

Supplementary data

Figure S1. Sociability test.

(A), (C) After 4 and 8 weeks of neonatal VPA exposure, the social index was measured by comparing the time spent in the empty zone and the stranger zone through a social interaction test.

(B), (D) The social preference index was measured by comparing the time spent in the stranger zone and the familiar zone. Values represent the mean \pm Standard deviation.

The significance level between the groups is indicated by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

((A), male control $n = 21$, male VPA $n = 22$, female control $n = 20$, female VPA $n = 15$;

(B), male control $n = 5$, male VPA $n = 6$, female control $n = 4$, female VPA $n = 6$;

(C), male control $n = 19$, male VPA $n = 20$, female control $n = 16$, female VPA $n = 15$;

(D), male control $n = 4$, male VPA $n = 6$, female control $n = 5$, female VPA $n = 6$)).

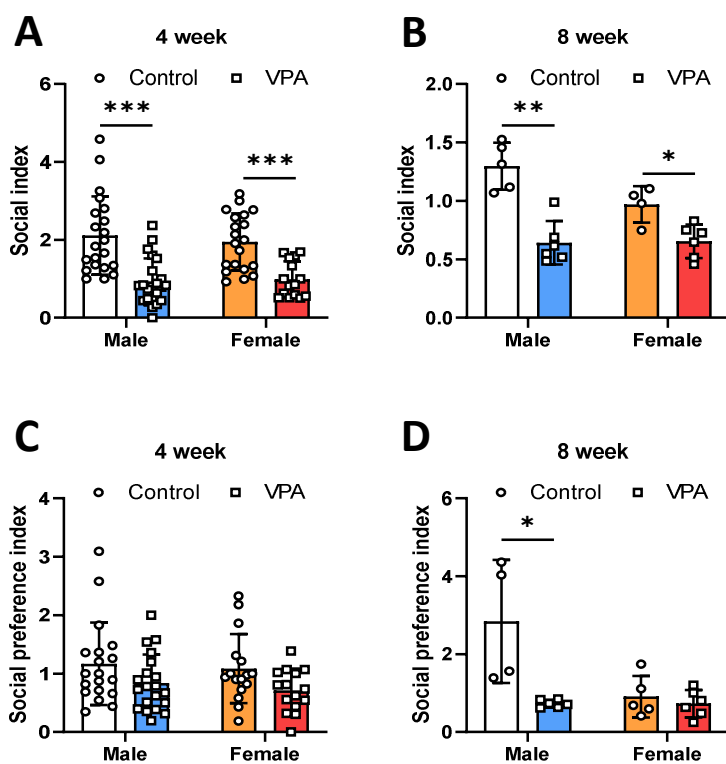


Figure S2. Neonatal VPA exposure have long-term effects on FOXO3 and Notch signaling protein expression in the 4 and 8 weeks old male hippocampus.

The protein levels of *Foxo3* (Fig. 3A; $t(21) = -2.090$, $p = 0.048$) and *Notch1* (Fig. 3C; $t(21) = -2.882$, $p = 0.009$, $p = 0.009$) were significantly increased only in the 4 weeks old male rats after VPA exposure.

Ascl1 protein levels were significantly decreased in 4 weeks old males (Fig. 3B; $t(21) = 2.260$, $p = 0.026$) and 8 weeks old males (Fig. 3B; $t(14) = 2.334$, $p = 0.035$).

No changes were observed in the females, and no sex-related differences were observed.

The significance level between the groups is indicated by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

((A) 4 weeks control $n = 12$, 4 weeks VPA $n = 11$, 8 weeks control $n = 8$, 8 weeks VPA $n = 8$;

(B) 4 weeks control $n = 12$, 4 weeks VPA $n = 11$, 8 weeks control $n = 12$, 8 weeks VPA $n = 12$;

(C) 4 weeks control $n = 12$, 4 weeks VPA $n = 11$, 8 weeks control $n = 8$, 8 weeks VPA $n = 8$).

