Science Advances
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Supplementary Materials for

Early-life environmental enrichment generates persistent individualized behavior in mice

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Published 26 August 2020, *Sci. Adv.* **6**, eabb1478 (2020)
DOI: 10.1126/sciadv.abb1478

The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/35/eabb1478/DC1)

Supplementary Figures

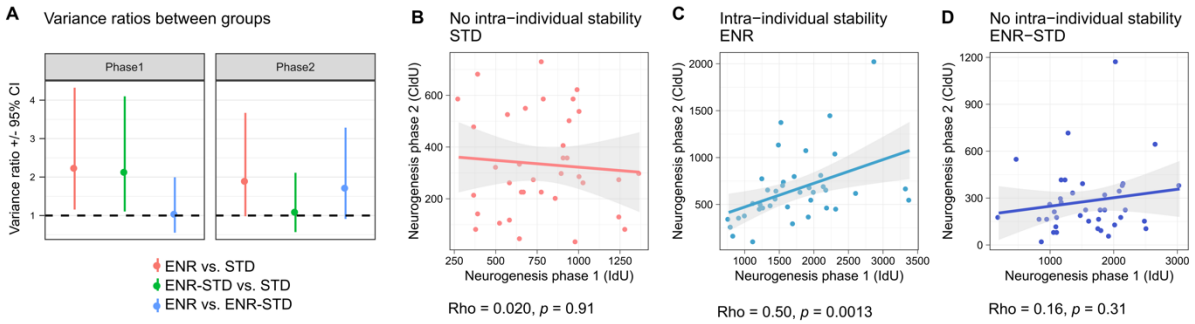

Fig. S1: ENR stabilized individual levels of adult hippocampal neurogenesis during aging. **A,** Pairwise variance ratios of adult hippocampal neurogenesis with 95 % confidence intervals (CI). Dashed line at unity indicates no differences in variance between the two groups. **B-D,** Correlations of individual levels of adult hippocampal neurogenesis between phase 1 and phase 2 (IdU vs CldU) in mice separated by groups. Depicted are Spearman's rho and *p*-value of the rank correlation. (n = 36 (STD); 39 (ENR); 40 (ENR-STD)).
Figure refers to content in Figure 3.

