*, *Maoa*, *Nos1*, *Nr3c1* promoter, and *Nr3c1* NGFI-A binding site) were analyzed in 6 brain regions (amygdala, frontal cortex, hippocampus, hypothalamus, raphe nuclei, and striatum) of adult male mice. In the bar diagrams (mean \pm SEM) for each gene and brain region, blue bars indicate prenatal CTRL and green bars prenatal EE conditions. MS, CTRL, and EE represent postnatal conditions. The six treatment groups are shown in the following order, preCTRL/postMS vs. preEE/postMS, preCTRL/postCTRL vs. preEE/postCTRL, preCTRL/postEE vs. preEE/postEE. Significant differences between preCTRL and preEE animals of a given offspring group are indicated by thin vertical lines



Supplementary Figure S2. Effects of prenatal conditions on adult female brain methylation. Nine loci (*Bdnf* promoter region 1, *Bdnf* promoter region 2, *Crhr1*, *Fkbp5*, *Htr1a*, *Maoa*, *Nos1*, *Nr3c1* promoter, and *Nr3c1* NGFI-A binding site) were analyzed in 6 brain regions (amygdala, frontal cortex, hippocampus, hypothalamus, raphe nuclei, and striatum) of adult female mice. In the bar diagrams (mean \pm SEM) for each gene and brain region, blue bars indicate prenatal CTRL and green bars prenatal EE conditions. MS, CTRL, and EE represent postnatal conditions. The six treatment groups are shown in the following order, preCTRL/postMS vs. preEE/postMS, preCTRL/postCTRL vs. preEE/postCTRL, preCTRL/postEE vs. preEE/postEE. Significant differences between preCTRL and preEE animals of a given offspring group are indicated by thin vertical lines

Supplementary Table S1. PCR and sequencing primers.

Gene	Primer	Sequence (5'-3') ^a	Amplicon length (bp)	Chromosomal localisation (bp) ^b	Gene region	No. of CpGs
<i>Bdnf-1</i>	Forward	GGGGTTAGGGTAGTTTTTTTGAG	316	Chr. 2: 109,674,340- 109,674,656	Promoter: <i>Bdnf</i> -201, -206, -207, -208, and -210	3
	Reverse	^a ACACAAATTCCTATAAAACAATAACT				
	Sequencing 1	TTTTTTTTTTATTATGATTAAGGGT				
Sequencing 2	AGTGATTTGTTTTAGGGAATA	3				
Sequencing 3	TTTTTTAGGGATTGATGATTAG	3				
<i>Bdnf-2</i>	Forward	AGGTAGAGGAGGTATTATATGATAGT	343	Chr. 2: 109,692,371- 109,692,714	Promoter: <i>Bdnf</i> -203, -204, -205, and -211 Intron 1: -201, -206, -207, -208, and -210	4
	Reverse	^a CCCCAAAATTCTAAACTCTTACTATA				
	Sequencing 1	AGGAGGTATTATATGATAGTT				
Sequencing 2	GAGGAGAGGATTGTT	4				
<i>Crhr1</i>	Forward	^a AGGAGGTTAGAGAAGAAAGGTTGTT	271	Chr. 11: 104,132,485- 104,132,756	Promoter: <i>Crhr1</i> -201	7
	Reverse	ATCCCCTCCTCTTTCTCCCT				
	Sequencing	TACTAAACCCCTCT				
<i>Fkbp5</i>	Forward	TGGGGGTTGGATAATAGGT	227	Chr. 17: 28,517,552- 28,517,779	Promoter: <i>Fkbp5</i> -202 and -206	5
	Reverse	^a AAACATCCTCCCCTTCCTCA				
	Sequencing 1	TTTAAAAAAGTTGGGTTTG				
Sequencing 2	GGGAATTTTTGGAGT	4				
<i>Htr1a</i>	Forward	GGGATGGAGATGAGAAAGTTAATG	245	Chr. 13: 105,444,517- 105,444,762	Exon 1: <i>Htr1a</i> -201	7
	Reverse	^a ATACTACCCATAACTACTCTATACCA				
	Sequencing	ATGAGAAAGTTAATGAGTTAAG				
<i>Maoa</i>	Forward	^a TGGAGAAGTTTAGTATTATAGGTTATATGT	124	Chr. X: 16,619,850- 16,619,974	Exon 1/Intron 1: <i>Maoa</i> -201	4
	Reverse	CAAATTCCTACCCCTAATCATCT				
	Sequencing	ACTACTATTA AAAATCCAAACTAAC				
<i>Nos1</i>	Forward	AGTTTTGAATTTGGTTAGGTTAGAG	254	Chr. 5: 117,842,043- 117,842,297	Promoter: <i>Nos1</i> -201 Exon1: -206 Intron 1: -203 and -205	3
	Reverse	^a ATCCAAA ACTAAAACTCACCTACT				
	Sequencing 1	GTTTTGGAAGTTTTTAT				
Sequencing 2	AAGAGGTTGGGAGGG	3				