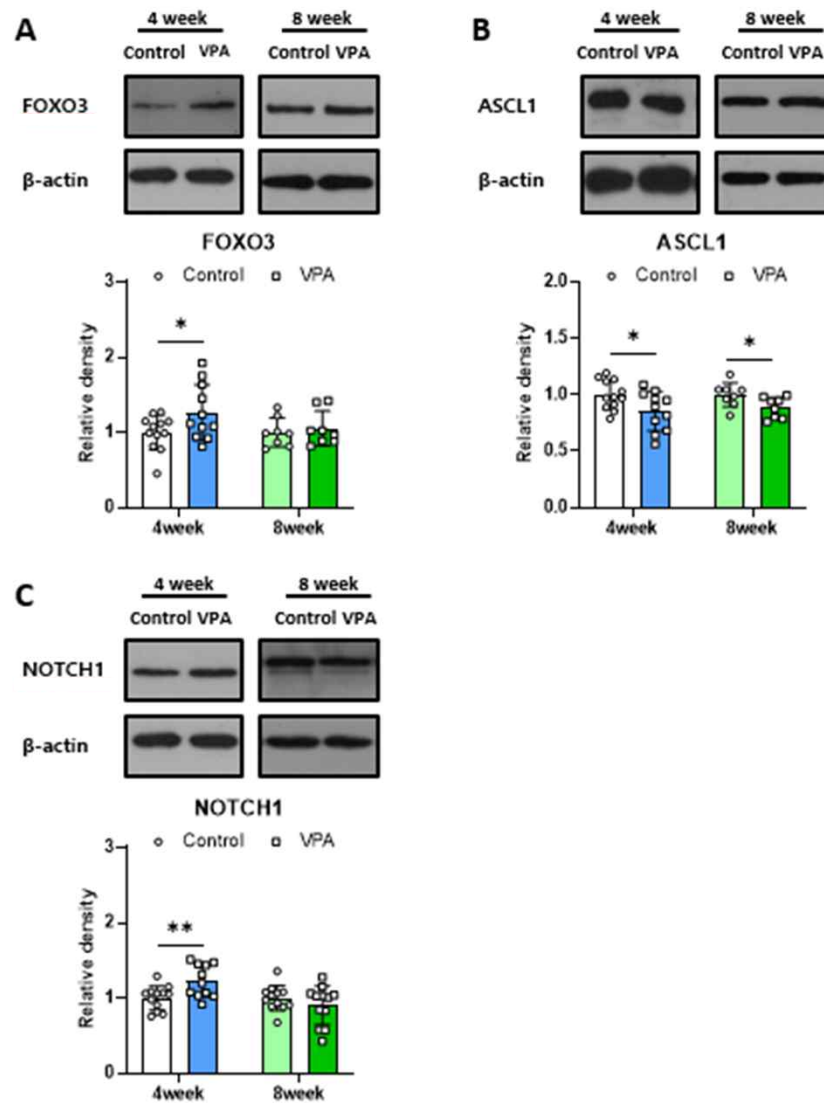


Table S1. Two-way ANOVA results Summary for VPA exposure and sex differences

Figure	Test	Gene	Sex		Treatment		Sex & Treatment	
			F	p-value	F	p-value	F	p-value
1	qPCR	<i>Ngn2</i>	5.355	0.026	0.074	0.787	4.694	0.037
		<i>Notch1</i>	64.787	0.000	13.525	0.001	3.249	0.081
		<i>Reln</i>	1.260	0.269	3.225	0.080	5.966	0.019
		<i>Lphn2</i>	0.874	0.356	3.636	0.064	1.441	0.237
3	Western blot	LC3-2/1	7.368	0.010	3.952	0.054	5.831	0.021
		P62	0.007	0.933	0.433	0.515	15.910	0.001
4	ChIP-qPCR	<i>Foxo3</i>	0.714	0.404	8.078	0.008	0.453	0.506
		<i>Ascl1</i>	5.563	0.024	0.007	0.936	2.298	0.139
5	DNA methylation & hydroxymethylation assay	5-mc	13.811	0.001	0.029	0.866	16.402	0.000
		5-hmc	4.670	0.037	0.711	0.405	0.080	0.778
	MERE-based qPCR	<i>Foxo3</i>	0.138	0.713	7.035	0.012	0.471	0.497
		<i>Ascl1</i>	5.004	0.031	2.472	0.124	1.613	0.212

Table S2. Primer list

Primers for RT-qPCR		
Gene name	Forward sequence (5'→3')	Reverse sequence (5'→3')
<i>Gapdh</i>	AGTGCCAGCCTCGTCTCATA	AGAGAAGGCAGCCCTGGTAA
<i>FoxO3</i>	CAGACCCTCAAACCTGACCGAA	GCTGCTAACAGTCTCTGCTGG
<i>Ascl1</i>	GGGATCCTACGACCCCCTTAG	AGACACAGGATCTCCTGCCAT
<i>Ngn2</i>	CGGGTAGGATGTTCGTCAAGT	ACCCAGCAGCATCAGTACCT
<i>Hes1</i>	GCGCCGGGCAAGAATAAA	TTCCAGAATGTCTGCCTTCTCC
<i>Hes6</i>	AGG CCA AGC TAG AGA ACG C	GGATGTAGCCAGCAGCGAA
<i>Notch1</i>	GTGCGCTCTAGGTGCCAA	TCT GAC ATG GGT TGG AGA TGC
<i>Reln</i>	AGTCCCTGGGGTTCTACCTC	CATGCTCGAGGCAAGTTTCG
<i>Lphn2</i>	TTTTCCCCTGCCAGAGAGAT	CTGTGGAAGCCATGCAGAGA
Primers for ChIP-qPCR		
Gene name	Forward sequence (5'→3')	Reverse sequence (5'→3')
<i>FoxO3</i>	AACCGGAAAAGCGTAGCTCA	GGTATAGGGTTGTCGCGGAG
<i>Ascl1</i>	TTTTCCAAGTTCTCAAGAGACTCC	GGTTTTAAAAGAGGAAAGGGAAAA
Primers for MSRE-based qPCR		
Gene name	Forward sequence (5'→3')	Reverse sequence (5'→3')
<i>FoxO3</i>	GAGGTGTATTTTCGCGGGGA	AGGTGATGAACGTGCTGGTC
<i>Ascl1</i>	CAGGTTAACCAACTTGACCCG	GAACTGATGCGCTGCAAACG

Table S3. Antibody list

Antibody	Dilution for WB	Manufacturer	Cat. No.
β -actin	1:3000	Cell signaling	4970
FOXO3	1:1000	Cell signaling	12829
ASCL1	1:1000	Abcam	ab74065
NOTCH1	1:1000	Cell signaling	3608
LC3	1:1000	Cell signaling	4108
P62	1:1000	Abcam	ab56416
H3K9me2	1:1000	Abcam	ab1220
H3K36me1	1:1000	Cell signaling	14111
H3K18ac	1:1000	Abcam	ab1191
H3K27ac	1:1000	Abcam	ab4729
HistoneH3	1:1000	Abcam	ab1791
DNMT1	1:1000	Abcam	ab13537
DNMT3a	1:1000	Abcam	ab2850
HRP-labeled rabbit	1:5000	Invitrogen	31460
HRP-labeled mouse	1:5000	Invitrogen	31430