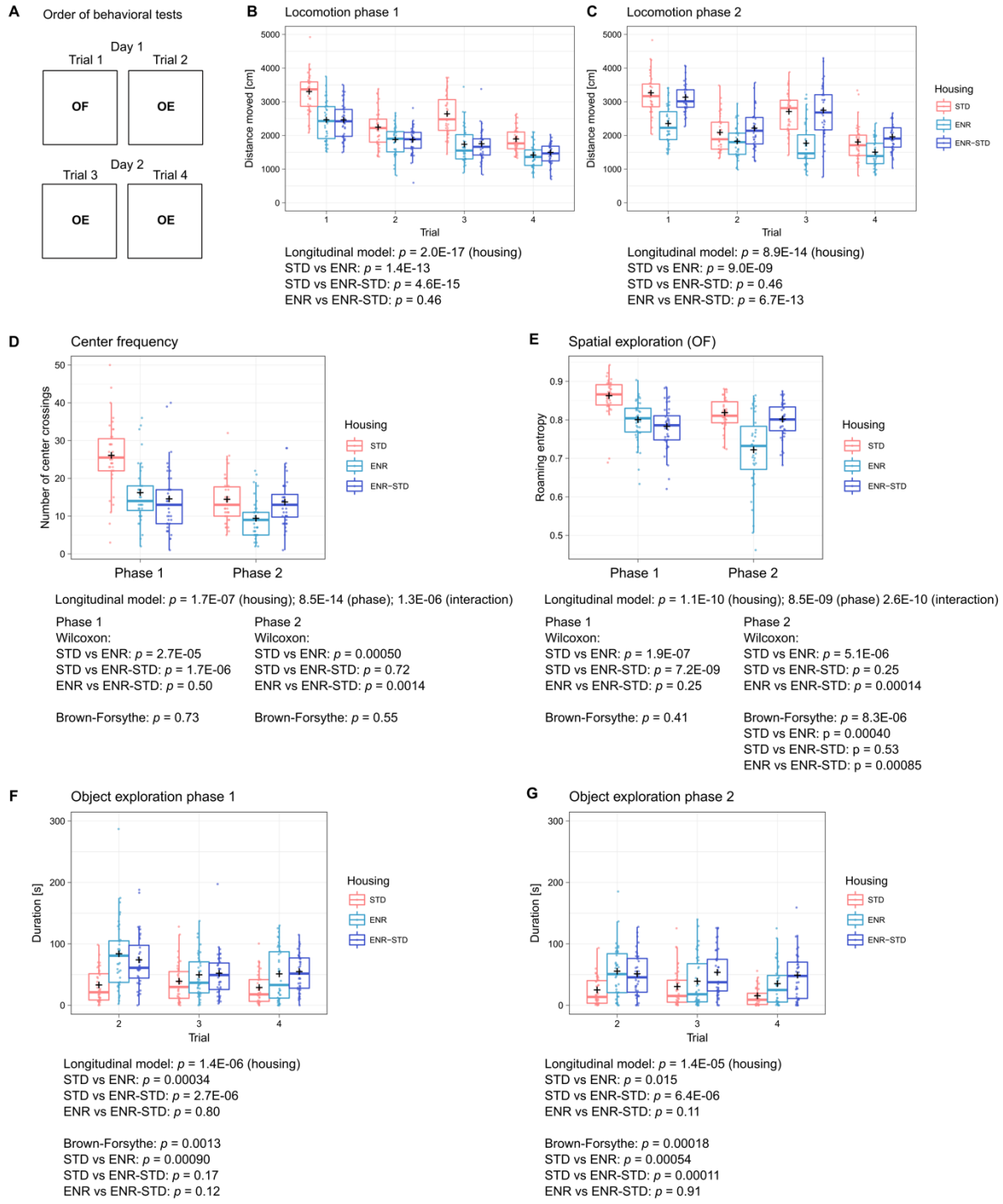

Fig. S2: Results of cross-sectional behavioral testing. ENR-induced behavioral changes in the open field are plastic towards environmental stimulation and reversed after withdrawal from ENR, while ENR-induced changes in object exploration are maintained after environmental change. **A,** Timeline for behavioral testing in phases 1 and 2. One trial of open field (OF) test and three trials of object exploration (OE) test were performed in the same arena on two consecutive days. **B,** Locomotion during individual trials of OF and OE tests. At the end of phase 1, ENR and ENR-STD mice traveled shorter distances compared to STD mice. **C,** At the end of phase 2, ENR mice maintained reduced levels of locomotion in OF and OE tests, while ENR-STD mice traveled distances similar to STD mice. **D,** ENR decreased the frequency of center passage in OF test. Decrease in frequency of center passage was not maintained in ENR-STD after withdrawal from ENR. **E,** ENR mice showed reduced roaming entropy in the open field compared to STD mice. Withdrawal from ENR increased roaming entropy in the open field to the levels observed in STD mice, while ENR mice showed reduced means and higher variances. **F-G,** Object exploration during all three trials of the OE test in phase 1 and phase 2. (n = 38 (STD); 39 (ENR); 40 (ENR-

STD)). Depicted *p* - values from Brown-Forsythe tests refer to housing effects. See Supplementary data 1 for detailed statistical results.
Figure refers to content in Figure 4.