<i>Nr3c1</i> pro- moter	Forward Reverse Sequencing	GGGAGAAGTTGTAAAGTAGAA ^a AAATACAAAAAACCCAACTC GAGTTTGAGAGGAGG	242	Chr. 18: 39,490,688- 39,490,930	Promoter: <i>Nr3c1</i> -201, -203, -206, and -208 Intron 1: -205, -207	7
<i>Nr3c1</i> NGFI-A	Forward Reverse Sequencing	^a GGGGGTTTTGGAGGTAGATTTA ATTTCTCTTCTCCCTAACTCCT CTCCCTAACTCCTC	95	Chr. 18: 39,490,248- 39,490,343	Promoter: <i>Nr3c1</i> -203, -205, and -207	4

^a primer biotinylated at the 5'end

^b according to Ensembl release 87 - December 2016

Supplementary Table S2. Two-way ANOVAs for postnatal environmental manipulations on adult brain methylation patterns of prenatal cohort 1 (standard environment).

Brain region	Gene	ANOVA		
		Sex	Postnatal	Sex * Postnatal
Amygdala	<i>Bdnf-1</i>	-	F(2,61)=4.00; p<0.05	-
	<i>Bdnf-2</i>	-	-	-
	<i>Crhr1</i>	F(1, 70)=16.32; p<0.0001	F(2, 70)=3.17; p<0.05	F(2, 70)=7.19; p<0.01
	<i>Fkbp5</i>	-	-	F(2, 65)=4.73; p<0.05
	<i>Htr1a</i>	F(1, 71)=7.49; p<0.01	-	-
	<i>Maoa</i>	F(1, 64)=2244; p<0.001	-	F(2, 64)=6.96; p<0.01
	<i>Nos1</i>	F(1, 78)=7.92; <0.01	-	-
	<i>Nr3c1</i>	F(1, 77)=25.43; p<0.0001	-	-
	<i>NGFI-A</i>	-	-	-
	Frontal cortex	<i>Bdnf-1</i>	-	-
<i>Bdnf-2</i>		F(1, 48)=16.80; p<0.001	-	-
<i>Crhr1</i>		-	-	-
<i>Fkbp5</i>		-	-	-
<i>Htr1a</i>		-	-	-
<i>Maoa</i>		F(1, 57)=1559; p<0.0001	-	-
<i>Nos1</i>		-	-	-
<i>Nr3c1</i>		-	-	-
<i>NGFI-A</i>		F(1, 69)=18.56; p<0.0001	-	-
Hippo-campus		<i>Bdnf-1</i>	-	-
	<i>Bdnf-2</i>	-	-	-
	<i>Crhr1</i>	F(1, 63)=21.95; p<0.001	-	-
	<i>Fkbp5</i>	-	-	-
	<i>Htr1a</i>	-	-	-
	<i>Maoa</i>	F(1, 64)=1860; p<0.0001	-	-
	<i>Nos1</i>	-	-	-
	<i>Nr3c1</i>	-	-	-
	<i>NGFI-A</i>	-	-	-
	Hypo-thalamus	<i>Bdnf-1</i>	-	-
<i>Bdnf-2</i>		-	-	-
<i>Crhr1</i>		F(1, 51)=15.12; p<0.001	-	-
<i>Fkbp5</i>		-	-	-
<i>Htr1a</i>		-	-	-
<i>Maoa</i>		F(1, 47)=1431; p<0.0001	-	-
<i>Nos1</i>		-	-	-
<i>Nr3c1</i>		-	-	-
<i>NGFI-A</i>		F(1, 52)=5.12; p<0.05	-	F(2, 52)=4.53; p<0.05
Raphe nuclei		<i>Bdnf-1</i>	-	-
	<i>Bdnf-2</i>	-	-	-
	<i>Crhr1</i>	-	-	-
	<i>Fkbp5</i>	-	-	-
	<i>Htr1a</i>	-	-	-
	<i>Maoa</i>	F(1, 68)=515.2; p<0.0001	-	-

	<i>Nos1</i>	-	-	-
	<i>Nr3c1</i>	-	-	-
	<i>NGFI-A</i>	-	-	-
Striatum	<i>Bdnf-1</i>	-	-	-
	<i>Bdnf-2</i>	-	-	F(1, 53)=5.02; p<0.05
	<i>Crhr1</i>	-	-	-
	<i>Fkbp5</i>	-	-	-
	<i>Htr1a</i>	F(1, 68)=9.23; p<0.01	-	-
	<i>Maoa</i>	F(1, 56)=592.6; p<0.0001	-	-
	<i>Nos1</i>	-	-	-
	<i>Nr3c1</i>	-	-	-
	<i>NGFI-A</i>	-	-	-

Supplementary Table S3. Two-way ANOVAs for postnatal environmental manipulations on adult brain methylation patterns of prenatal cohort 2 (enriched environment).