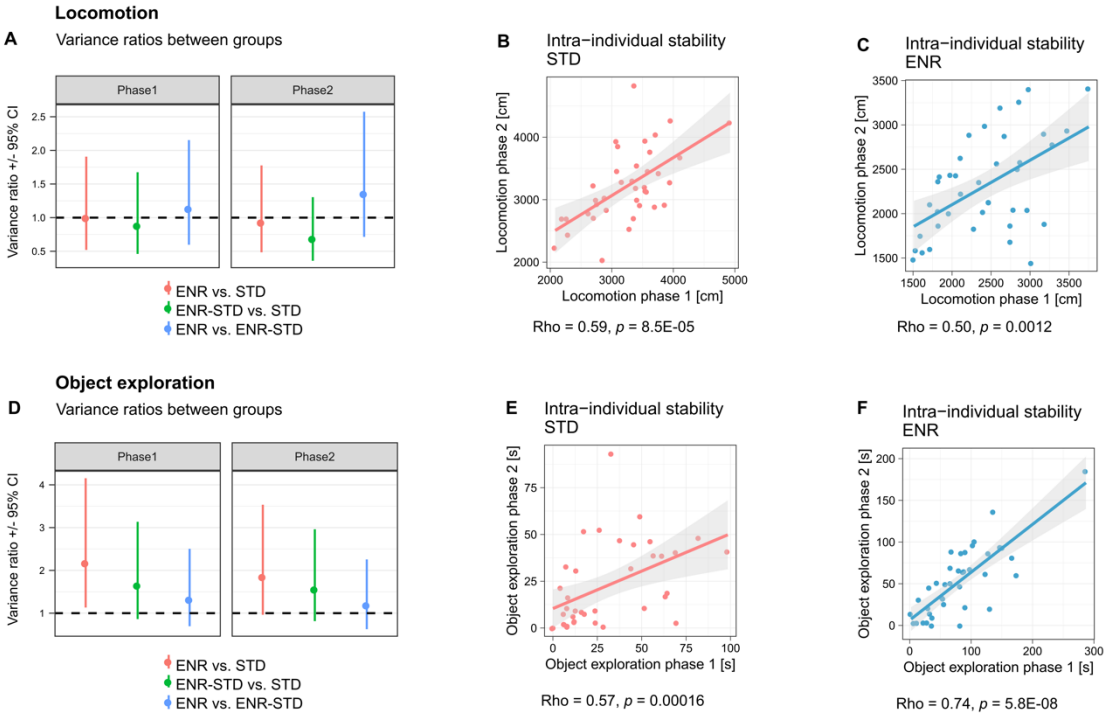

Fig. S3: Variance ratios and intra-individual stability of object exploration and locomotion. **A,** Confidence intervals (CI, 95 %) for pairwise variance ratios of locomotion in the open field test. Dashed line at unity indicates no difference between groups. **B-C,** Correlations of individual locomotion in open field test between phase 1 and phase 2. **D,** Confidence intervals for variance ratios of object exploration (time around objects). **E-F,** Correlations of object exploration in the first trial of the object exploration test between phase 1 and phase 2. Depicted are Spearman's rho and *p* - values of the rank correlation. (n = 38 (STD); 39 (ENR); 40 (ENR-STD)).
Figure refers to content in Figure 4.

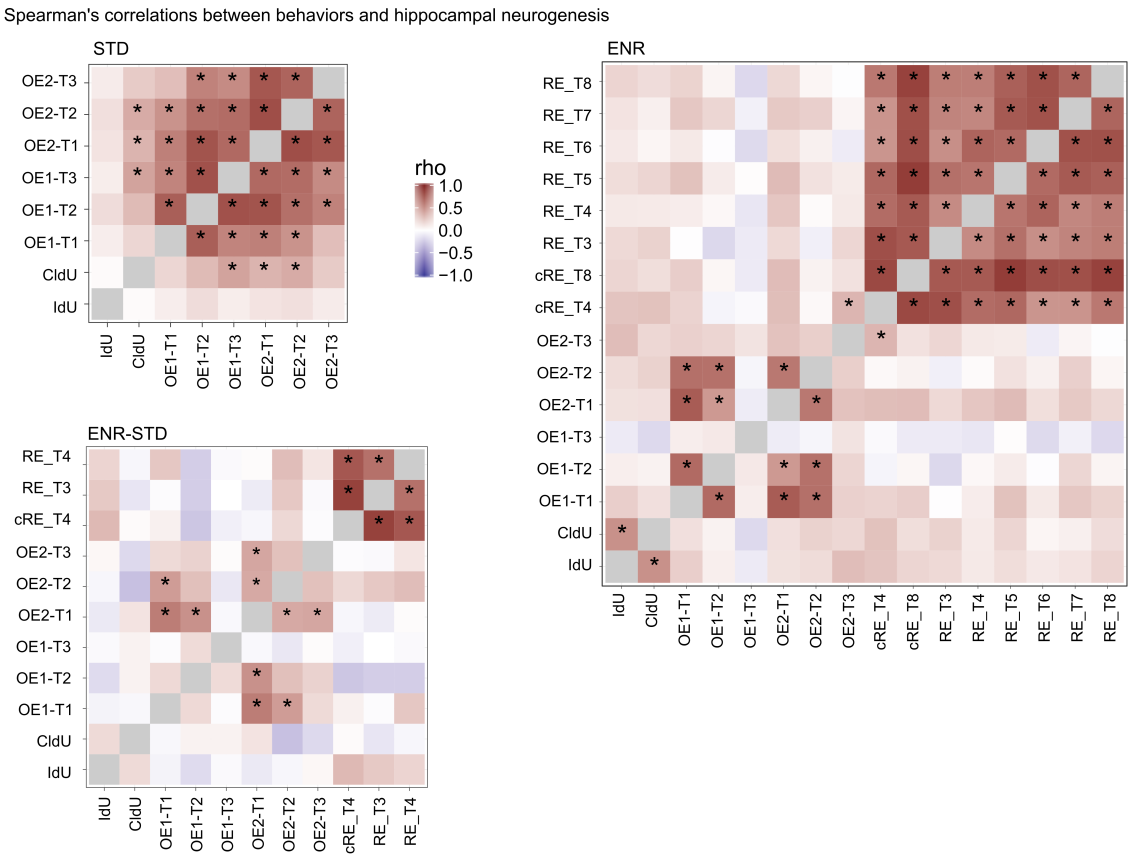

Fig. S4: Associations between exploratory behavior and adult hippocampal neurogenesis. Depicted are matrices of Spearman's correlations between behavioral traits and adult neurogenesis in STD, ENR and ENR-STD mice. Asterisks highlight significant correlations with $p < 0.05$. OE1-T1/2/3 – object exploration phase 1 trial 1/2/3, OE2-T1/2/3 – object exploration phase 2 trial 1/2/3, RE_T1-T8 – roaming entropy in time blocks 3-8, cRE_T4/8 – cumulative roaming entropy time blocks 4/8.