Brain region	Gene	Sex	ANOVA Postnatal	Sex * Postnatal
Amygdala	<i>Bdnf-1</i>	-	-	-
	<i>Bdnf-2</i>	-	-	-
	<i>Crhr1</i>	F(1, 62)=29.95; p<0.0001	F(2, 62)=4.61; p<0.05	-
	<i>Fkbp5</i>	-	-	-
	<i>Htr1a</i>	F(1, 70)=14.51; p<0.0001	-	F(2, 70)=7.25; p<0.01
	<i>Maoa</i>	F(1, 62)=779.3; p<0.0001	-	-
	<i>Nos1</i>	-	-	-
	<i>Nr3c1</i>	F(1, 71)=42.53; p<0.0001	-	-
	<i>NGFI-A</i>	-	-	-
Frontal cortex	<i>Bdnf-1</i>	F(1, 47)=6.17; p<0.05	-	-
	<i>Bdnf-2</i>	-	-	-
	<i>Crhr1</i>	-	-	-
	<i>Fkbp5</i>	-	-	-
	<i>Htr1a</i>	-	-	-
	<i>Maoa</i>	F(1, 58)=1739.85; p<0.0001	-	-
	<i>Nos1</i>	F(1, 63)=24.99; p<0.0001	F(2, 63)=6.96; p<0.01	-
	<i>Nr3c1</i>	-	-	-
	<i>NGFI-A</i>	F(1, 70)=18.89; p<0.0001	-	-
Hippo-campus	<i>Bdnf-1</i>	-	-	-
	<i>Bdnf-2</i>	-	-	-
	<i>Crhr1</i>	F(1, 54)=11.39; p<0.001	-	-
	<i>Fkbp5</i>	F(1, 65)=16.75; p<0.0001	-	-
	<i>Htr1a</i>	-	-	-
	<i>Maoa</i>	F(1, 67)=2032.11; p<0.0001	-	-
	<i>Nos1</i>	-	-	-
	<i>Nr3c1</i>	-	-	-
	<i>NGFI-A</i>	-	-	-
Hypo-thalamus	<i>Bdnf-1</i>	F(1, 60)=17.72; p<0.0001	-	-
	<i>Bdnf-2</i>	-	-	-
	<i>Crhr1</i>	F(1, 68)=19.67; p<0.001	-	-
	<i>Fkbp5</i>	-	-	-
	<i>Htr1a</i>	-	-	-
	<i>Maoa</i>	F(1, 51)=2942; p<0.0001	-	-
	<i>Nos1</i>	-	-	-
	<i>Nr3c1</i>	-	F(2, 75)=5.04; p<0.01	-
	<i>NGFI-A</i>	-	-	F(1, 66)=4.35; p<0.05
Raphe nuclei	<i>Bdnf-1</i>	F(1, 41)=7.76; p<0.01	-	-
	<i>Bdnf-2</i>	-	F(2, 49)=6.26; p<0.01	-
	<i>Crhr1</i>	-	-	-
	<i>Fkbp5</i>	-	-	-
	<i>Htr1a</i>	-	-	-
	<i>Maoa</i>	F(1, 63)=234.4; p<0.0001	-	-

	<i>Nos1</i>	F(1, 39)=12.60; p<0.01	-	-
	<i>Nr3c1</i>	-	-	-
	<i>NGFI-A</i>	-	-	-
Striatum	<i>Bdnf-1</i>	-	-	-
	<i>Bdnf-2</i>	-	-	-
	<i>Crhr1</i>	-	-	-
	<i>Fkbp5</i>	-	-	-
	<i>Htr1a</i>	F(1, 62)=17.05; p<0.0001	-	-
	<i>Maoa</i>	F(1, 45)=290.0; p<0.0001	-	-
	<i>Nos1</i>	-	-	-
	<i>Nr3c1</i>	-	-	-
	<i>NGFI-A</i>	F(1, 63)=23.30; p<0.0001	F(2, 63)=3.88; p<0.05	-