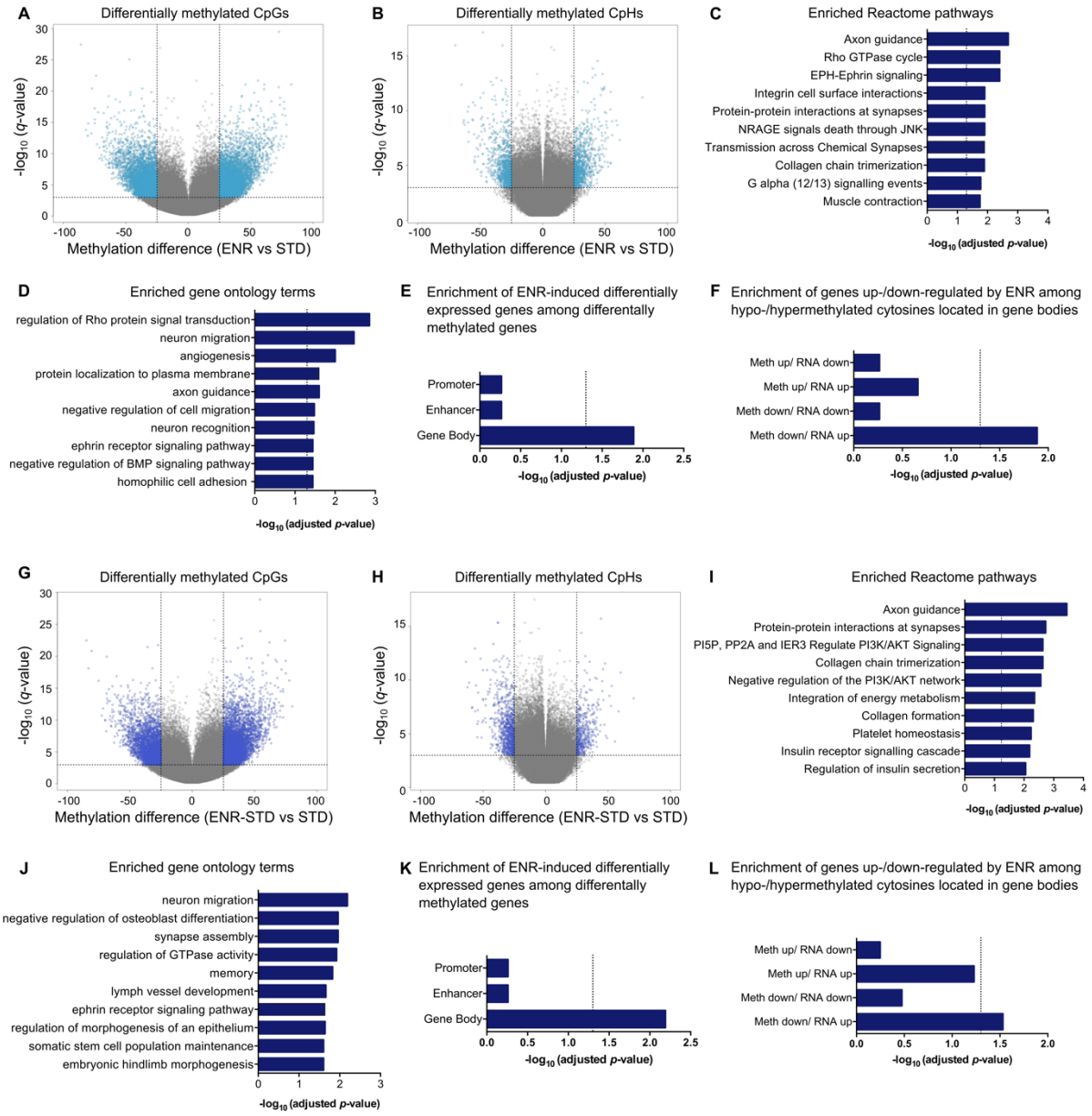

Fig. S5: DNA methylation differences between ENR and STD mice as well as ENR-STD and STD mice. **A-B,** Volcano plots depicting significantly differentially methylated CpGs and CpHs ($q < 0.001$, methylation difference > 25 %) between ENR and STD mice in pale blue. **C-D,** Genes with ENR-induced differentially methylated cytosines (CpGs or CpHs) are enriched at neuronal plasticity pathways. **E,** Genes with previously reported ENR-induced gene expression changes in the dentate gyrus (*21*) are enriched among genes with ENR-induced differentially methylated cytosines located in gene bodies, but not among genes with ENR-induced promoter or enhancer methylation changes. **F,** Genes with ENR-induced hypomethylation (Meth down) of gene bodies, significantly overlap with transcripts upregulated (RNA up) by ENR. Depicted are results from hypergeometric testing. **G-H,** Significantly differentially methylated cytosines between ENR-STD and STD mice depicted in dark blue. **I-J,** Gene ontology and pathway enrichment of genes differentially methylated between ENR-STD and STD. **K-L,** Gene body methylation changes in ENR-STD mice were detected at genes with up-regulated expression after ENR.
Figure refers to content in Figure 5.

Supplementary Tables

Table S1: Variance estimates from linear mixed models with 95 % confidence intervals.

Phenotype	Housing	Vind	Vind CI_low	Vind CI_up	Vres	Vres CI_low	Vres CI_up	R	R CI_low	R CI_up
RE T1	ENR	0.00	0.00	0.14	0.99	0.81	1.19	0.00	0.00	0.13
RE T2	ENR	0.14	0.00	0.33	0.45	0.33	0.64	0.26	0.03	0.46
RE T3	ENR	0.10	0.05	0.19	0.51	0.44	0.58	0.19	0.09	0.38
RE T4	ENR	0.14	0.07	0.24	0.66	0.59	0.74	0.22	0.11	0.37
RE T5	ENR	0.20	0.11	0.34	0.63	0.56	0.71	0.30	0.18	0.56
RE T6	ENR	0.28	0.15	0.48	0.84	0.75	0.93	0.33	0.19	0.59
RE T7	ENR	0.12	0.06	0.23	0.75	0.67	0.84	0.17	0.08	0.31
RE T8	ENR	0.23	0.14	0.42	0.81	0.71	0.91	0.30	0.16	0.52
Neurogenesis	STD	0.00	0.00	0.03	0.11	0.08	0.16	0.01	0.00	0.23
Neurogenesis	ENR	0.17	0.00	0.40	0.32	0.20	0.54	0.38	0.04	0.63
Neurogenesis	ENR-STD	0.01	0.00	0.16	0.36	0.24	0.52	0.02	0.00	0.34
Locomotion	STD	15.07	7.35	29.55	9.84	6.45	16.29	0.64	0.39	0.79
Locomotion	ENR	16.35	5.32	33.12	15.11	9.57	25.58	0.55	0.26	0.74
Locomotion	ENR-STD	0.52	0.00	9.36	17.01	11.07	25.15	0.02	0.00	0.39
Initial object exploration	STD	3.38	1.52	6.67	2.40	1.53	4.03	0.60	0.35	0.78
Initial object exploration	ENR	7.23	4.13	12.69	2.71	1.74	4.36	0.75	0.56	0.86
Initial object exploration	ENR-STD	4.03	1.56	7.96	3.61	2.23	5.78	0.59	0.29	0.74

Vind – inter-individual variance component; Vres – residual variance component; R – repeatability; CI_low – lower confidence interval; CI_up – upper confidence interval.

Table S2: Comparison of models with heterogeneous inter-individual and residual variances with models assuming homogeneous variance. Reported are values of deviance information criterion (DIC) for the full and simplified models and the difference between simplified and full models. Reduction of ΔDIC by at least 2 units suggests better fit to the data.

Phenotype	**Full model**	**Homogeneous Vind**	**ΔDIC (full – simpler model)**	**Homogeneous Vres**	**ΔDIC (full – simpler model)**
Neurogenesis	373.5	387.1	13.6	380.1	6.6
RE	9856.9	9962.0	105.1	9907.0	50.1
Locomotion	1365.1	1369.9	4.9	1371.6	6.6
Initial object exploration	1017.2	1016.6	-0.6	1016.8	-0.4

Vind – inter-individual variance component; Vres – residual variance component.

Table S3: Posterior mode of inter-individual correlation of RE between time blocks with 95 % confidence intervals.

Time blocks	Pearson's r	Lower confidence interval	Upper confidence interval
PeriodT1:PeriodT1	0.99903806	1	1
PeriodT2:PeriodT1	0.1050056	-0.6417686	0.76093968
PeriodT3:PeriodT1	0.06890519	-0.782984	0.88019508
PeriodT4:PeriodT1	0.06739862	-0.7943345	0.85552608
PeriodT5:PeriodT1	-0.0353727	-0.8474715	0.96942138
PeriodT6:PeriodT1	-0.1458702	-0.9285464	0.86032641
PeriodT7:PeriodT1	-0.0286419	-0.9233632	0.87755538
PeriodT8:PeriodT1	0.04904015	-0.8793511	0.91660039
PeriodT1:PeriodT2	0.1050056	-0.6417686	0.76093968
PeriodT2:PeriodT2	0.99903806	1	1
PeriodT3:PeriodT2	0.62675542	0.15179004	0.89196265
PeriodT4:PeriodT2	0.27451313	-0.1665017	0.75833962
PeriodT5:PeriodT2	0.51584507	0.03470389	0.87748919
PeriodT6:PeriodT2	0.2503437	-0.1693521	0.79855866
PeriodT7:PeriodT2	0.43645247	-0.0507094	0.86714146
PeriodT8:PeriodT2	0.43357102	-0.0532153	0.84537966
PeriodT1:PeriodT3	0.06890519	-0.782984	0.88019508
PeriodT2:PeriodT3	0.62675542	0.15179004	0.89196265
PeriodT3:PeriodT3	0.99903806	1	1
PeriodT4:PeriodT3	0.67141489	0.32522859	0.8908061
PeriodT5:PeriodT3	0.86125272	0.61712202	0.97355785
PeriodT6:PeriodT3	0.7469976	0.44112478	0.92995947
PeriodT7:PeriodT3	0.80697785	0.52671953	0.96993771
PeriodT8:PeriodT3	0.81531877	0.52616635	0.95047219
PeriodT1:PeriodT4	0.06739862	-0.7943345	0.85552608
PeriodT2:PeriodT4	0.27451313	-0.1665017	0.75833962
PeriodT3:PeriodT4	0.67141489	0.32522859	0.8908061
PeriodT4:PeriodT4	0.99903806	1	1
PeriodT5:PeriodT4	0.80075574	0.59216473	0.94572527
PeriodT6:PeriodT4	0.81534247	0.58450869	0.94840719
PeriodT7:PeriodT4	0.79930587	0.50348182	0.94713855
PeriodT8:PeriodT4	0.78018085	0.53527346	0.93517763
PeriodT1:PeriodT5	-0.0353727	-0.8474715	0.96942138
PeriodT2:PeriodT5	0.51584507	0.03470389	0.87748919
PeriodT3:PeriodT5	0.86125272	0.61712202	0.97355785
PeriodT4:PeriodT5	0.80075574	0.59216473	0.94572527
PeriodT5:PeriodT5	0.99903806	1	1
PeriodT6:PeriodT5	0.89999346	0.73213161	0.97462696
PeriodT7:PeriodT5	0.93310049	0.7656326	0.98704948
PeriodT8:PeriodT5	0.92159991	0.76446734	0.98884355
PeriodT1:PeriodT6	-0.1458702	-0.9285464	0.86032641
PeriodT2:PeriodT6	0.2503437	-0.1693521	0.79855866
PeriodT3:PeriodT6	0.7469976	0.44112478	0.92995947
PeriodT4:PeriodT6	0.81534247	0.58450869	0.94840719
PeriodT5:PeriodT6	0.89999346	0.73213161	0.97462696
PeriodT6:PeriodT6	0.99903806	1	1
PeriodT7:PeriodT6	0.9176525	0.77199618	0.98363845
PeriodT8:PeriodT6	0.92546987	0.77807902	0.98505228
PeriodT1:PeriodT7	-0.0286419	-0.9233632	0.87755538
PeriodT2:PeriodT7	0.43645247	-0.0507094	0.86714146
PeriodT3:PeriodT7	0.80697785	0.52671953	0.96993771
PeriodT4:PeriodT7	0.79930587	0.50348182	0.94713855
PeriodT5:PeriodT7	0.93310049	0.7656326	0.98704948
PeriodT6:PeriodT7	0.9176525	0.77199618	0.98363845
PeriodT7:PeriodT7	0.99903806	1	1
PeriodT8:PeriodT7	0.91430631	0.73417398	0.98550421
PeriodT1:PeriodT8	0.04904015	-0.8793511	0.91660039
PeriodT2:PeriodT8	0.43357102	-0.0532153	0.84537966
PeriodT3:PeriodT8	0.81531877	0.52616635	0.95047219
PeriodT4:PeriodT8	0.78018085	0.53527346	0.93517763
PeriodT5:PeriodT8	0.92159991	0.76446734	0.98884355
PeriodT6:PeriodT8	0.92546987	0.77807902	0.98505228
PeriodT7:PeriodT8	0.91430631	0.73417398	0.98550421
PeriodT8:PeriodT8	0.99903806	1	1

Captions of Supplementary data files 1-3

Supplementary Data 1: Detailed results of statistical analysis of behavioral data and adult hippocampal neurogenesis. Data related to Figures 3-4 and S2.

Supplementary Data 2: Lists of differentially methylated cytosines between ENR and STD mice (sheet 1), differentially methylated cytosines between ENR-STD and STD (sheet 2) and list of ENR-induced differentially methylated cytosines maintained after withdrawal from ENR (sheet 3). Data related to Figures 5 and S5.

Supplementary Data 3: R code for mixed linear models and repeatability calculation related to results in Figures 2-4.